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AN INTRODUCTION TO BIOINORGANIC CHEMISTRY

Presa Universitară Clujeană

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This text is an updated translation of a book originally published in Romanian in 2011 under the title „Metalele în sistemele vii". While the book served primarily as lecture material for Bioinorganic Chemistry classes, our aim is generally to provide some basic notions of bioinorganic chemistry, written in such a way so as to be accessible to undergraduates or graduates interested in the topic as well as to members of a more general audience interested in knowing more about the roles of metals in living systems.

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Chapter 1. Introduction

Living systems, as defined in biochemical terms (highly organized entities in which each part has a function, where there is a direct relationship between function and structure, capable to extract energy from the environment, capable to replicate and capable to adapt to the environment),[1] have a greatly variable chemical composition depending on species or even on individual, as well as on the environment. The living systems known so far tend to feature certain common structural elements, as illustrated in Figure 1. Thus, even the simplest systems are organized into cells delimited by a membrane. This membrane is formed by a physical aggregation of lipids. The complex aqueous chemical mixture enclosed inside the membrane is called cytosol. The cytosol typically contains most of the molecules required for survival, growth and cell reproduction. These so-called 'biomolecules' tend to belong to a small number of classes, common to all species. In general the most abundant molecule in a cell is water. Its role is essential in living organisms - as solvent, as stabilizing agent for biomolecules, and as a direct participant in essential reactions for cell functioning.

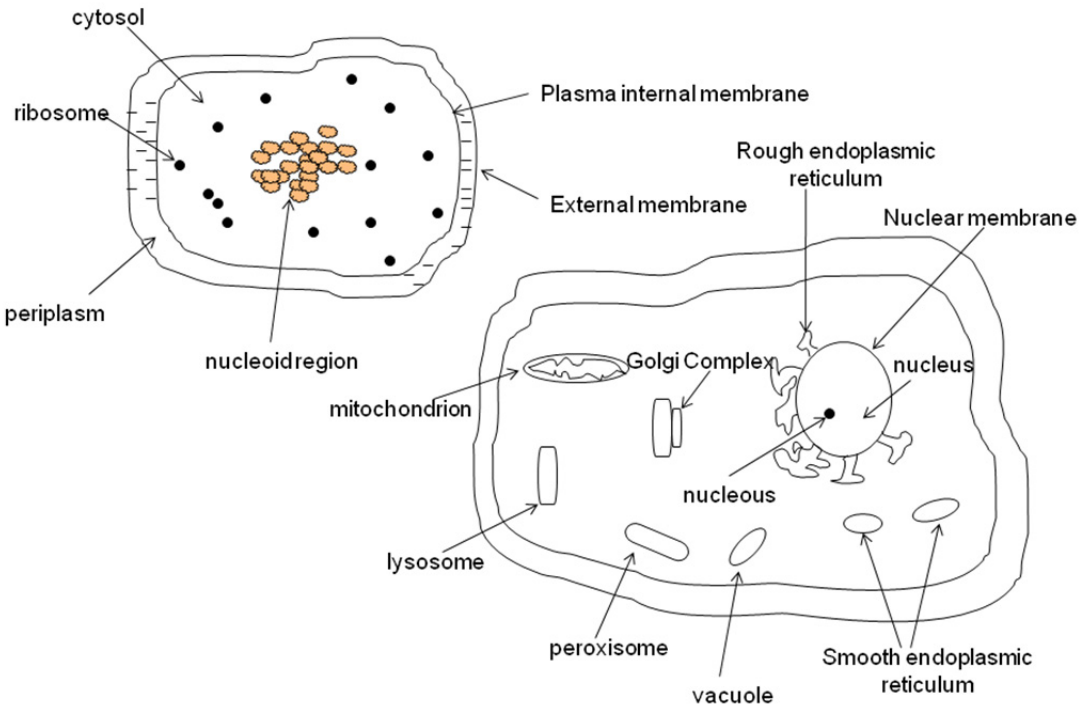


Figure 1. Schematic representations of some living cells. Top: prokaryotic cell type. Bottom: higher organized cell (eukaryotic).

In a typical cell, the second abundant class of compounds are the proteins (usually at least several thousand kinds of proteins, differing in chemical composition and concentration, are present in a cell), followed by nucleic acids (DNA and RNA, the latter also being present as several thousand molecules distinct from each other) and then lipids. Also present in the cell are several hundred small molecules whose utility is either as temporary storage for energy (e.g., adenosine triphosphate, ATP, synthesized to store in a readily chemical accessible the energy released in various chemical reactions, or starch synthesized as storage form of glucose), or as direct energy source (e.g. glucose), or source of atoms needed to build other molecules for the proper functioning of the cell (e.g. carbon dioxide in plant leaves), or with structural roles (e.g., trehalose, a sugar used to adjust the osmotic pressure in some organisms). With the notable exception of water, all molecules above are, in chemical terms, organic (i.e., constructed on carbon skeletons and using other nonmetals as accessories). It should be noted however that many of them are not able to maintain the structure or to execute their function *in vivo* without the assistance / presence of metal ions. Thus, to the best of our knowledge, there is no living system able to work in the absence of all metals. This book aims to present an overview, not intended to be exhaustive, of the types of processes involving metals in living systems. This area of interest, at the intersection of biochemistry and inorganic chemistry, is defined by various experts under various names: bioinorganic chemistry, inorganic biological chemistry, or inorganic biochemistry.[2-6] Throughout the book we will assume that the reader has basic knowledge of chemistry and biology to the level they are taught in most high-schools. For completion of certain passages in detail, also recommended is a summary knowledge of biochemistry.[1, 7] In the first chapter we summarize some basic methodology concepts on spectroscopy, useful primarily for students wishing to devote time to practical work in bioinorganic chemistry. The remaining chapters present various classes of processes in living systems where metals play an important role, with emphasis on notions like oxygen-transport proteins, sensor-proteins, selective activation of molecular oxygen, xenobiotic metabolism, electron-transport proteins, cytochrome oxidases, processes involving sodium, potassium and calcium, proteins involved in the nitrogen cycle, hydrogenases, methanogenesis, life in absence of oxygen, hydrolases, anhydrases, bioorganometallic chemistry, photosynthesis, elements of medicinal bioinorganic chemistry, oxidative stress, free radicals.

Chapter 2. Experimental methods in bioinorganic chemistry

In most cases, biologically relevant metal complexes (whether metallo-proteins, synthetic compounds, or of other kind) are available in research laboratories in limited quantities, usually in relatively dilute solutions (concentrations in the μM-mM range). The purification methods for these compounds are similar to those applied to most biomolecules, and are discussed in detail in references 1 and 7. In this chapter we briefly present the most common spectroscopic methods applicable to metalloproteins and related complexes, and also discuss x-ray diffraction (a non-spectroscopic experimental technique of utmost importance to the field).

2.1. Electronic absorption spectroscopy and related methods (UV-vis, CD, MCD, VT / VH-MCD, fluorescence)

The operating principle of this class of methods is probably already known to students who have followed a specialization in chemistry, physics or biology. We resume briefly: one sends a beam of light from the UV-vis-NIR domains (ultraviolet - visible near infrared, ~ 200 nm - 1500 nm) through the sample of interest, after which one measures to the light after passing through the sample. Responsible for this absorption are the electrons placed in the outer ("valence") orbitals of the molecules in the sample; by absorbing the energy of the photons, these electrons are promoted to higher, previously unoccupied, orbitals. In the case of fluorescence spectroscopy, one measures the light emitted at a 90°-angle with respect to the incident light; this light is generated as a result of processes subsequent to the absorption. Figure 2 illustrates schematically the operating principles described above. The extent to which the sample absorbs light is quantified in various ways, including absorbance (describing the percentage of light absorbed by the sample) which is most often used, while transmission (describing the percentage of light is not absorbed by the sample, essentially inversely proportional to absorbance) is relatively rarely used in current experiments in bioinorganic laboratories.

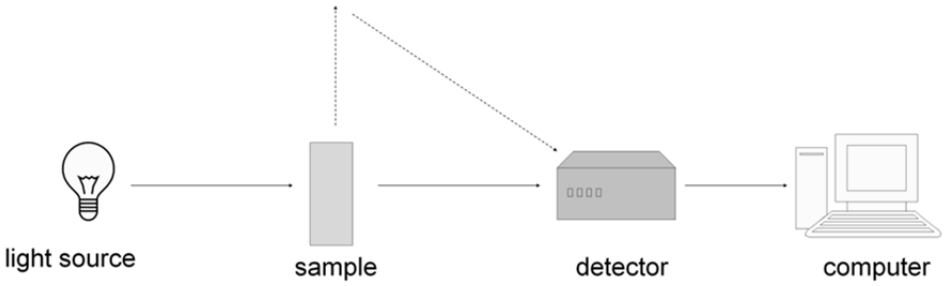


Figure 2. Operation scheme of UV-VIS electronic absorption spectroscopies; for fluorescence spectroscopy the detector is at a 90 ° angle with respect to the incident beam (pathway shown in dotted line).

UV-vis spectroscopy

Results for typical electronic absorption spectroscopy measurements, using light from the 200 nm-800 nm energy domain (the ultraviolet and visible domains, making for the experiment being usually denoted by the term "UV-vis spectrum") are typically presented graphically as a plot absorbance = function of energy, where the energy is expressed in nanometers (i.e., the wavelength of the light). The absorbance at a given wavelength obeys the Lambert-Beer law, namely $A = \varepsilon * b * c$, where b is the length of path traversed by light through the sample (typically 1 cm for measurements performed on liquid samples), c is the molar concentration of the sample and ε is molar extinction coefficient or absorptivity expressed in $M^{-1}cm^{-1}$ (so that the absorbance is a dimensionless quantity). ε is a unique property of the given substance for a particular wavelength, and the value reflects, in a predictable way, elements of the electronic structure of the substance. As mentioned above, the incident photon is able to be absorbed by one of the electrons in the valence shell of a substance in the sample; in order for this phenomenon of absorption to take place, it is necessary that the photon energy be equal to the energy difference between the orbital where the electron already is and the orbital where the electron will move after photon absorption. Therefore, the position and number of bands (absorption maxima) observed in the spectrum of a compound reflects the relative energies of the frontier orbitals of that compound. The efficiency with which a compound absorbs light of a certain energy is empirically described by the extinction coefficient ε, and is determined by the nature of the orbitals involved in the process (symmetry type, occupancy, similarity degree). Thus, among the weakest intensity bands (magnitude of ε up several hundred) observable in bioinorganic chemistry are those characteristic to transition metal ions, where the electron undergoes a transition from one *d* orbital

to another *d* orbital. On the other hand, when electrons migrate from a molecular orbital predominantly localized on a ligand to an orbital predominantly localized on the metal, the movement of the electron between the two orbitals ipon absorbtion of light will formally be a charge migration phenomenon (charge transfer from ligand to metal, with LMCT acronym from "ligand to metal charge transfer" currently used in the literature); in such situations usually extinction coefficients reach values of several thousands. In the same area tend to fall the extinction coefficients of some common cofactors as NAD(P) (nicotinamide adenine dinucleotide) or FMN / FAD (flavin adenine mono/di-nucleotide), which means that such centers will often obscure the absorption bands due to metal centers. Not of the same problem suffer a class of metal centers found in hemoglobin: here, an extended conjugated system (porphyrin, which coordinates the central iron, forming a so-called heme type structure) has available a large number of π and π * orbitals, with compatible symmetries, giving rise to bands ranging in intensity up to 200 000 M^{-1} cm^{-1} - which are most the intense possible in a biological relevant metal complex. Figure 3 shows spectra of three classes of metal complexes, illustrating the differences in extinction coefficients caused by the different nature of ligands present at the metal.

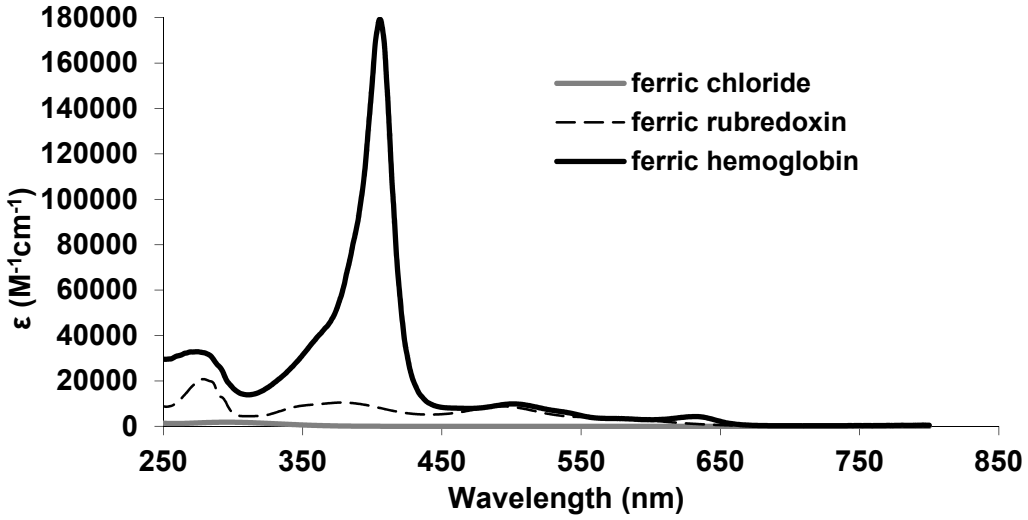


Figure 3. UV-vis spectra of a heme group, an iron-sulfur protein, and of Fe(III) chloride, illustrating (in this order) transitions: π-> π * (about 400 nm in the hemoglobin spectrum), LMCT (350 -600 nm in the rubredoxin spectrum) and respectively d-> d (ferric chloride).

UV-vis devices are among the most common tools in bioinorganic chemistry, requiring relatively small amounts of sample and having much lower costs than the

other tools discussed in this chapter; moreover, their operation is very simple and therefore does not require the presence a dedicated operator (or limited set thereof).

Circular dichroism spectroscopy (CD)

If the sample has chiral properties (which is the case of any protein even in the absence of a metal center), when the incident beam is polarized (light waves oscillate in a well-defined plane, unlike in normal light where the photons of the beam oscillate in planes at an infinite number of different angles to each other), the chiral sample will rotate the polarized light plane. CD spectra profit of this property; they are used relatively rarely in metalloproteins field, usually as a tool to investigate the protein part (for instance, in order to investigate the relative content of α or β-type structures, or the degree of denaturation); an illustration is given in Figure 4.

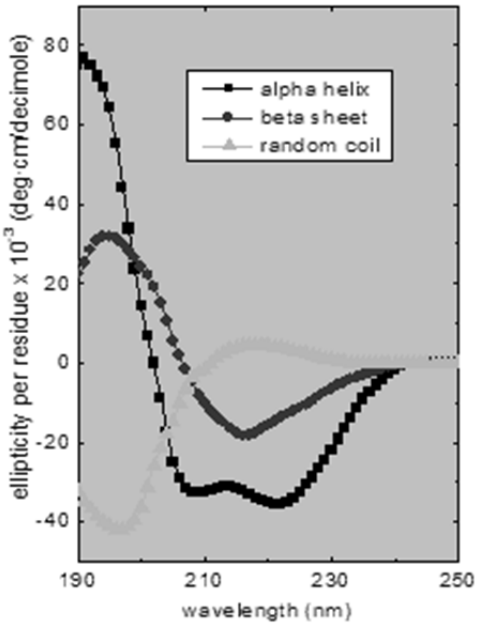


Figure 4. Examples of DC spectra, illustrating the manner in which they can be used to determine the secondary structure type of a protein.[8]

Magnetic circular dichroism (MCD, VT/VH-MCD)

If a CD experiment is performed with the sample in a magnetic field, one can selectively observe the signals due to paramagnetic centers – including thus certain transition metal centers. Moreover, the different behavior in terms of temperature dependence (measuring in the range of ~ 4-300 K) and magnetic field dependence for organic paramagnets compared to transition metals allows one to discern between these two classes of centers. Spectra measured at variable temperature and/or variable

field, VT / VH allow, after application of standard algorithms, for identification of the number of unpaired electrons and also for finding information about the nature of the ligand field present around the metal. Solomon and colleagues were among those who have shown how, with a database of reference spectra available, MCD spectroscopy may indicate the oxidation state, nuclearity degree and coordination number of important metal centers otherwise inaccessible to structural investigations of such detail.

Fluorescence

If the UV-vis experiment changes so that the detector is placed at a 90° angle to the incident beam, one can estimate the degree to which the electron excited by light can return to the fundamental energy level by emitting light instead of heat (i.e., fluorescence). This phenomenon is most commonly seen in the organic part of metalloproteins (aromatic amino acids as tryptophan, aromatic cofactors as FMN), and is therefore an indirect tool for investigating biological metal centers. Moreover, the method is at best semi quantitative, as laws such as Lambert-Beer do not apply in this case.

2.2 Vibrational spectroscopy (IR, Resonance Raman)

Vibrational spectroscopy in the infrared (IR) uses light in infrared domain (~ 100-4000 cm-1). The energy of the absorbed photons is used not for an electron jump, but rather for entrance of a chemical bond (or groups of bonds) into vibrational resonance. Therefore, the position of a band in the IR spectrum provides direct information on the strength of the bond involved in that vibration, or on the level/type of symmetry of the center (depending on the number of atoms involved in that vibration). Laws such as Lambert-Beer type can be applied to a lesser extent here, for technical reasons concerning sample preparation. Thus, whereas in the UV-vis spectroscopy liquid samples are quite common (usually as diluted solutions, in a standard solvent - preferably water for proteins), and these are usually put in a standard size cuvette transparent to UV-vis incident light (with the cuvette being made of glass, quartz, or plastic), there are much fewer materials transparent to IR light; most IR instruments use either potassium bromide pills into which the dry solid sample is dispersed by grinding, or suspensions of the sample in nujol oil

(hydrophobic) placed as a film between potassium bromide polished windows - which means that either optical path, or concentration, are difficult to control with precision and hardly reproducible. As far as we are concerned we will remark that the procedures described above are not applicable to proteins: the aqueous environment necessary for proteins is incompatible with potassium bromide. Where however a spectrum can be measured (by using special cuvettes, or by depositing the sample on a surface and measuring the reflection, with so-called ATR devices), spectra are extremely complicated due to overlapping vibrations of thousands of atoms with present metal. One of the possibilities of simplifying the spectrum is to measure the differences between the spectra of two different states of the protein, which differ only at the level of the metal center; thus protein bands essentially cancel each other out, allowing for selective detection of those bands whose positions are affected by the reaction connecting the two states of the protein. An attractive alternative for metal centers, since they have a UV-vis spectrum, is resonance Raman spectroscopy. In this experiment a Raman spectrum is measured (conceptually providing the same information as the classical IR spectrum, with some differences in the extent to which a band is visible - differences pertaining to the elements of symmetry), with the observation that the sample is excited with a laser beam of a wavelength similar to the absorption maxima from the UV-vis spectrum of the same sample. If this UV-vis band involves orbitals located on the metal, the laser tuned to that wavelength will cause selective excitation at the metal center and at the other atoms involved in the UV-vis transition; this selective excitation will translate into a significant increase of the relative intensity of the vibrational bands associated with these atoms, compared to all other bands, thus allowing selective observation of metal-associated vibrations even in molecules with hundreds of thousands of atoms, where the classical IR spectroscopy is not feasible. On the other hand, the laser used in the sample can induce unwanted photoreactions, which is why in bioinorganic one often seeks to obtain resonance Raman spectra chemistry using frozen samples.

Figure 5 shows a typical resonance Raman spectrum.

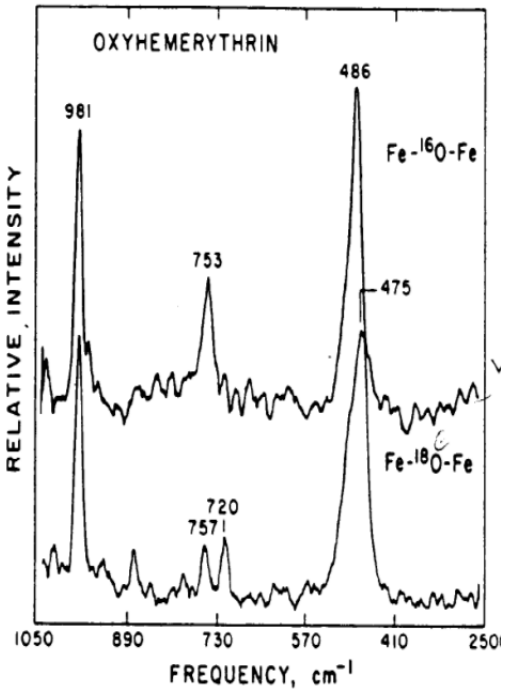


Figure 5. Raman resonance spectra of oxy-hemerythrin recorded with natural isotopes and with deuterium water or water marked with ^{18}O respectively.

2.3 Mössbauer Spectroscopy

This type of spectroscopy applies almost exclusively to iron, since it requires special isotopes, of which the most accessible happens to be ^{57}Fe; much rarer are examples of application Mössbauer spectroscopy for other elements as for example tin. This limitation, beyond the obvious disadvantage, has the advantage of a unique selectivity between the methods discussed in this chapter. The signals obtained, especially if measured at temperatures down to 4 K, allow identification of oxidation states, spin, and metal center nuclearity (including, for bi- or multinuclear centers, the coupling constants and implicitly the nature of bridging ligands between the two metals); also, by comparison with already measured spectra, one can draw conclusions about the nature of the ligands at the iron. The method is very costly, both in terms of the instrument itself, and because it detects only the iron isotope 57. Since this isotope is present in very low concentrations in nature, one will often seek to prepare samples starting from iron enriched in isotope 57.

A typical Mössbauer spectrum is shown in Figure 6. Note that a Mössbauer signal is described by parameters δ and ΔEq (isomers shift and quadrupole splitting),

whose values are referred to the metallic iron standard; the signal intensity is proportional to the concentration of iron in sample.

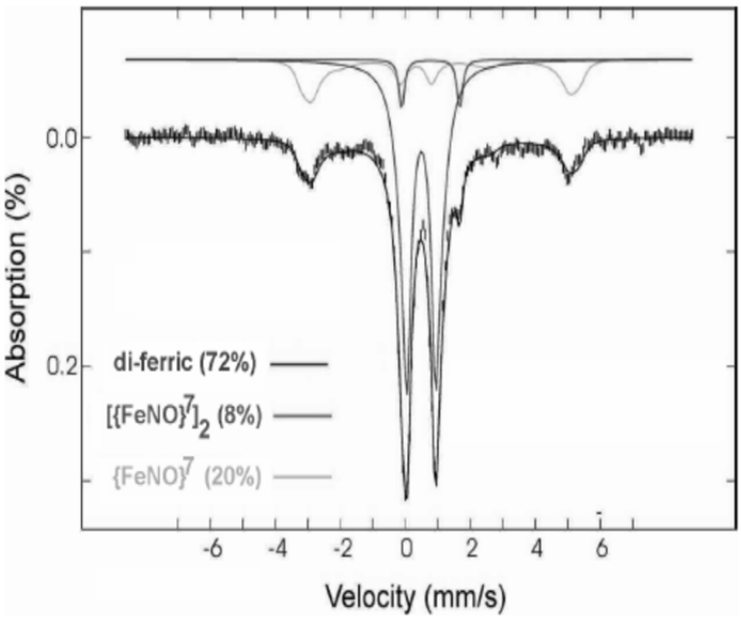


Figure 6. A representative Mössbauer spectrum. The dotted line represents experimental data while the continuous represent simulations, and a weighted sum of these, for the iron centers types shown in Figure.[9]

2.4 Electronic paramagnetic resonance spectroscopy (EPR, ESR)

Electronic paramagnetic resonance spectroscopy (EPR), also called electronic spin resonance (ESR) allows for a selective investigation of paramagnetic centers - especially of those which have an *odd* number of unpaired electrons. The interaction between the magnetic field and the electron in this case is based on the property of the electron to spin around its axis, which, considering that the electron is electrically charged, transforms the electron into a magnet. Under normal conditions, the relative orientation of the magnet in space is not subject to any *a priori* constraints in the molecule; on the other hand, if the molecule is placed into an external magnetic field, one can define two possible orientations of the electron: with its own magnetic field aligned parallel or antiparallel to the orientation of the externally-applied magnetic field. The energy difference between the two states is, by consequence, proportional to the magnetic field strength. By analogy with other types of spectroscopy, an electron can jump from the lower-energy state to the higher-energy state as illustrated in Figure 7, when the molecule is provided with external electromagnetic energy in the form of quanta whose energy is equal to the difference between the two levels shown in Figure; this energy is in the microwave region. In principle, the ESR

experiment would then involve placing the sample in a fixed-magnitude magnetic field and then probing it with a microwave beam of variable wavelength, tracking the wavelengths where the sample absorbs. However, for practical reasons, ESR instruments instead use varying magnetic field intensity while maintaining the microwave radiation at constant wavelength.

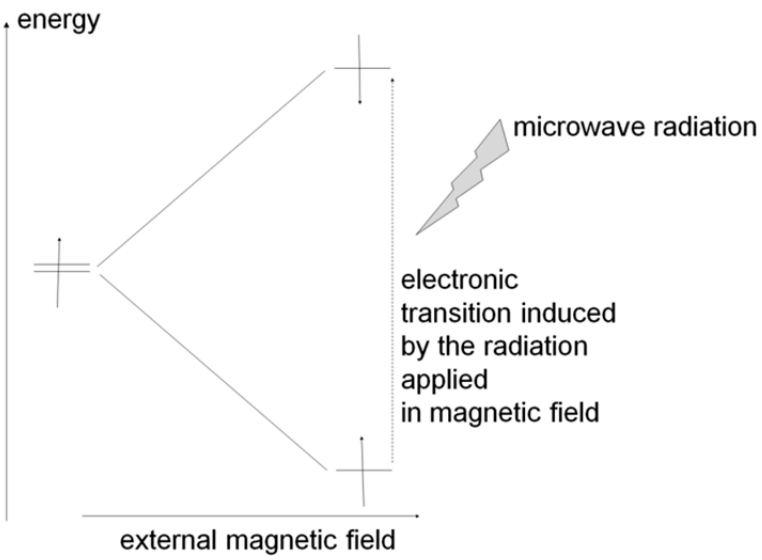


Figure 7. Operation principle of ESR spectroscopy.

Figure 8 illustrates a typical ESR spectrum, featuring signals due metal centers as well as to a non-metallic free radical.

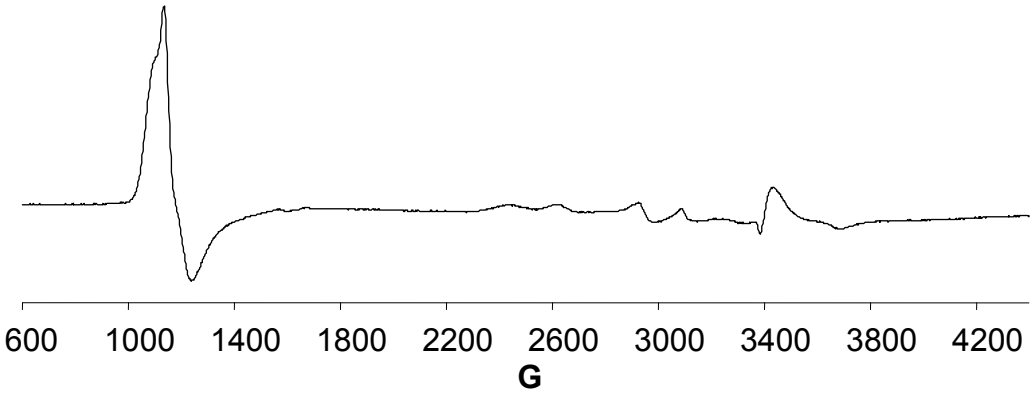


Figure 8. ESR spectrum of a reaction mixture containing metal centers: high-spin (S = 5 / 2, 1000 to 1400 G), low-spin (S = 1 / 2, 2000-3400 G) and a non-metallic free radical (NO, ~ 3300 G).

2.5. Nuclear magnetic resonance spectroscopy (NMR)

NMR operates on a similar principle as ESR. While the key in the ESR experiment was the magnetic property of the electron, in NMR the key is the magnetic property of the atomic nucleus. Similarly to the electron, the nucleus gives rise to a magnetic field. Similarly to the EPR case, the orientation of the nuclear magnetic field relative to the rest of the molecule is not submitted to any limitations under normal conditions, but is limited to two feasible quantum positions when the molecule is placed inside a magnetic field: namely, the nuclear magnetic dipole will be oriented parallel or antiparallel to the externally-applied magnetic field. Again as in the case of ESR, the sample will be exposed to microwave electromagnetic radiation, with absorption taking place only if the energy of the entering quantum is equal to the difference between the energies of the two states accessible to the nucleus, similarly to the situation shown in Figure 7 for ESR.

The NMR experiment can be applied to a large number of nuclei, ^{1}H and ^{13}C being by far the most common. The main condition is that the nuclear spin be different from zero. The energy necessary for the nuclear transition will be, for a particular type of nucleus, influenced by the electron density around it, and therefore will inform the researcher about the local structure of that molecule around the respective nucleus. Specific to bioinorganic chemistry would be applications involving detection of metallic nuclei (Zn, Co), or investigations on how the presence of metal influences other nuclei - primarily hydrogen and carbon. This latter influence is controlled by the distance between the nuclei – a parameter which will influence the extent of magnetic and electrostatic/electronic interaction between them. On the other hand an indirect utility for understanding the metal centers of proteins or nucleic acids comes from the fact that modern NMR methods allow for the determination of the three-dimensional structure of biopolymers by analysing the carbon, nitrogen and hydrogen signals. Figure 9 shows a typical NMR spectrum, measuring ^{1}H nuclei.

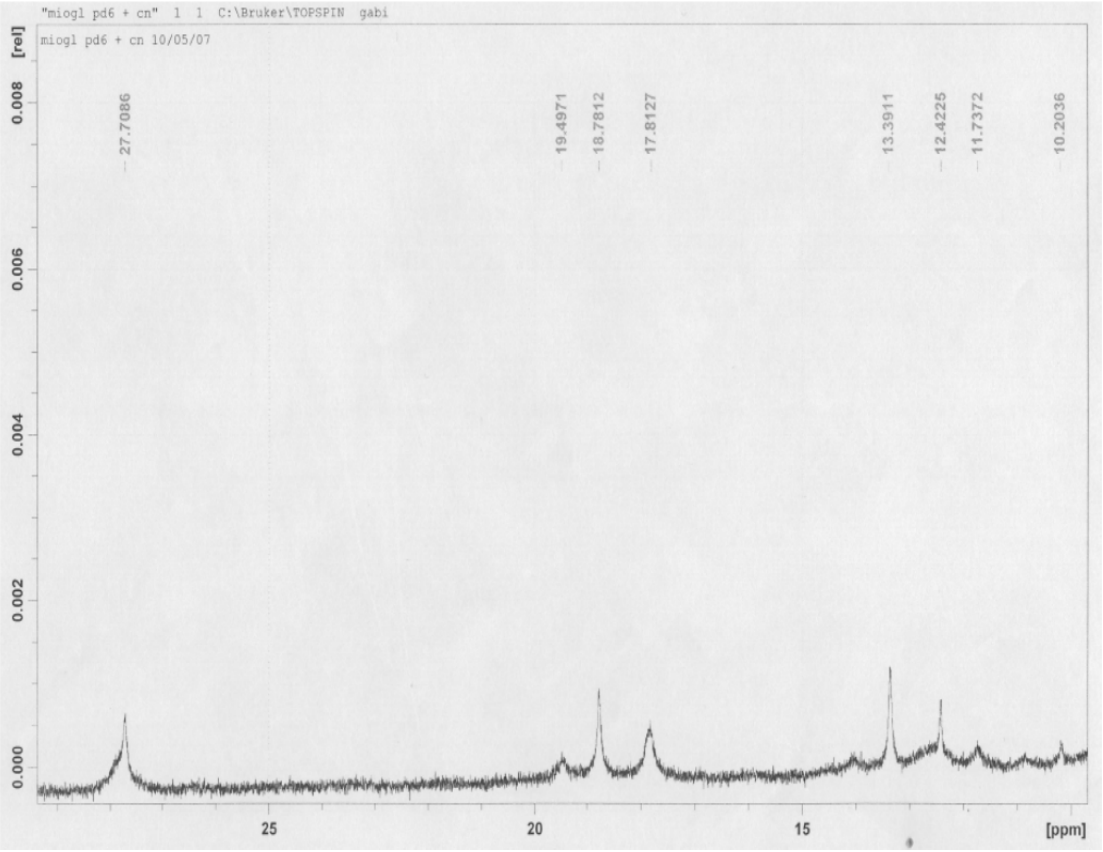


Figure 9. Myoglobin proton NMR spectrum, reflecting organic protons deshielded because of the metal center (by comparison, typical signals of protons in organic compounds are in the range of 0-10 ppm).

By coupling NMR and ESR techniques, systems that meet the criteria of both methods can be tested in more detail; the method is known under the acronym of ENDOR (electron-nuclear double resonance).

2.6 X-ray absorption spectroscopy (XAS, EXAFS)

X-ray absorption spectroscopy (XAS) involves interaction of this radiation with electrons close to the nucleus, as opposed to those found in the outer layers that were involved in UV-vis experiments. Moreover, unlike in the UV-vis, where energy absorption results in promotion of an electron to higher-energy level retaining it in the molecule, in XAS the electron is eventually permanently expelled from the electronic shell of the respective atom. The energy required for this ionization phenomenon will be influenced, in a detectable measure, by the identity of the element, by its oxidation state, and by the symmetry elements around the element.

Once expelled from the atom as described above, the electron undergoes a separate phenomenon from what happened so far: it travels to neighboring nuclei,

from which it bounces off rejected by their electron cloud; if the ensuing trajectory takes the electron back to the original element, a resonance phenomenon can be established. The XAS detection system will perceive not only the initial ionization phenomenon, but also the resonance phenomena ensuing from it. As illustrated in Figure 10, the two sides of the XAS spectrum - the absorption and the reflection of the electron between nuclei, are not only well separated in terms of the energy domain where they are observed, but are also separately interpretable because they provide largely disjoint information: the absorptive area ("edge") describes the oxidation state and symmetry, while the reflection area (“fine structure”, often described as EXAFS – extended x-ray absorption fine structure) offers, as expected from the fact that the electron is reflected by the surrounding atoms, information on the types of atoms around the examined nucleus and on the distances to these atoms. These distances are thus in principle measurable with a 0.01-0.02 Å accuracy, by far the largest among the available structural methods in biology, allowing one to determine how many ligands are around the central atom (usually metal), at what the distance and of what kind. A limitation of this is method that it cannot distinguish between similar nuclei in the coordination sphere; for instance, oxygen and nitrogen behave similarly and they cannot be distinguished, and a similar situation is seen with sulfur and chlorine.

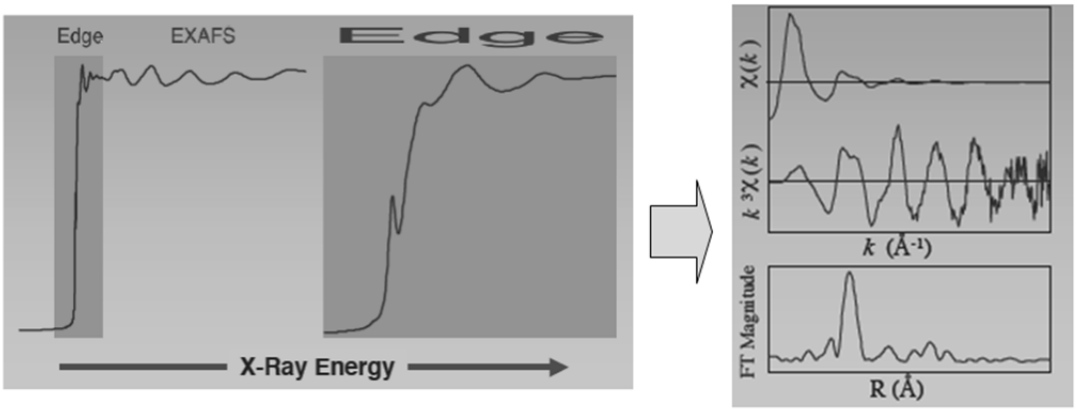


Figure 10. Right: an illustration of an XAS spectrum; the 7110-7130 region is described in the text as “edge”. By mathematical transformations, the spectrum recorded beyond ~ 7130 eV can be transformed into a form that reflects directly the metal-ligand distances (note that the x-axis is eventually converted into Å).[10]

From a practical point of view, XAS is applied especially to larger nuclei like those of the transition metals or sulfur; nevertheless, the experiment is also feasible on smaller nuclei.

2.7 X-ray diffraction

X-ray diffraction is by far the most demanding of the methods discussed until now, especially in terms of the fact that the sample needs to be presented as a high purity crystal - which for many biomolecules is very difficult to achieve. Furthermore, data work-up is significantly more complex than in other experimental techniques, involving extensive computational efforts and time. When the sample is placed in an x-ray beam, the regular arrangement of the molecules inside the crystal leads to a diffraction of this beam by the electrons around the atoms present in the crystal. Rotating the crystal and recording the diffraction patterns for a wide range of angles under which it is hit by the beam, using mathematical transformations, the three-dimensional structure of the electron density inside an elementary cell of the crystal can be determined. Atomic models are then built, that match within this electron density; the model that best fits is usually presented under the term of "x-ray crystal structure of molecule". Besides NMR, X-ray diffraction is probably the most spectacular method of analysis, due to the fact that it allows accurate determination of the complete three-dimensional structure of a molecule; it is nevertheless important to note that the true experimental result provided by this method is the electron density map, the identity and positions of atoms inside this map remain a *model* proposed by the researcher.

Chapter 3. Oxygen transporters and related sensor proteins

The oxygen molecule (O_2) is essential to the human body and to most of the other types of organisms known to us in everyday life. Therefore, these organisms have developed proteins specialized in storage and transport of molecular oxygen through the body. From a structural point of view, there are three classes of such proteins: globins (e.g., hemoglobin, with the active center consisting of an iron ion), hemerythrins (with an active center composed of two iron ions) and hemocyanins (active center composed of two copper ions).

3.1. Globins[11, 12]

Hemoglobin is arguably by far the best known metalloproteins. It is found in blood, and located inside cells specialized exactly in its transport and maintenance: the red blood cells, also known as erythrocytes. The role of hemoglobin is to carry oxygen from the lungs to the body. Structurally related to hemoglobin, myoglobin is localized in muscle cells and its function is taking the oxygen from the blood and storing it until the mitochondria need it for respiration (see also Chapter 6).

The part responsible for binding oxygen in globin is an iron center coordinated by the porphyrin; this iron-porphyrin complex, shown in Figure 11, is called heme.

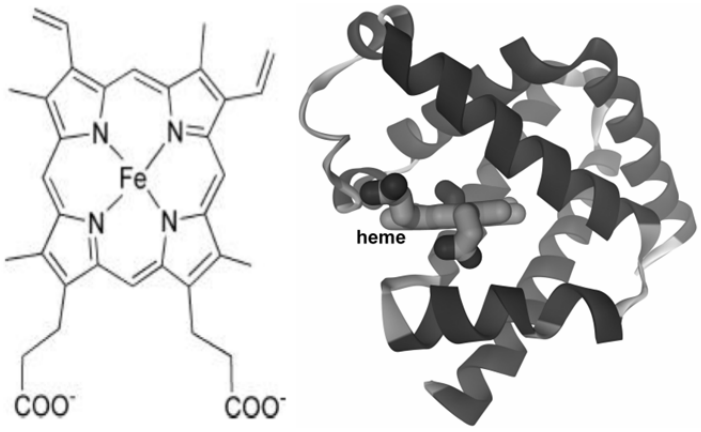


Figure 11. Heme - an iron porphyrin complex, and the manner in which it is embedded in the structure of myoglobin.

In myoglobin, heme is surrounded by a polypeptide chain, which folds to close the iron inside, limiting the access of the potential ligands from outside. Moreover, an

imidazole system belonging to the side chain of one the of histidines of the polypeptide chain coordinates to the iron, which thus becomes pentacoordinated, with only one free coordination position left; this position is especially kept free for binding molecular oxygen. The histidine coordinated the iron is called, because of its proximity to the metal, “proximal histidine”. In the trans position to the proximal histidine, the protein a defines a limited-size cavity over the iron, large enough to allow molecular oxygen binding at the iron but too small to allow easy access for molecules larger than O_2. Essential in defining the cavity is again one of the side chains of the histidines of the polypeptide chain. This second histidine is called, by contrast to the proximal histidine, "distal histidine". Its role is proposed to be not only steric, in limiting the volume of the cavity, but to form a hydrogen bond with molecular oxygen after it binds to iron (see also Figure 13). We note at this point that the active center, as described here for myoglobin, is also found in the same form in hemoglobin. Moreover, the polypeptide chain of hemoglobin is very similar to that of myoglobin at the primary level (the amino acid composition) as well as at the secondary and tertiary levels (spatial organization). The main structural difference between human hemoglobin and myoglobin is that hemoglobin contains four of these polypeptide chains, each wearing their own heme, so that one can describe this protein as a tetramer whose four subunits are each similar to myoglobin. In most organism, hemoglobin is, as the human one, tetrameric; exceptions involve organisms with dimeric hemoglobins, or with hemoglobins containing more than four monomers (24, for example). In tetrameric hemoglobins the four chains are usually slightly different to each other. Thus, human hemoglobin contains two chains called α and two called β; differences between α and β are minor, at the primary structure, and lead to minor differences, sometimes even non-detectable, in the reactivity of the iron centers housed by the two types of chains.

The reason why hemoglobin adopts a more complicated structure than myoglobin (tetramer, compared with monomer) can be understood if we follow in Figure 12 the oxygen affinity profile of the two globins. Thus, Figure 12 reveals that given level of occupancy with O_2 ligands at the iron centers can be attained at a higher concentration of oxygen in solution for hemoglobin than for myoglobin. In the particular case of O_2-binding proteins, the oxygen concentration, expressed in terms of partial pressure, required to achieve binding to 50% of the centers available in the solution (50% of the iron, in this case) is defined as "p50". Thus, hemoglobin's p50 is

larger than that of myoglobin, which translates into the fact that hemoglobin has lower affinity for molecular oxygen than myoglobin. The reason why myoglobin should have a higher affinity to oxygen than hemoglobin is that myoglobin must be able to attract into muscle tissue the oxygen molecule that circulates in blood already bound to hemoglobin. An additional difference between the two globins can be seen in the shape of two curves in Figure 12: if for myoglobin the form is the typical of curve of a ligand binding to a protein, described by an exponential equation, for hemoglobin the curve adopts a sigmoid shape. The sigmoid aspect is in fact largely responsible for the fact that at the levels of O_2 concentration typically present inside the human body hemoglobin's affinity to O_2 is lower than myoglobin's. On the other hand, at the lungs, where the oxygen concentration is much higher than in the rest of the body, the hemoglobin affinity is much better and allows for loading oxygen at all four sites. The differences in shape and in affinity (in fact mathematically connected to each other) between the two curves of the two globins are consequences of the tetrameric structure of hemoglobin, since, as noted above, this is the most notable structural difference between the two globins.

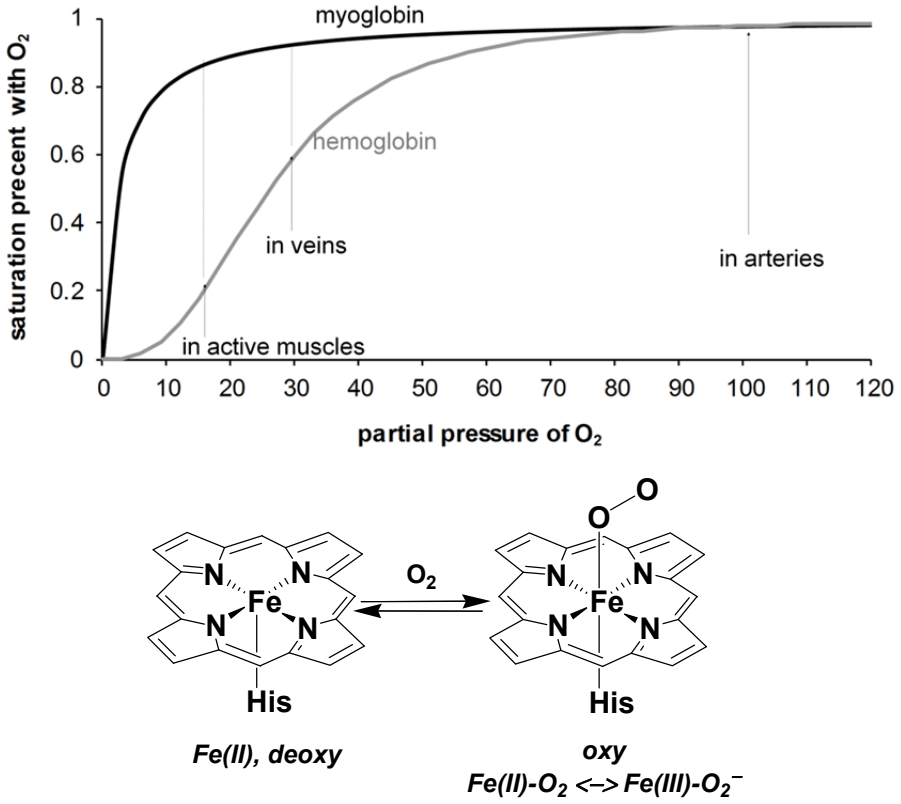


Figure 12. Oxygen affinity profile of hemoglobin and myoglobin, with an illustration of the chemical equilibrium involved (deoxy - oxy).

To understand the mechanism by which the tetrameric structure can affect the oxygen affinity of hemoglobin, we will return to the active center, following the effects of binding molecular oxygen to it. In general the iron inside the globins iron is found *in vivo* in two forms, both in the formal oxidation state Fe^{2+}: pentacoordinated as described above (traditionally called "deoxy") and hexacoordinated bearing as the sixth ligand an oxygen molecule (traditionally known as "oxy"). In the deoxy form the system of ligands around the iron is asymmetric: the iron placed in the center of porphyrin has as axial ligand only the proximal histidine, while in the trans position to the histidine there is no ligand. This asymmetry dictates that the iron, instead of being placed exactly in the plane defined by the porphyrin, is slightly displaced outside this plane towards the proximal histidine. An additional argument for the iron to leave the porphyrin plane is its high-spin electronic configuration, which means that its 6 d electrons are so arranged that four of them remain unpaired. High-spin systems by definition feature a larger volume of the electron cloud compared to low-spin systems. For this reason the iron has an advantage in being outside of the porphyrin plane in the deoxy form, as this alleviates some of the steric constraints imposed by the limited size of the metal-binding site at the center of the porphyrin. When the oxygen molecule binds to the hemoglobin iron as a sixth ligand, it establishes a symmetry between the two sides of the heme (each of them now has a ligand). In addition, the oxygen, forming a strong bond to the iron, leads to the transition of the metal to a low spin state (0 unpaired electrons), which has a smaller volume of the electronic cloud that the high spin deoxy form. These two elements (the volume of the electron cloud and the appearance of an additional ligand trans to histidine) cancel the reasons why iron was placed outside the porphyrin plane in deoxy form, and thus cause a movement of the iron away from the proximal histidine and back into the porphyrin plane, where the metal remains as long as the oxygen remains bound to it. This iron movement does not have a particular importance for myoglobin, but it is the origin of the sigmoidal appearance of the oxygen binding curve to hemoglobin. Thus, the movement of the iron into the porphyrin plane also entails a movement of the proximal histidine side chain, as a consequence of the coordinative bond connecting them. In turn, the side chain of the proximal histidine can only move together with the entire fragment of α helix to which it is attached, which by necessity implies subtle structural changes in the rest of polypeptide chain. Furthermore, these changes are felt in the neighboring polypeptide chains of the hemoglobin tetramer. One may question

the possibility that the simple binding of a small molecule such as O_2 can lead to changes, even subtle, in the *entire polypeptide chain and even in the neighboring protein subunits*. In fact, the protein is so built that it can adopt two major conformations: one in which the proximal histidine is placed further, and one where it is placed closer to the heme, corresponding to deoxy and oxy structures, respectively. The energy difference between the two conformations of the protein is very low, so that indeed the simple binding of the oxygen molecule to iron can trigger the transition from one conformation to another. The significance of the existence of two conformations is understood if we remember the position of iron in each of the two conformations. Thus, in the conformation typical of the oxy form, the iron is placed closer to the position in which oxygen will be bound whereas in the other one (typical of the deoxy structure) the iron will be less accessible to the oxygen; this makes oxygen binding to be easier in a conformation than in the other, or, in other words, the two conformations have different affinities for oxygen. These two conformations are traditionally called T and R respectively (tense and relaxed respectively).

The nature of the chemical bond between the globin iron and molecular oxygen has been a matter of debate. One of the most powerful arguments considered was the vibrational spectrum (resonance Raman) of oxy-hemoglobin, in which the frequency of vibration of the oxygen-oxygen bond of O_2 ligand coordinated to iron is characterized by a much smaller value than of the molecular oxygen and is in fact very similar to what is seen in well-characterized compounds featuring a superoxo ligand (O_2^-) coordinated to a metal. In concordance to this, the Mössbauer spectrum of oxy-hemoglobin presents clear features characteristic to Fe (III) and not to Fe (II). Thus, it is generally accepted today that upon binding O_2 to the Fe (II) in hemoglobin, at least a partial charge transfer from iron to oxygen takes place, so that the resulting oxy complex is described as featuring a strong Fe(III)-superoxo character. This phenomenon, whereby a molecule adopts two or more electronic structures which differ from each other by the position of one or more electrons, is called electromerism, by analogy with the well-known phenomenon from organic chemistry, tautomerism. Thus, the oxy form of hemoglobin can access two electromers: one describable as Fe(II) coordinated with molecular oxygen, and one describable as Fe(III) coordinated with superoxide. The actual structure of the system is accepted as being intermediate between these two electromers, but with a more important contribution coming from the Fe(III)-superoxo.[13] It is important to emphasize that

upon release of the oxygen from the iron, the two chemical bond partners recover their initial structure - namely Fe (II) and molecular oxygen.

In addition to molecular oxygen, hemoglobin interacts *in vivo* with many other small molecules or ions. One of the most popular of these is carbon monoxide (CO), present as pollutant in air mainly a result of incomplete combustion. CO is produced in very low concentrations (hence, non-toxic) even inside our bodies, in the first stage of the catalytic cycle of heme-oxygenase (an enzyme that destroys heme); furthermore, recent experimental results certify some possible therapeutic applications of compounds that release in organism CO at low concentrations. The toxicity of CO is explained by the fact that it has a remarkable affinity for Fe (II) in principle and for the heme iron in particular; this affinity is much higher than that of oxygen, which makes CO to be preferred by hemoglobin at the expense of molecular oxygen, and strongly bound even when present in much lower concentrations than molecular oxygen. The stability of the Fe (II)-CO adducts of globins is in fact so great, that once bound CO is no longer released from hemoglobin, hindering it from exercising its function as physiological transport of molecular oxygen. Globins limit the toxic effect of carbon monoxide via two mechanisms, both involving the distal histidine. As shown in Figure 13, Fe-C≡O adducts prefer a linear geometry, bringing carbon monoxide and the distal histidine in a slight steric conflict - unlike the case of the oxygen molecule, whose Fe-O=O adduct adopts a non-linear geometry, with the third atom located away from the distal histidine and avoiding this way the steric conflict. On the other hand, as noted above, in the oxy adduct the O_2 ligand bears a strong superoxide character, with an excess of negative electric charge on both oxygen atoms. This negative charge enables the superoxo ligand to form a hydrogen bond with the distal histidine; on the other hand, carbon monoxide does not carry a net electric charge and therefore is much less capable of forming a strong hydrogen bond with the distal histidine. Thus, the steric factor and the one involving hydrogen bonding contribute to a partial destabilization of the Fe(II)-CO adduct compared to the oxy one.

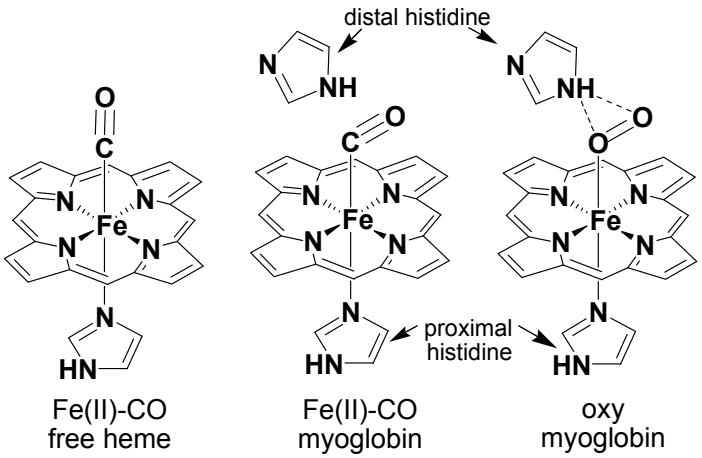


Figure 13. Schematic representations of the structures of the ferrous heme adducts with carbon monoxide and oxygen respectively, illustrating the tendency of steric conflict brought by the distal histidine (His E7) of globin. It is however important to note that in reality even in the globin the Fe(II)-CO adduct remains linear – with the Figure illustrating only the tendency of the distal histidine to impose a different geometry.

A second important molecule that interacts *in vivo* with hemoglobin is CO_2. Unlike O_2 and CO, when interacting with hemoglobin the carbon dioxide binds not to iron but to the nitrogen atom of an amino acid - namely the N-terminal amino group (the first amino acid in the sequence); the reaction is illustrated in Figure 14. This phenomenon is important because it allows the transport of CO_2 from various tissues in the body (where it appears as a product of cellular metabolism) to the lungs (where it is excreted).

$$R{-}NH_2 + CO_2 \rightleftharpoons R{-}NH{-}CH_2OO^- + H^+$$

Figure 14. CO_2 binding reaction to hemoglobin

It is important to note that the binding of carbon dioxide causes a local change in the protein structure, resulting in a slight decrease in oxygen affinity. The effect is that at the CO_2-rich tissues, which are highly metabolically active and thus require increased amounts of O_2, hemoglobin releases more O_2 due to its binding CO_2 – thus allowing the carbon dioxide to efficiently function of a messenger molecule that controls tissue oxygenation. An indirect way of CO_2 affecting hemoglobin derives from the well-known hydration reaction in which carbon dioxide is involved in water,, and whose product is carbonic acid. Thus, in the vicinity of CO_2-rich tissues the pH of the blood will decrease slightly, due to the production of carbonic acid; the affinity of hemoglobin for oxygen is in fact pH-dependent, decreasing at acidic pH due to subtle structural changes following protonation of some amino acids (this phenomenon is known as the "Bohr effect"). The net effect is a delivery of increased amounts of O_2 to the respective tissue.

As shown above, carbon dioxide and protons are able to affect molecular oxygen binding to iron, although they not interact directly with iron or its ligands. This kind of interaction, in which a molecule A binds to a protein P at a particular location ("site") and affects in this way the binding of another molecule B to another site of the same protein is generically called “allostery”. Molecule A in the above definition is called in this context the “allosteric effector”. Thus carbon dioxide and protons are allosteric effectors for molecular oxygen binding to iron. There are other allosteric effectors with similar effects, which have as common feature the anionic character. All of them (2,3-bis-phosphoglycerate - BPG, inositol phosphates, chloride) have a well-defined binding site at the junction between the four subunits of hemoglobin, in the tetramer center (as illustrated in Figure 15). Hemoglobins found in various organisms have different affinities towards these allosteric effectors. Thus, human hemoglobin uses 2,3-BPG as allosteric effector; the red cell hosts, in addition to hemoglobin, important quantities of 2,3-BPG. By contrast, bovine hemoglobin is not sensitive to 2,3-BPG and uses instead chloride as effector. In humans the hemoglobin produced in adults differs slightly, at the primary structure level, from the one produced by the fetus (before birth); this difference in primary structure leads to a slight difference in affinity towards 2,3-BPG - sufficient for the fetal hemoglobin to manifest a higher affinity towards oxygen than adult hemoglobin - which in turn allows efficient transfer of the oxygen from mother's blood to fetal blood.

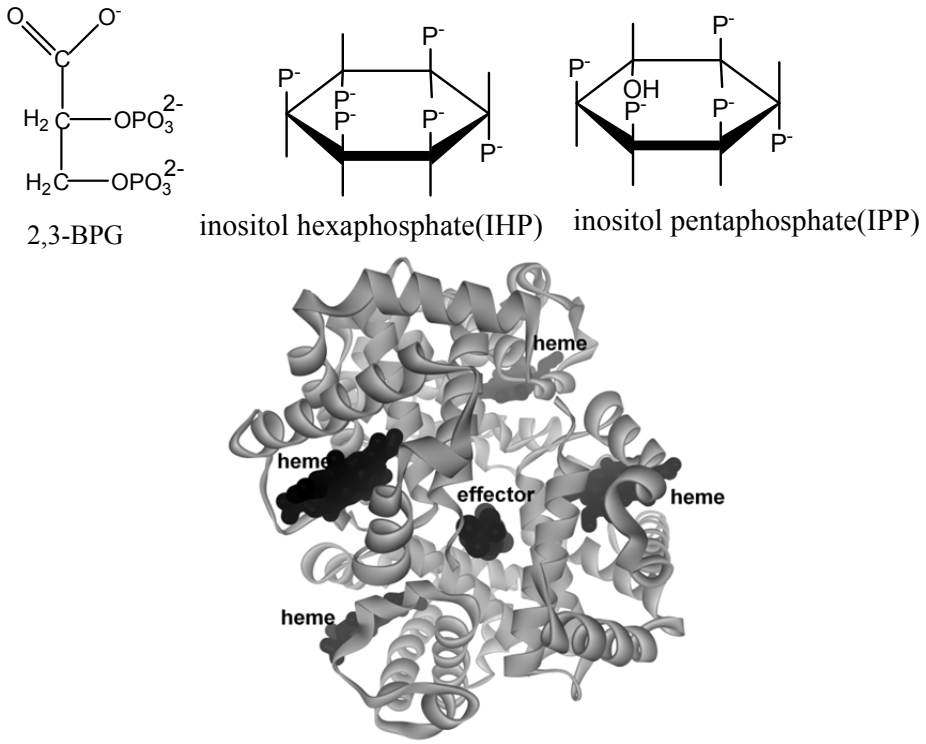


Figure 15. Allosteric effectors of hemoglobin with anionic character, and their binding site.

An important reaction in globins is autooxidation. In principle, the oxy adduct of a heme, either embedded in a protein or free in solution, has two decomposition pathways. The first one predominates in globins and involves releasing molecular oxygen from Fe(II). The second pathway, called autooxidation, involves releasing superoxide, O_2^-, and leaving behind Fe(III). The oxy adducts of free hemes, especially when dissolved in polar solvents, display a strong preference for the autooxidation reaction. Conversely, the hydrophobic environment created by the globin around its heme strongly disfavors the separation of electric charge involved in the autooxidation reaction and, instead, favors the release of the neutral ligand, O_2; however, the autooxidation reaction is not entirely averted. Since the Fe(III) form of the globins (traditionally called "met") is unable to bind the oxygen molecule, met-Hb is useless for the organism (as will be seen below, met-Hb is in fact toxic) and therefore its transformation back to Fe(II) is necessary, through a reduction reaction involving transfer of one electron to the iron. Red cells contain an enzyme dedicated to this reduction process of met-hemoglobin to deoxy-hemoglobin (Fe(III) to Fe(II)): methemoglobin reductase; due to the action of this enzyme, the Fe(III)-hemoglobin is maintained, in a healthy individual, to maximum 0.1% of total hemoglobin. Genetic deficiencies in the methemoglobin reductase can lead to an excess of methemoglobin in the blood, which will result in a bluish aspect of the blood, observable in extreme cases even on the skin. This discovery was a milestone in medicine, being the first case where a medical condition (abnormally blue color of patient's skin) was explained by a well-defined genetic mutation, at the level of a single enzyme. Chemical agents, such as nitrites present as pollutants in drinking water, can also induce autoxidation, leading for example to the "blue baby syndrome" (observed in newborns whose diet was affected by nitrate-rich waters).

Another class of molecules that interact *in vivo* with the globins are the peroxides. They are present in blood and have the property to react with Fe(III), including methemoglobin, in a reaction that produces free radicals and leads to irreversible degradation of the globin. Hemoglobin's degradation products are detectable in the blood of a healthy individual and appear in higher concentrations under conditions as physical effort or certain diseases.[14]

Nitric oxide, NO, is a highly toxic, unstable gas, mainly because of its free radical character. Many organisms have built sets of specialized enzymes in the fight against NO. It was therefore a surprise when it was discovered that NO is generated in

the walls of blood vessels, at very low concentrations, as a messenger that controls the contraction of the smooth muscles surrounding the blood vessel, thereby controlling blood pressure. Oxy-hemoglobin reacts extremely rapidly with NO, a fact that can be explained by the Fe(III)-superoxo character of oxy-Hb; superoxide, as NO, has a free radical character, which leads to a very facile reaction in which the unpaired electrons of NO and O_2^- form a chemical bond, yielding a Fe(III)-OO-NO^-(peroxynitrito) adduct that subsequently isomerizes very quickly to Fe(III)-nitrate. In the human blood this reaction is avoided under normal conditions, due to the fact that NO is found in very low concentrations and only near the blood vessel wall, while hemoglobin is protected inside the red cell. There are, however, organisms where hemoglobin has precisely the role to react with NO in the manner described above. Thus, *Escherichia coli* contains a protein called flavohemoglobin, similar to myoglobin and whose physiological function seems to be nitrogen monoxide destruction via its reaction with the oxy form, in order to protect the cell under conditions of exposure to an excess of NO. The catalytic cycle is completed by re-reducing the iron to Fe(II), facilitated by the fact that in the immediate vicinity of the heme the protein contains a flavin (known for its ability to transfer electrons to and from metal centers). In other bacterial organisms enzymes similar in structure and function to flavohemoglobin are found, but where the flavin and the globin part evolved as separate proteins; in this case we speak of truncated hemoglobins (tHb).[15]

In close connection with nitric monoxide, hemoglobin is able to interact with nitrite. Deoxy Hb reduces nitrite to NO, while oxy-Hb suffers in the presence of nitrite an autooxidation reaction. Nitrite's ability to generate NO in blood towards its reaction with hemoglobin has been proposed, in recent years, as a reason for approving nitrite for therapy related to the cardiovascular system,.

As partially illustrated above with flavohemoglobin and truncate hemoglobins, the globin family extends far beyond hemoglobin and myoglobin. Thus, all human cells contain, in very small amounts, a globin called cytoglobin, whose role is still unknown. Additionally, neurons contain neuroglobin - another protein with unclear role - but most probably related to oxidative stress (excess of oxidizing agents and free radicals). Plants contain a globin called leghemoglobin (particle "leg" from "leguminous"), whose role, most likely, is similar to that of myoglobin - oxygen storage inside the cell. Considering that hemoglobin relatives from inferior organisms do not serve as molecular oxygen transporter but rather as redox enzymes, it is very

likely that animal hemoglobin and myoglobin have evolved from enzymatic systems that had initially had completely different roles and that had existed before the Earth's atmosphere developped from a predominantly anaerobic one to the oxygen-rich one where animals subsequently evolved.

3.2. Hemerythrins[11]

Hemerythrins (Hr) serve as oxygen transporters a in narrow range of inferior organisms - such as marine worms. Their active center, where molecular oxygen binding occurs, contains two iron ions, as illustrated in Figure 16.

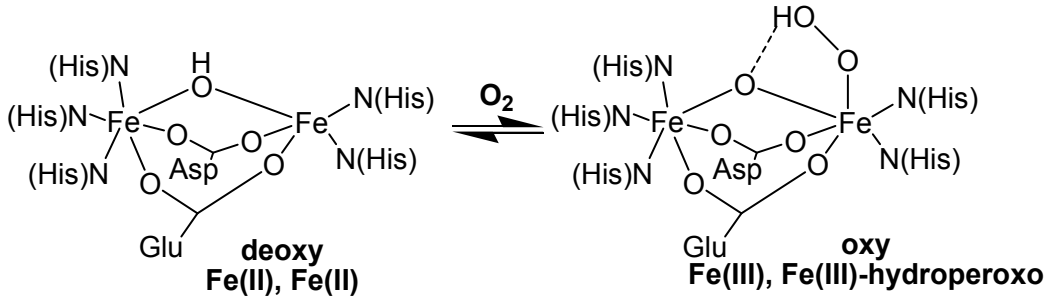


Figure 16. Structure of the active center of hemerythrins, and the oxygen binding reaction.

Similarly to hemoglobin, there are three possible states of the active center in hemerythrin: deoxy (di-ferrous), oxy (formed by binding molecular oxygen to the deoxy form) and met (di-ferric). In addition, because of the bimetallic nature of the active center in hemerythrin, here a so-called semi-met form (or "mixed valence") is also defined, in which one metal is Fe(II) and the other Fe(III). As in case of hemoglobin, Mössbauer measurements have demonstrated that oxy-hemerythrin contains two Fe(III) ions; this implies that upon binding of the oxygen molecule to the di-ferrous center of hemerythrin a two-electron transfer towards O_2 occurs, producing peroxide, O_2^{2-}. Furthermore, as shown in Figure 16, the active center provides a proton that stabilizes this chemically-reduced form of the ligand. Vibrational measurements (resonance Raman) have confirmed the existence of a much weaker bond between the two atoms of the dioxygen ligand in oxy-Hr, in accordance with the simple bond existent in a peroxo ligand and in contrast to the formally double bond in O_2. Like hemoglobin, the oxy form of hemerythrin is red. The nature of this color is, however, completely different. Thus, the color of hemoglobin is extremely intense and exclusively caused by π-π * electronic transitions within the iron-bound

porphyrin, with extinction coefficients around 100000-200000; by contrast, the color of oxy-Hr is due LMCT electronic transitions (ligand-to-metal charge transfer), that have extinction coefficients about 10 times smaller than those of the porphyrin. This difference explains why the *deoxy* form of hemoglobin is also strongly colored, while deoxy-Hr, where the metal is reduced and therefore much less willing to engage in LMCT transitions, is colorless.

Hemerythrins also differ from hemoglobins in terms of the structure of the protein matrix, even though in both classes of proteins the α-helix type of secondary structure is predominant and the molecular weights of the monomers are close. While hemoglobin is present as monomer, dimer, tetramer or oligomers with up to 180 subunits, hemerythrin may be organized as monomer or as oligomer with 2, 3, 4 or 8 subunits.

3.3. Hemocyanins[11]

Hemocyanins (Hc) serve as oxygen transporters in certain types of crustaceans, and differ from hemerythrins and globins in two ways: their active center contains copper instead of iron, and in the protein matrix β sheet structures dominate now instead of α-helices; also, the molecular weight of Hc is about four times larger than in globins.

Binding of molecular oxygen to the active center of hemocyanins is illustrated in Figure 17. The active center is, as in the hemerythrin case, binuclear, with the deoxy form containing Cu(I) and the oxy form containing Cu(II). As in hemerythrin, dioxygen binding involves its reversible transformation to peroxide; this time, however, the peroxide is bound in a bidentate bridging manner, as shown in Figure 17. This binding mode, where both oxygen atoms interact simultaneously with two metals, leads to a significant weakening of the oxygen-oxygen bond, as confirmed by vibrational spectra of oxy-hemocyanin featuring an O-O vibration frequency lower than in most peroxo-metal complexes (implying one of the weakest oxygen-oxygen bonds).

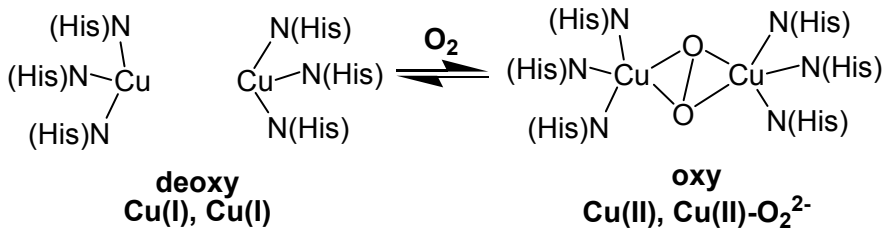


Figure 17. Structure of hemocyanins active center and the oxygen binding reaction.

3.4. Sensor-proteins for O_2 and related molecules

As seen above, O_2 binding to hemoglobin triggers structural changes that are propagated on long distances in the polypeptide component of these metalloproteins. Of such changes take advantage hemoproteins whose role is detection of the oxygen molecule: structural changes within such proteins will be transmitted to other cellular components, signaling the presence of O_2 in the environment. Thus, the FixL protein is produced in nitrogen-fixing bacteria, and its role is in regulating precisely the production of the genes involved in transformation of N_2 to NH_3 (these proteins being very sensitive to oxygen). In other bacteria the HemAT hemoprotein is found, which has a structural domain related to globins, and which is designed to detect molecular oxygen as part of a chemotactic mechanism by which bacteria choose to move in the environment along a concentration gradient of O_2.

Soluble guanylate cyclase (sGC) also has a hemoprotein domain with a sensor role for a diatomic gas - nitric oxide. NO itself is a messenger molecule and its binding to sGC triggers structural changes that, in another domain of the sGC, lead to synthesis of another messenger molecule, cGMP (cyclic guanosine monophosphate), with roles in smooth muscle relaxation or nerve impulses transmission. The ability of NO to trigger blood vessel dilation and therefore an increased local influx of blood was exploited by some insects that feed on the blood of animals. These insects release during their bite a hemoprotein that transports NO bound to Fe, and which releases this gas in the victim's blood, allowing a more efficient feeding of the insect.

CooA is a hemoprotein found in bacteria capable of using carbon monoxide as energy source (unlike, for example, more complex organisms that use as energy source much more complex classes of molecules - lipids, sugars, proteins). This protein serves as a sensor for CO.

DcrH is the only gas sensor with a non-heme nature that has been discovered so far. Its function is similar to that of HemAT, with the distinction, at structural level, that DcrH binds the oxygen molecule in a domain related to hemerythrin both in terms of protein matrix and in terms of the di-ferrous active center.[16]

Chapter 4. Proteins activating molecular oxygen

The reaction between molecular oxygen and an organic substance is generally strongly exothermic. However, almost all organic molecules are stable in the presence of O_2. Molecular oxygen has two unpaired electrons, whereas organic substances are generally diamagnetic and their "combustion" products (CO_2 and water, ultimately) are also diamagnetic. This involves a reaction between O_2 and the organic compound, where the total number of unpaired electrons changes from 2 to 0; a change like this is strongly disfavored by the rules of quantum mechanics and provides an important kinetic barrier for the reaction. On the other hand, living systems need to use molecular oxygen, for a variety of reasons – e.g. to selectively oxidize some cellular components. For this purpose metalloenzymes are often employed, which, as seen in Chapter 3, can use the metal to profoundly change the electronic structure of the O_2 ligand (e.g. transforming it into superoxide or peroxide) - thus avoiding the problem of the kinetic barrier.

This chapter will be discussed the reactions in which metalloproteins attract molecular oxygen and its congeners - superoxide and peroxide. An overview of these processes is shown in Figure 18.

Superoxide dismutase:	$2O_2^- \rightarrow H_2O_2 + O_2$
Superoxide reductase:	$2O_2^- + e^- \rightarrow H_2O_2$
Catalase:	$2H_2O_2 \rightarrow H_2O + O_2$
Peroxidase:	H_2O_2 + substrate $\rightarrow$ H_2O + oxidized substrate
Peroxygenase:	H_2O_2 + substrate $\rightarrow$ H_2O + substrate(O)
Oxidase:	O_2 + substrate $\rightarrow$ $2H_2O$ + oxidized substrate
Monooxygenase:	O_2 + substrate $\rightarrow$ H_2O + substrate(O)
Dioxygenase:	O_2 + substrate $\rightarrow$ substrate(O,O)

Figure 18. The definitions of some types of reactivity involving metalloproteins and molecular oxygen or its reduced forms.

4.1. Superoxide dismutase[17]

As shown in Figure 18, superoxide dismutases (SOD) catalyze superoxide O_2^-, dismutation, to peroxide and molecular oxygen. Their role is a protective one, superoxide being a free radical and therefore able to affect extremely rapidly and in unwanted ways the structures of many compounds inside a living organism. Along

superoxide, other particularly dangerous agents are its congeners, hydrogen peroxide, organic peroxides, and singlet oxygen (the excited state of O_2, where there are no unpaired electrons). Along organic free radicals, this series of substances is responsible for what is generically called oxidative stress. A number of enzymes discussed in this chapter (SOD, but, as well as others) function *in vivo* precisely in combating oxidative stress.

All SOD use a redox-active metal on catalytic site; a general scheme for the mechanism of all SOD is shown in Figure 19. The starting point is the reduced form of the metal center, M^{n+} (depending on the metal, it can be Ni(II), Fe(II), Mn(II) or Cu(I)). The metal reacts with a first molecule of superoxide via a mechanism still incompletely elucidated. Thus, one possibility is direct outer-sphere oxidation of the metal to M^{n+1} (Ni(III), Fe(III), Mn(III), Cu(II)), without forming a coordinative bond between superoxide and metal - thus reducing superoxide to peroxide. A second possibility is formation of a superoxide-metal complex M^{n+}-O_2^-, which will present an electromerism phenomenon very similar to the one seen upon binding molecular oxygen to the iron in globins; thus, Ni(II)-superoxo, Fe(II)-superoxo, Mn(II)-superoxo and Cu(II)-superoxo complexes could be described as peroxide adducts of Ni(III), Fe(III), Mn(III) or Cu(II), respectively. H_2O_2 would then be produced by protonation of such complexes. Irrespective of the mechanism, at the end of this first phase of the catalytic cycle the metal center is found in the oxidized form, M^{n+1}, which then reacts with a second molecule of superoxide, again via two possible mechanisms: an outer-sphere one and an inner-sphere one – both of them releasing a molecule of oxygen and closing the catalytic cycle.

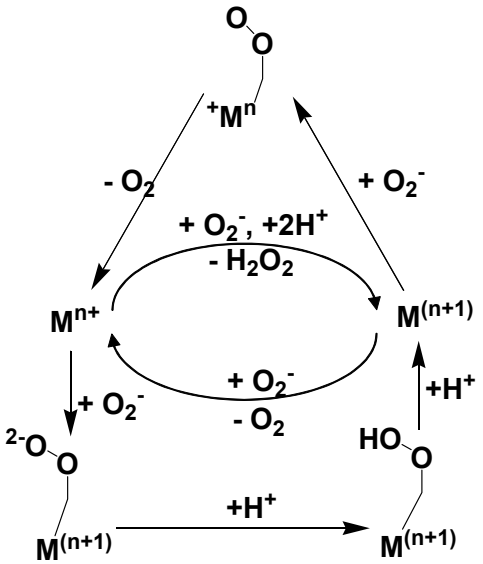


Figure 19. Mechanisms proposed for superoxide dismutase

Figure 20 illustrates the structures of active sites of the three main types of superoxide dismutase. Ni-SOD presents an unusual way of coordination, in which one of the ligands is an amide group (-CO-NH-) that is deprotonated to nitrogen; generally in biochemistry the cases where a peptide bond is deprotonated under physiological conditions are extremely rare. Superoxide dismutases with Fe and those with Mn have essentially identical catalytic sites, differing only in terms of the metal. Cu, Zn-SOD present a structural feature unique in bioinorganic chemistry: the imidazole system of a histidine serves as a bridging ligand between copper and a Zn(II) center. A notable feature of all three structural classes shown in Figure 20 is that metal is not coordinatively saturated by the protein; the remaining open positions are either retained as they are (e.g., in Ni-SOD) or are occupied by water molecules (e.g., Fe-SOD or Mn-SOD). This feature leaves open the possibility of binding the superoxide to metal during the catalytic cycle, water being a ligand easy to replace.

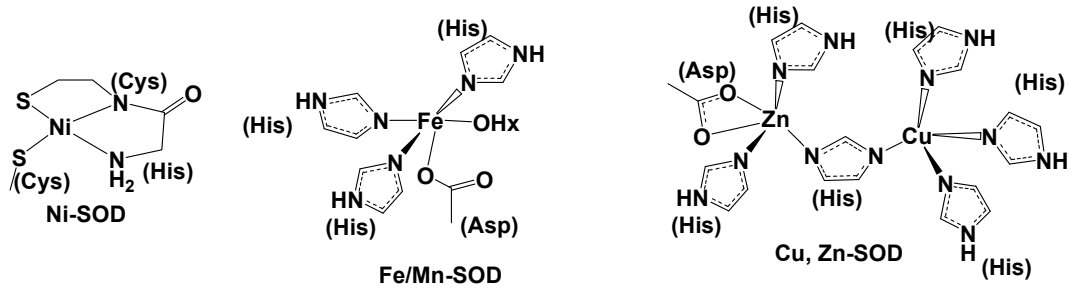


Figure 20. Active sites of the three types of superoxide dismutase discussed in the text.

4.2. Superoxide reductase[18, 19]

Like SOD, superoxide reductases (SOR) have a protective role. This time, however, the process is purely reductive, peroxide being the only product - unlike SOD where both peroxide and molecular oxygen were produced. This difference correlates with the fact that SOR are found exclusively in anaerobic organisms, the production of O_2 (as done by SOD) would be counterproductive. The catalytic cycle proposed for SOR is illustrated in Figure 21. The required electrons to complete the catalytic cycle are provided by the cell through specialized electron-transport proteins (rubredoxins, see Chapter 5).

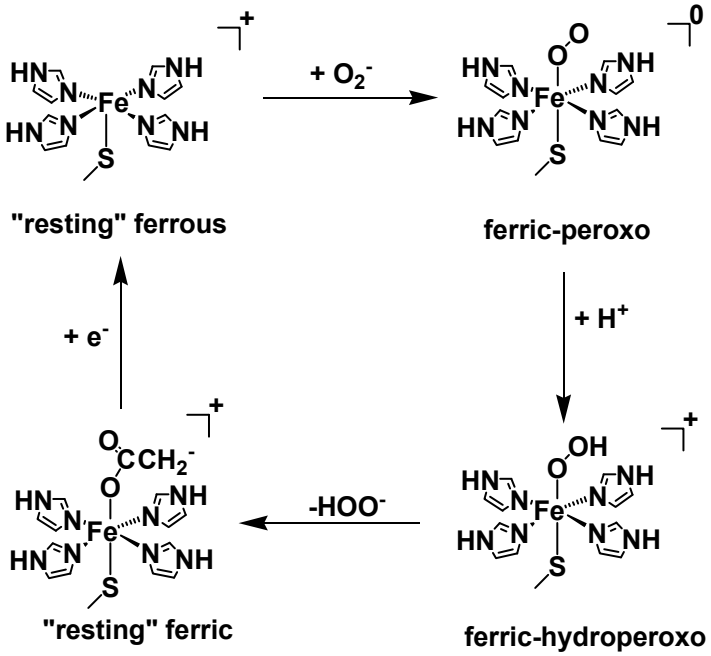


Figure 21. The reaction mechanism proposed for superoxide reductase.

4.3. Peroxidases[18, 20, 21]

Four types of peroxidases are known, depending on the type of active center: heme, non-heme iron, vanadium, and non-metal (with selenium or sulfur). Figure 22 presents the catalytic cycle of heme-containing peroxidases. Notable are the forms called Compound I and Compound II, that are produced by binding peroxide to iron, and where the formal oxidation state of the iron is Fe(IV); Compound I presents in addition a radical cation located on the porphyrin, so that it formally has two electrons less than the stable ferric form. Compound I is the result of heterolytic cleavage of the oxygen-oxygen bond from a ferric heme adduct with hydrogen peroxide, with the release of a water molecule. This reaction involves the reorganization of some protons, and therefore it is not surprising that the amino acids around the sixth coordination position of the iron, where hydrogen peroxide binds, tend to be polar or electrically charged - therefore capable of acid-base catalysis (histidine, arginine, tryptophan, glutamate).

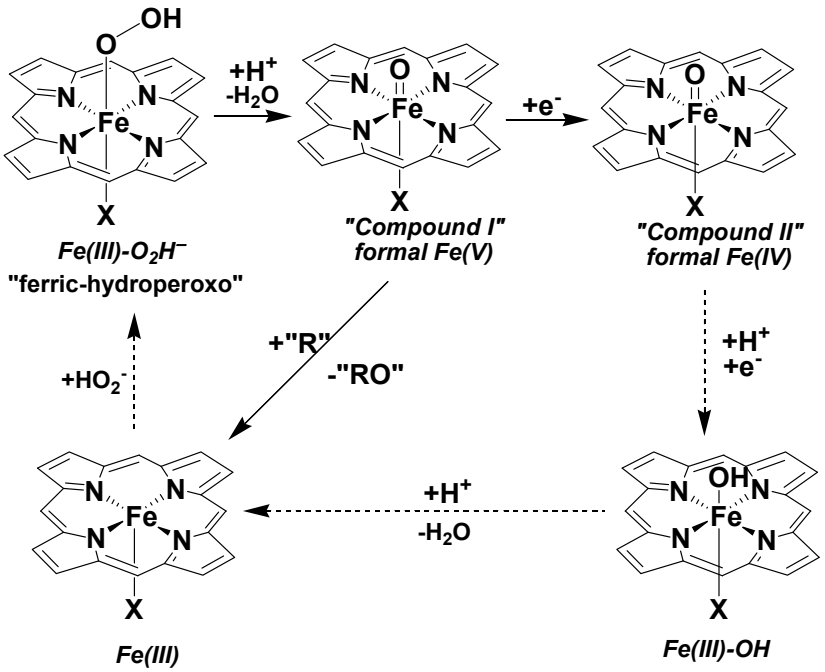


Figure 22. The catalytic of heme peroxidases. "X" is usually histidine.[22, 23]

Compounds I and II are, as expected, very strong oxidants. The physiological functions of heme-containing peroxidases imply in fact precisely the oxidation of organic or inorganic compounds by Compound I and/or by Compound II - either with synthetic purposes, either as with a protective role to protect the organism by decomposing hydrogen peroxide. In some cases, Compound II is not a distinct part of the catalytic cycle: thus, the Compound I of myeloperoxidases and chloroperoxidases oxidizes chloride in a reaction that requires a simultaneous two-electron transfer, yielding HOCl with a role in the organism's defense mechanisms (for myeloperoxidase and related enzymes) or yielding organo-halogen compounds (in chloroperoxidase). Structurally, the active sites of peroxidases are similar to those found in globins: pentacoordinated iron with a free position for binding hydrogen peroxide and with an amino acid of the protein coordinated trans to the hydrogen peroxide binding position - histidine in most cases, the most important exception being chloroperoxidase, whose axial ligand is a cysteinate.[24]

Vanadium-containing peroxidases function as haloperoxidases (like chloroperoxidases); their active site structure is illustrated in Figure 23, and the mechanism is very different from that of heme peroxidases in that it involves no equivalent of the Compound I and Compound II states: most probably it involves a

direct reaction of the halide with the peroxo adduct of vanadium – the metal being from the beginning in the maximum oxidation state accessible.

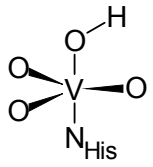


Figure 23. Active site of vanadium-containing peroxidase.

Non-heme iron peroxidases (rubrerythrin and its relatives, such as nigerythrin) have a protective role, often found in tandem with SOR in the same types of anaerobic organisms. The active center is similar to that of hemerythrins, except that the contribution of the nitrogen-based ligands is lower here than in hemerythrin. The mechanism, illustrated in Figure 24, is drastically different from that of heme peroxidase: it starts with a di-ferrous state, which means that the binding of peroxide, which involves a two electron-oxidation, will produce directly a diferric state, avoiding high-valent iron – unlike in heme peroxidases where a two-electron oxidation is seen from Fe(III) to (formally) Fe(V) (Compound I). This mechanism avoids the formation of strong oxidants like Compound I or Compound II, which for an enzyme with a protective role constitutes an advantage. Structurally, rubrerythrin present a remarkable feature, in that one of the two iron ions changes its position in space twice during each catalytic cycle, traveling ~ 3 Å each time; the main difference between its two positions in space is a ligand switch at of one the metals: a histidine (softer ligand), preferred in the ferrous form, is lost in the ferric form, and replaced by a glutamate (a harder ligand, just as Fe(III) is harder than Fe(II)).

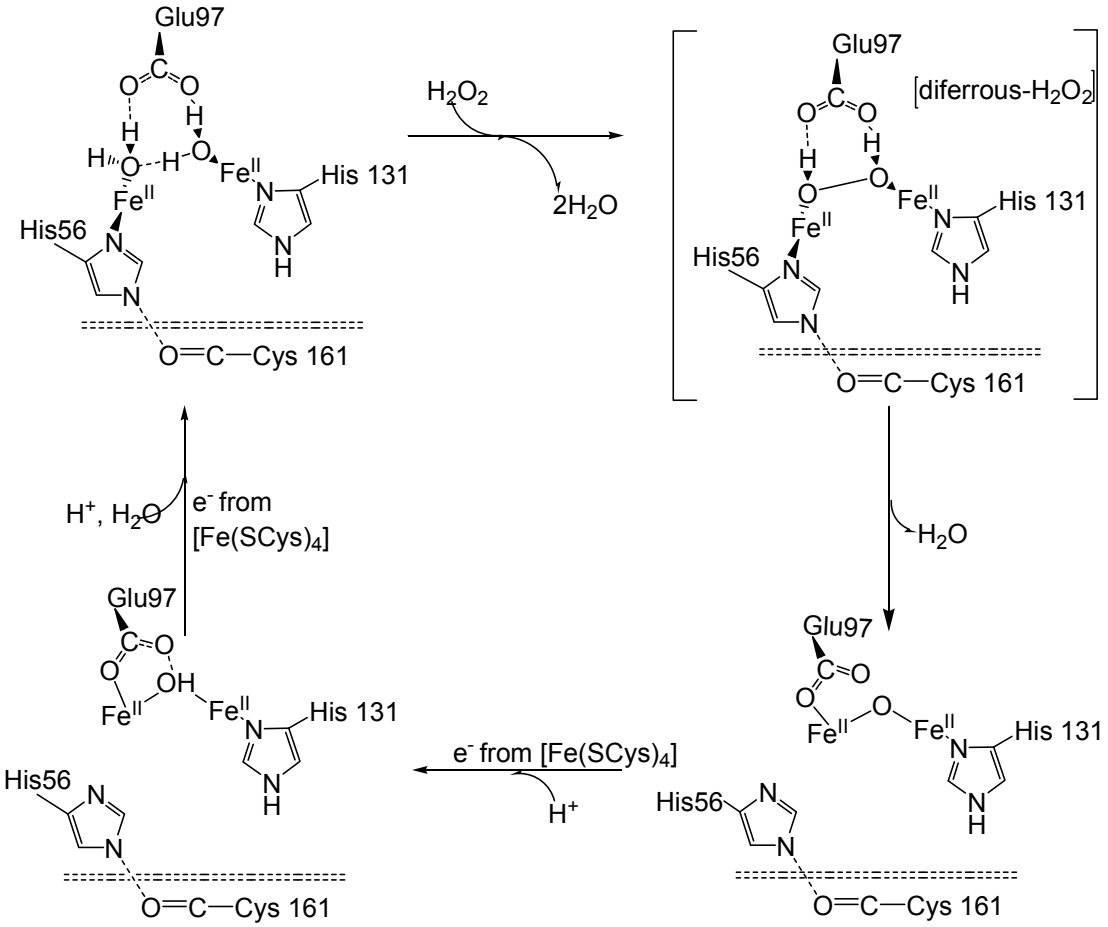


Figure 24. The reaction mechanism of non-heme peroxidases. The dotted line at the bottom of each structure illustrates inter-subunit hydrogen bonds.[18]

Non-metal peroxidases feature selenium or sulfur based active centers, which cycle between -S-S- (oxidized) and -SH HS- (two-electrons reduced) forms. Their role is protective, destroying peroxide with electrons supplied by low-molecular weight thiols such as cysteine or the cysteine-based peptide, glutathione.

4.4. Catalase

Most of the catalases work after the same principle and use a very similar active site to those of heme peroxidases, as illustrated in Figure 25. Thus, to a ferric heme, axially coordinated by a tyrosinate (instead of the cysteinate or histidine from peroxidases), hydrogen peroxide binds and cleaves in order to form Compound I. In the next step of the catalytic cycle a second molecule of hydrogen peroxide is oxidized by Compound I to O_2, the iron being re-reduced to Fe(III). The function of such enzymes is to protect the organism against hydrogen peroxide. A particular class of enzymes, named catalase-peroxidases, feature an axial histidine instead of the

tyrosinate, and exhibit both catalases and peroxidase activity. A more recently discovered class of catalases presents a binuclear manganese active center, possibly also involving a Mn(IV) state.

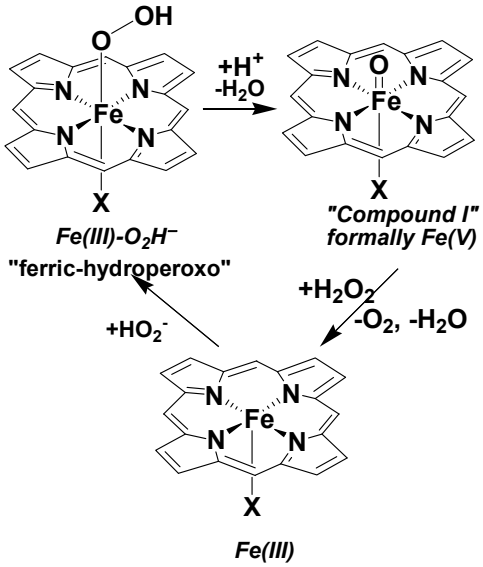


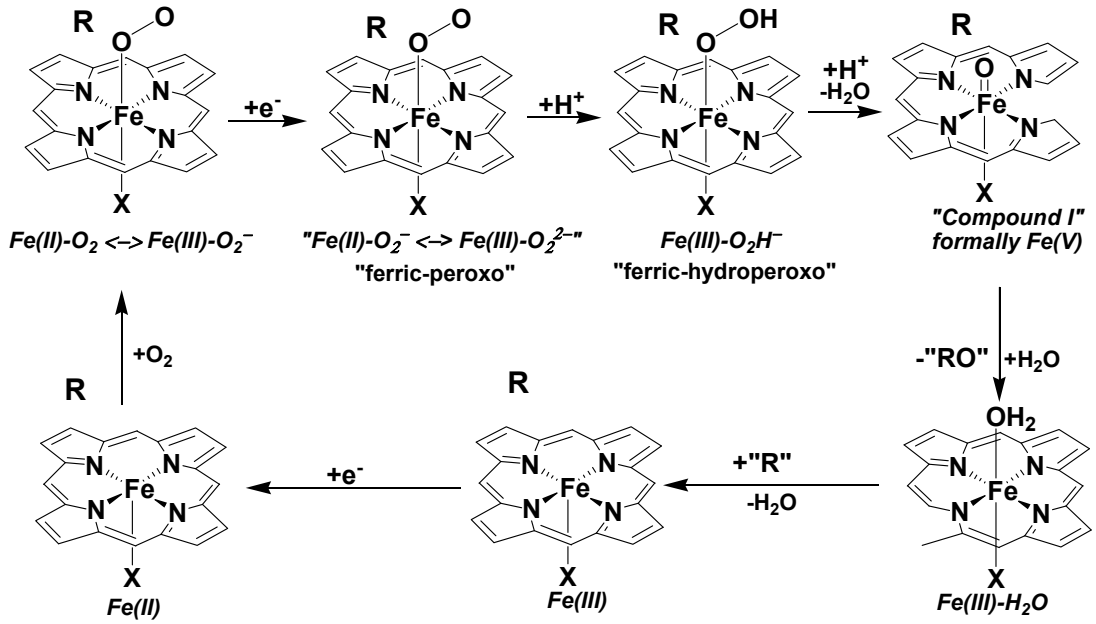
Figure 25. The mechanism of action of heme-containing catalases.

4.5. Oxygenases

4.5.1. Heme monooxygenases - cytochromes P450[25, 26]

Cytochromes P450 catalyze the insertion of an oxygen atom from an O_2 molecule into various organic compounds. Their roles vary from a synthetic one, which seeks to produce a useful compound, to a detoxification one, where substances foreign to the organism ("xenobiotics") are chemically modified in order to facilitate their elimination from the organism or to limit their toxicity. The P450 active site is similar to that discussed above for chloroperoxidases - a heme coordinated axially by cysteinate. In the stable ferric form trans to the cysteinate is found a water molecule. An important difference is that in P450 the heme is placed in a more hydrophobic than in chloroperoxidases. The catalytic cycle (Figure 26) is initiated by binding of a substrate molecule in the neighborhood of the iron-coordinated water. The steric impediments brought by this event, and the change in the polarity of the local environment in the cavity above the heme, lead to expulsion of the water molecule coordinated to the iron, leaving the metal pentacoordinate. Due to this weakening of the ligand field, the iron spin state changes from S = 1/2 (doublet) to S = 5/2 (sextet).

These changes also bring a change in the redox potential of the metal, which becomes easier to be reduced, accepting an electron brought by a specialized protein (cytochrome P450 reductase). The Fe(II) form produced in this way will immediately bind molecular oxygen similarly to the situation seen in globins, yielding an adduct describable as Fe(II)-oxygen or as Fe(III)-superoxo. Unlike in globins, the oxy form of cytochrome P450 suffers an immediate monoelectronic reduction, whose product may be described as Fe(II)-superoxo or as Fe(III)-peroxo. The peroxo species is extremely basic, and therefore rapidly protonated (in some cases the phenomenon cannot be stopped even at temperatures as low as 4 K), most likely resulting in a Compound I (but still undetected). Compound I would then insert its oxygen atom into the organic substrate by direct transfer or, in what has been called a 'rebound mechanism', by extracting a hydrogen atom from the substrate and then attaching the oxygen atom in the position from which the hydrogen was extracted, as illustrated in Figure 26. It is important to note that all the reductive steps downstream of the initial Fe(III) -> Fe(II) are very fast and thermodynamically favored, but they cannot take place in the absence of substrate, as the substrate is responsible for the elimination of the water molecule initially linked to Fe(III). In this manner, the protein ensures that Compound I cannot be produced unless its target (the substrate molecule) is already present; in the absence of such a safety mechanism there is a risk of producing Compound I in an uncontrolled manner, with unwanted, destructive effects.



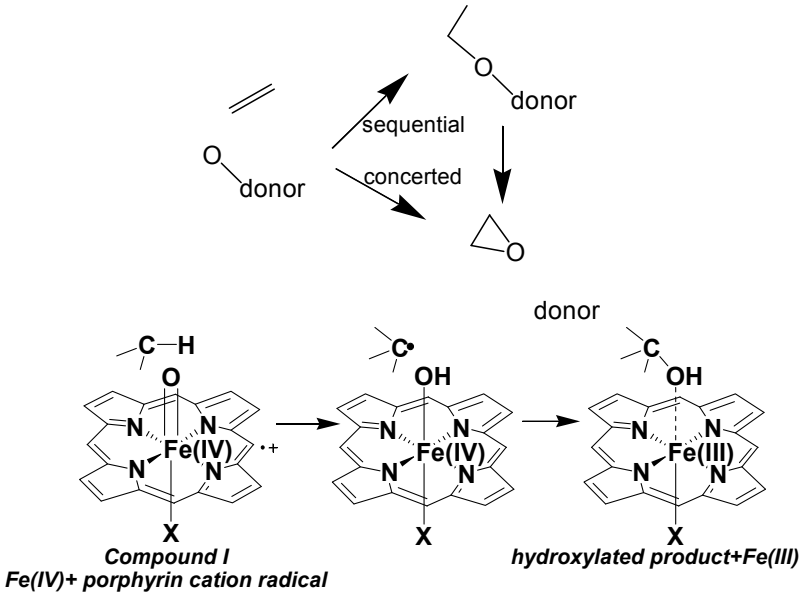


Figure 26. Catalytic cycle of cytochromes P450, "R" is any organic substrate. The bottom of the figure illustrates the mechanistic details of the occurrence of the species "RO" for cases in which R reacts to the carbon-carbon double bonds of or at the level of oxygen-oxygen links.

4.5.2. Non-heme iron oxygenase

Non-heme iron oxygenases present an interesting problem, to the extent that they follow similar catalytic cycles with hemoproteins such as P450: in the absence of the porphyrin coordinated to iron, the species that would correspond to Compound I should contain two extra oxidizing equivalents compared to Fe(III), apparently implying the problematic necessity of creating an Fe(V) center.

Rieske type dioxygenases (RDO) [27]

Dioxygenases in this class are characterized by the presence, in the proximity of the active center, of a second metal center, specialized in electronic transfer, called Rieske (see Chapter 5 for more on Rieske centers). RDO substrates tend to be aromatic compounds, and are generally oxidized to diols (an exception regarding the nature of the product is benzene dioxygenase, which catalyzes in the first instance a mono-oxygenation reaction). The catalytic cycle proposed for these enzymes is shown in Figure 27. It is still unclear which species is responsible for the direct reaction with the substrate: the main candidates are Fe(V) and Fe (III)-hydroperoxo.

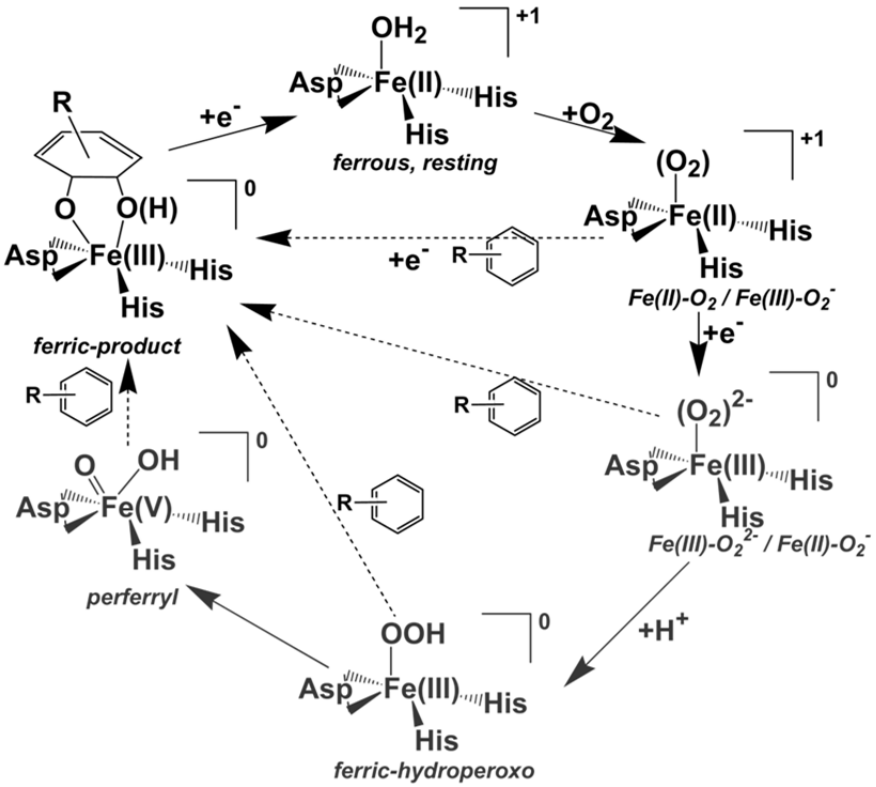


Figure 27. Proposed catalytic cycle Rieske dioxygenases. Species shown in gray have not yet been directly detected in experiments; the dotted lines indicate possible final steps to the product of the reaction.[28]

Diol dioxygenases[29]

Diol dioxygenases, classified, as shown in Figure 28 in extradiol and respectively intradiol dioxygenase, use a non-heme iron center to insert an oxygen molecule in the organic substrate, but without involving high-valent iron forms. Their catalytic cycles, illustrated in Figure 28, involve binding the oxygen in a bridge between iron and a carbon atom of the substrate; this situation implies a distinct weakening and eventually breaking of the oxygen-oxygen bond. This offers a contrast to the oxygenases, peroxidases and catalases discussed so far, where the oxygen-oxygen bond cleavage was the result of acid-base catalysis completed by the elimination of one of the two oxygen atoms as water.

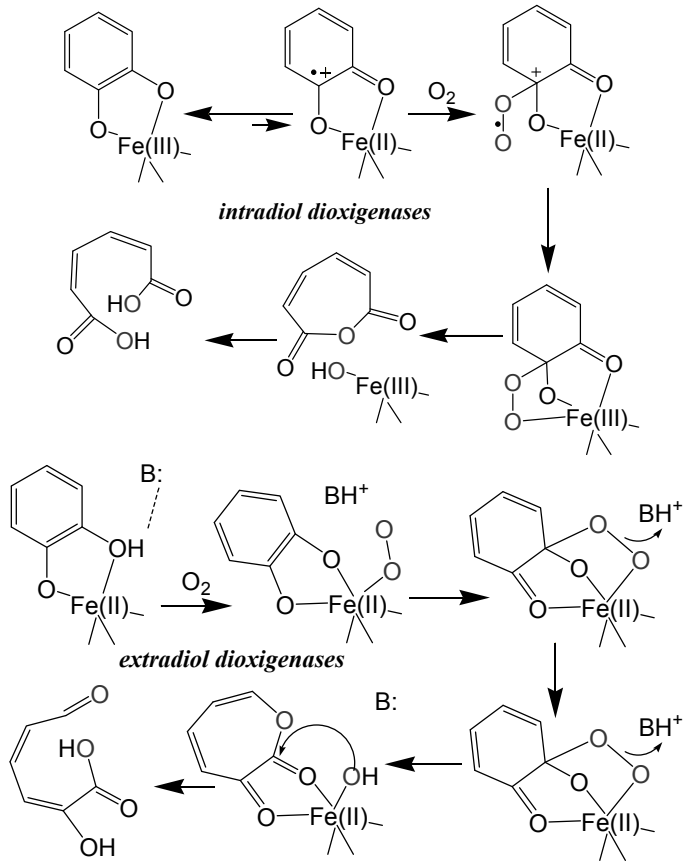


Figure 28. Reactions catalyzed by diol dioxygenases.

Oxygenases dependent on organic compounds [29, 30]

Following the model of diol dioxygenase, where oxygen-oxygen bond cleavage was facilitated not by a proton but by a carbon atom, there is a series of oxygenases that use an organic accessory molecule to weaken the oxygen-oxygen bond. One of the classes in this category is that of the pterin-dependent oxygenases. As shown in Figure 29, they use a pterin, regenerated at the end of catalytic cycle, to homolytically cleave the oxygen-oxygen bond, generating a Fe(IV) center which then oxygenates the substrate.

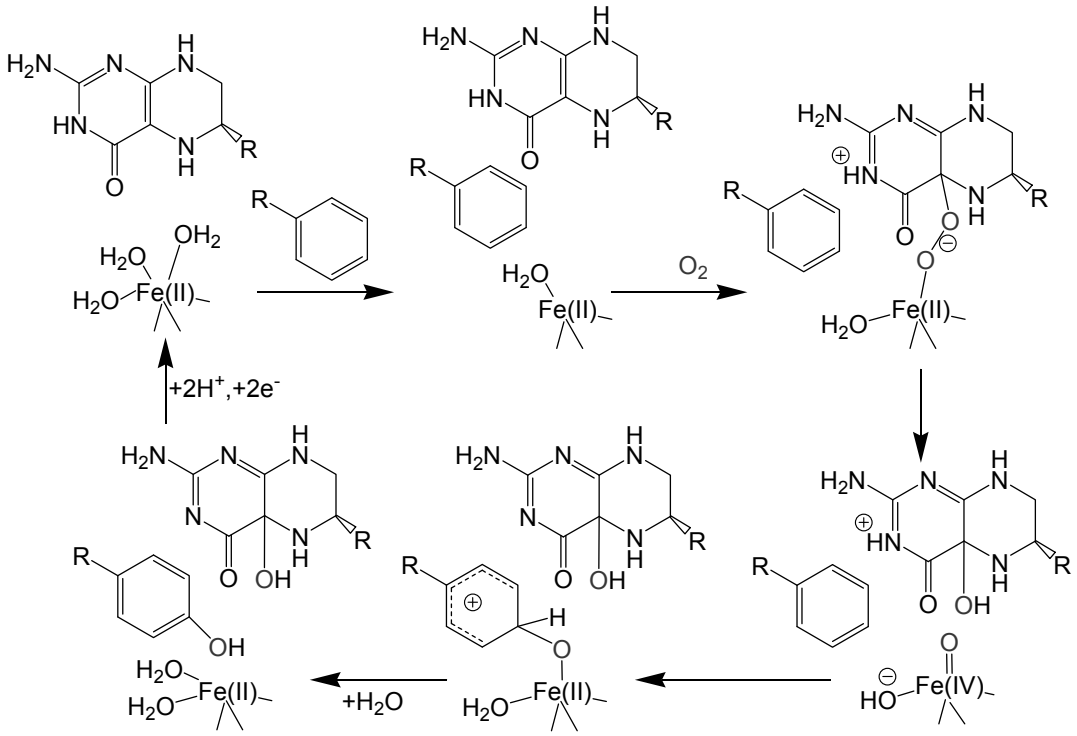


Figure 29. Catalytic cycle of pterin-dependent oxygenases.

Another class of enzymes that use this philosophy are those dependent on α-ketoglutarate (αKG). In this case however, as shown in Figure 30, αKG cannot be regenerated at the end of catalytic cycle, acting thus as a sacrifice molecule. Besides α-KG, one also requires an accessory reducing agent, to bring the enzyme to the starting point, Fe(II). For some oxygenase enzymes involved in connective tissue synthesis, this accessory reducing agent is vitamin C, and its absence from the body affects primarily this oxygenation pathway, with disastrous results on connective tissue in the disease called scurvy, whose victims were not only many of the great explorers but also entire armies in the Middle Ages - all due to non-recognition of the importance of fresh fruits and vegetables in nutrition.

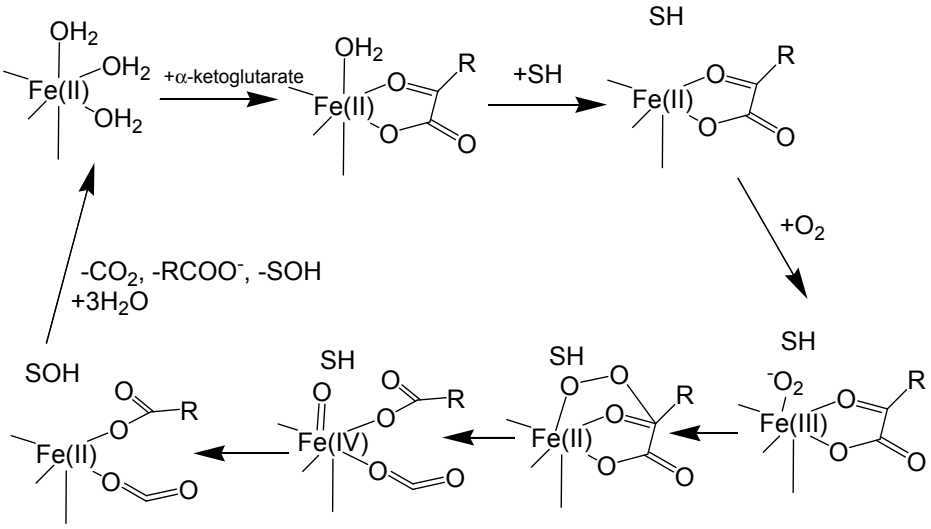


Figure 30. Catalytic cycle of α-ketoglutarate (α-KG) dependent oxygenases. „SH" generically represents an organic substrate.

Iron-containing methane monooxygenase [31]

Selective oxidation of methane to methanol is difficult to achieve, CH_4 presenting one of the least reactive carbon-hydrogen bonds among organic compounds. The enzyme capable of this reaction, methane monooxygenase, presents a catalytic site similar to the one found in hemerythrin or rubrerythrin, but much richer in oxygen-based ligands (glutamate/aspartate). The structure and proposed mechanisms for this active center are presented in Figure 31. Due to the presence of two iron ions, the maximum oxidation state that this site can reach in the reaction with molecular oxygen and its congeners is Fe(IV) and not Fe(V).

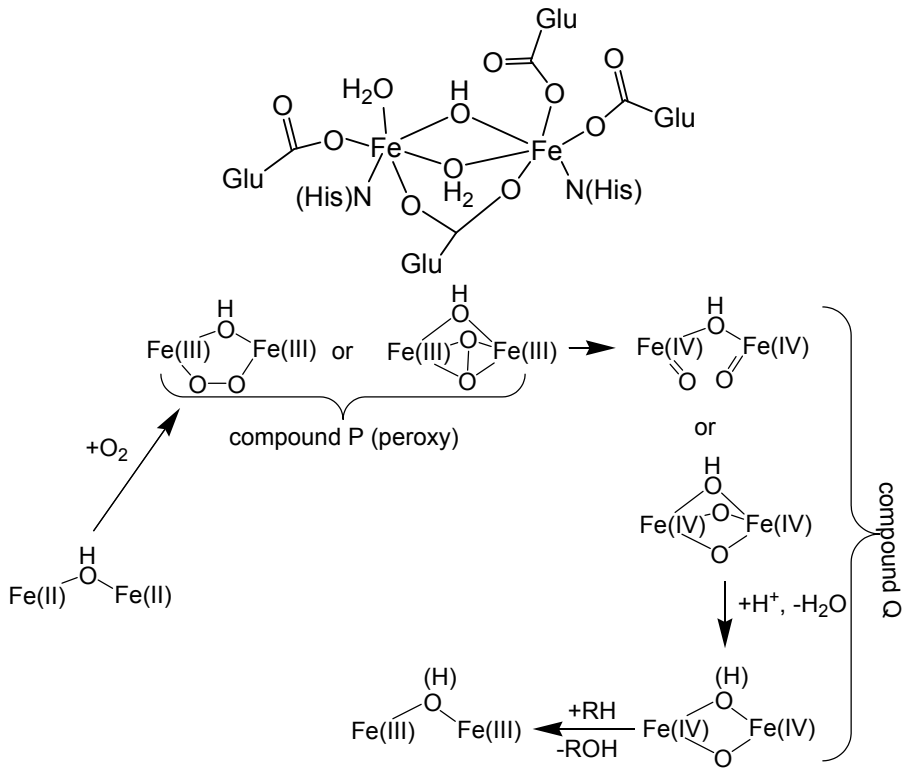


Figure 31. Structure and proposed mechanisms for iron methane monooxygenase. Concerning the structure, it is notable that depending on temperature and on the oxidation state of the metals, the oxygen-based ligands undergo rearrangements (e.g., water molecules may be lost, carboxylates can change their hapticity).

Ribonucleotide reductase[31, 32]

Ribonucleotide reductases have the role of transforming sugar units from nucleotides in deoxy sugars (deoxynucleotides). Their active site contains a tyrosyl radical, generated however by a reaction taking place far away within the same protein, between molecular oxygen and a di-iron site similar to that of methane monooxygenase. A schematic representation of the proposed mechanism of ribonucleotide reductase is shown in Figure 32.

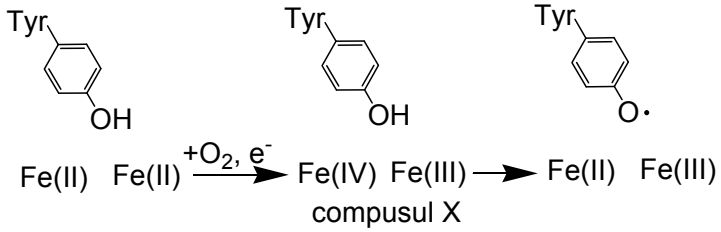


Figure 32. Ribonucleotide reductase reaction at the metal site.

4.5.3. Copper-containing oxygenases [33]

Most types of reactions discussed so far in this chapter can also be catalyzed by copper-based enzymes. Figure 33 illustrates some examples, and Figure 34 illustrates types of copper-based active sites involved in the reactions of Figure 33. Up to a point, iron and copper oxygenase work by the same principles: the reduced state of the metal - Cu(I) this time - bind molecular oxygen, forming a Cu(I)-O_2 adduct that presents a Cu(II) -superoxo electromer, and which can be reduced to Cu(II)-peroxo and protonated to Cu(II)-hydroperoxo in order to facilitate oxygen-oxygen bond breakage. The major difference is that, unlike iron, on the biological copper-based centers the general rule is not to form higher-valence states (e.g., Cu (III)), but to use directly the metal-coordinated peroxide in order to execute the oxidation reaction.

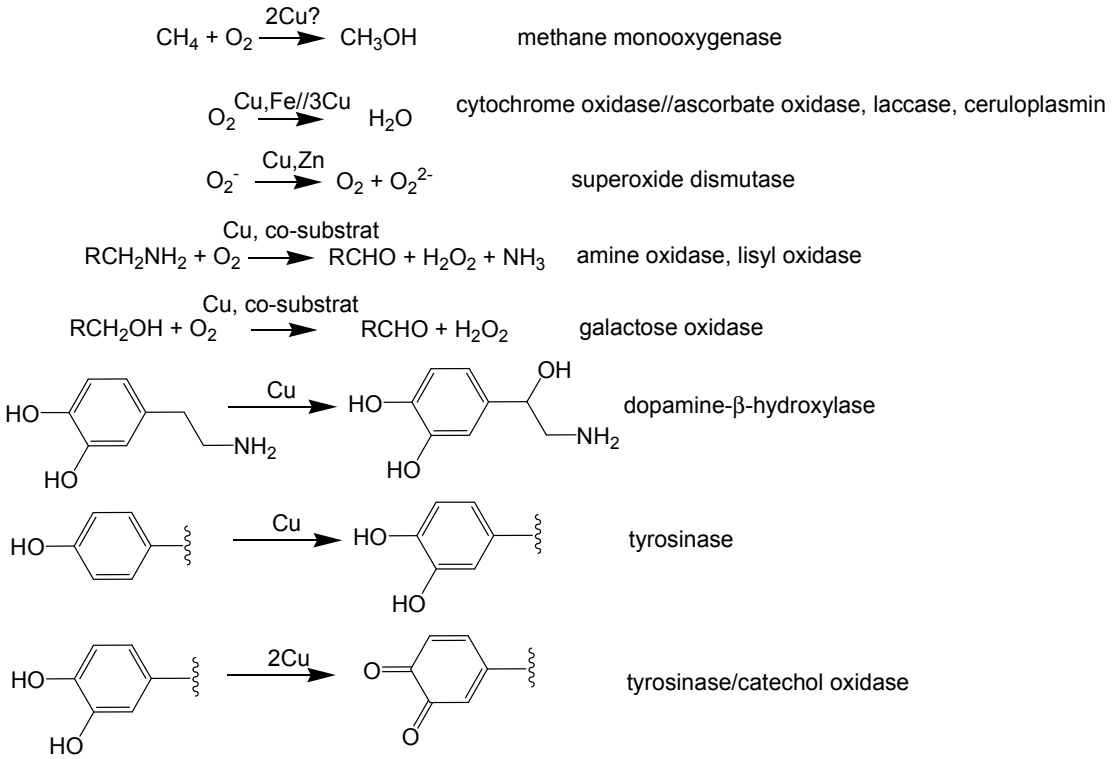


Figure 33. Processes catalyzed by copper-based metalloproteins and involving molecular oxygen or its congeners.[34]

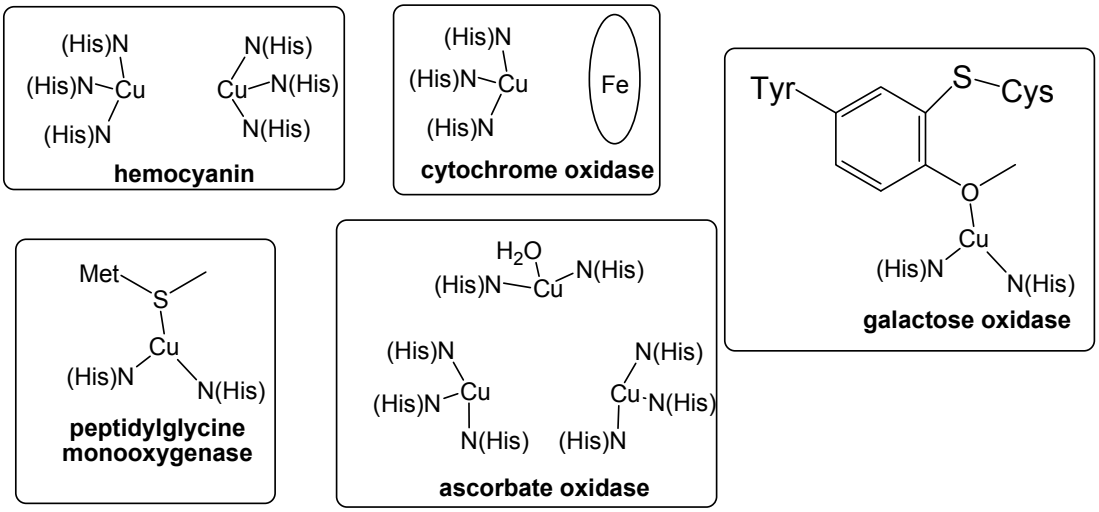


Figure 34. Representative copper active sites.[34]

4.5.4. Manganese oxygenases

Such oxygenases are much less known; they appear to function under principles very similar to those of non-heme iron oxygenases – the ligand system being also similar in the two classes of enzymes.

4.5.5. Non-metal oxygenases

There are enzymes, which do not form the subject of study for this text, in which molecular oxygen is activated by purely organic centers; an example of such a center are flavins - redox active aromatic compounds, with three accessible oxidation states (two-electron reduced, oxidized, and one-electron-reduced free radical).

Chapter 5. Electron-transport proteins and related enzymes

A wide range of metabolic processes, some of which were mentioned in previous chapters, require an input or removal of electrons to/from the active site. Therefore, living organisms have developed specialized proteins for electron transport. Here we discuss four main classes of such proteins, three of which contain metals.

5.1. Iron-sulfur proteins

The active centers of iron-sulfur proteins contain predominantly these two elements. Examples are illustrated in Figure 35. In the simplest representative, rubredoxin, the iron is tetrahedrally coordinated by four cysteinate ligands. Ferredoxins contain dimeric and multimeric versions of the rubredoxin site, where beside cysteines two or more sulfido bridges are also found between the metals. Rieske-type centers are related to those of ferredoxins, containing two histidines instead of two cysteinate ligands. Although many of these iron-sulfur clusters are in principle available in several oxidation states (e.g. a ferredoxin binuclear center can occur as Fe(II)-Fe(II), Fe(II)-Fe(III), or Fe(III)-Fe(III)), only a small part of these states are accessible and used in vivo.

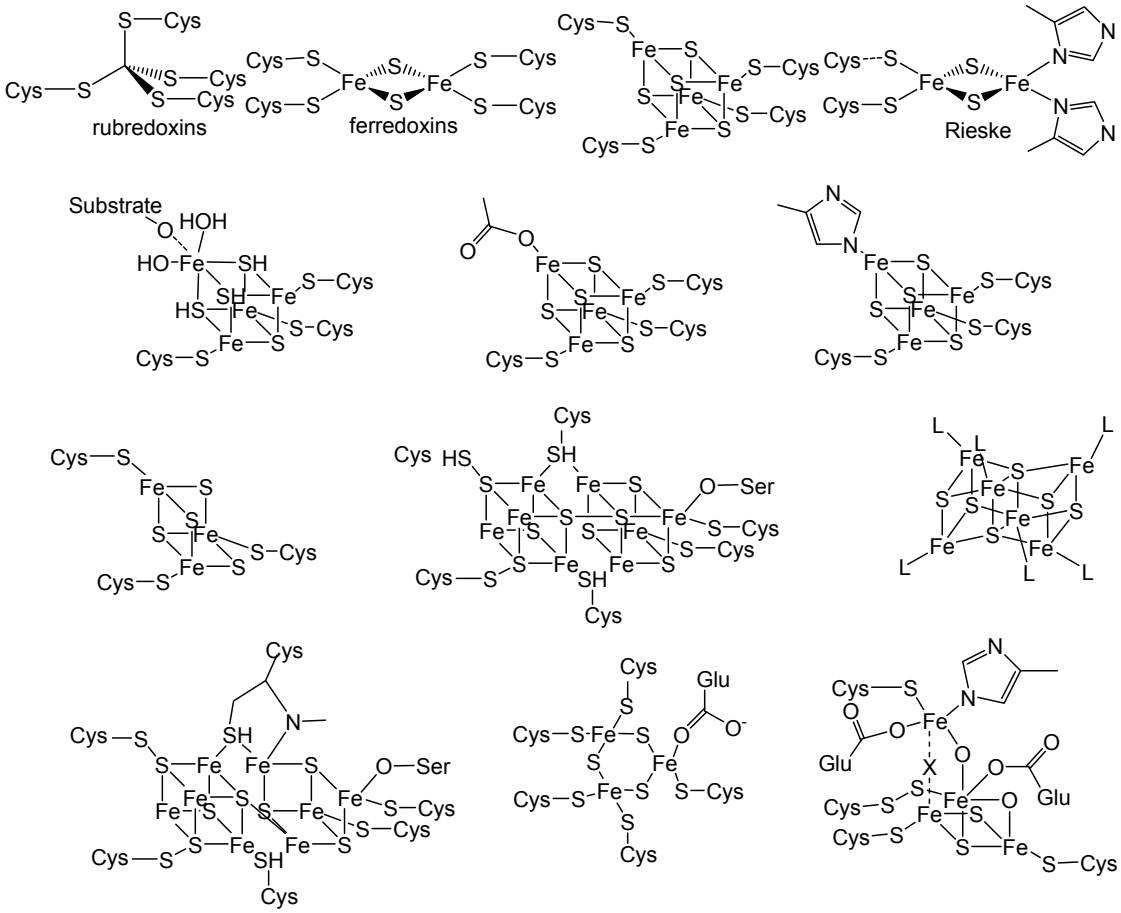


Figure 35. Centers with iron and sulfur in proteins.[35]

Although most centers with iron and sulfur have only electron transport roles, there are also exceptions. Among them the most considerable is the aconitase, whose structure is illustrated in Figure 36, which catalyzes the citrate-isocitrate isomerization using aconitate as intermediate in the tricarboxylic acid cycle (see also Chapter 6 for the importance of the latter). Additionally, aconitase also controls iron metabolism.

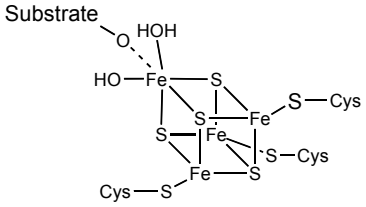


Figure 36. Active site of aconitase.[35]

5.2. Cytochromes

The name "cytochrome" generally denotes hemoproteins in biology. Hemoproteins that function as electron transporters tend to be hexacoordinate (cf.

Figure 37), and are named after the type of heme they contain (*a, b, c,* etc., see Figure 38) and sometimes by also denoting certain wavelengths where the characteristic absorbance spectrum is observed in UV-vis (example - cytochrome c_{552}).

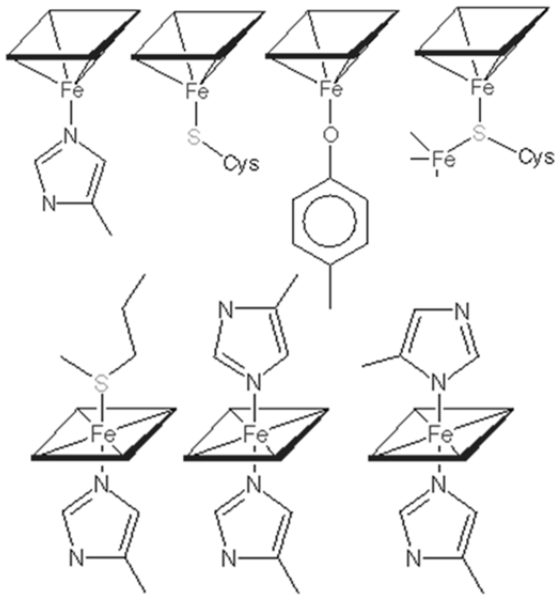
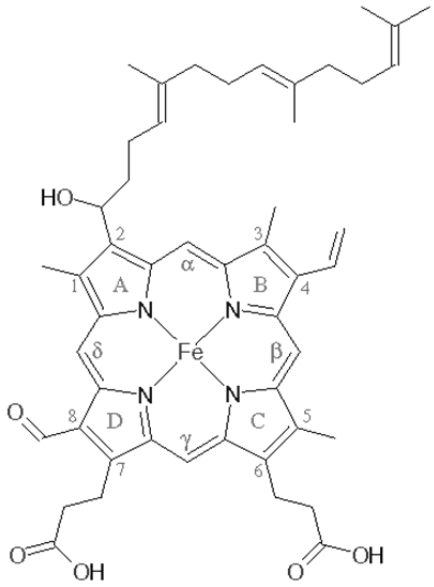
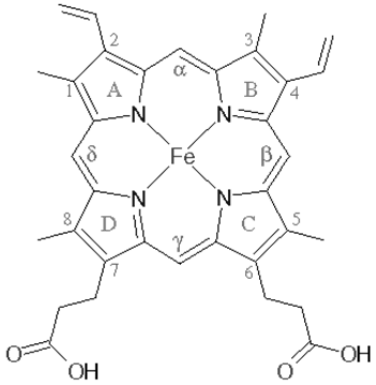


Figure 37. Heme coordination modes in hemoproteins. Electron transport proteins follow the coordination modes situated in the row below, while the top row structures are specific to enzymes.[35]

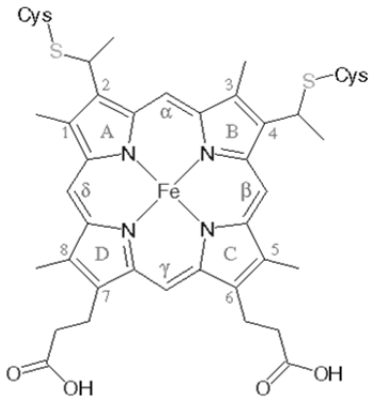
Some electron transporters, by virtue of their involvement in essential metabolic pathways are important targets for control mechanisms. For example, cytochrome *c* is involved as messenger in the process of apoptosis - programmed cell death.



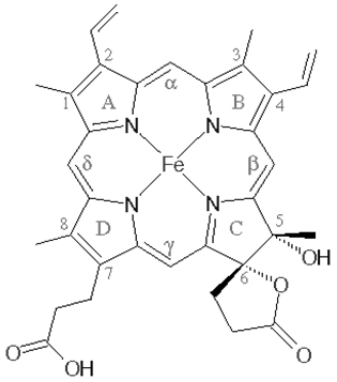
heme *a*
cytochrome *c* oxidase



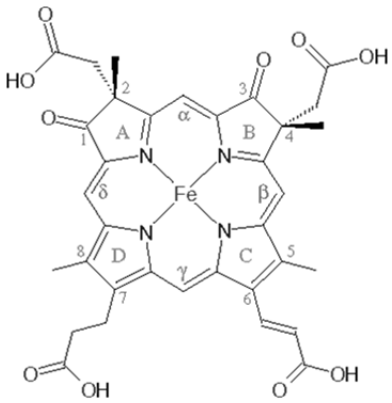
heme *b*
catalase, cytochromes *b*, globins, guanylate cyclase, cytochromes P50, nitroforine, peroxidase



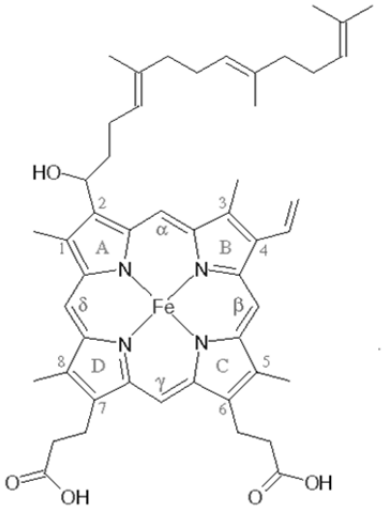
heme *c*
cytochromes *c*,
hydroxylamine oxidoreductase



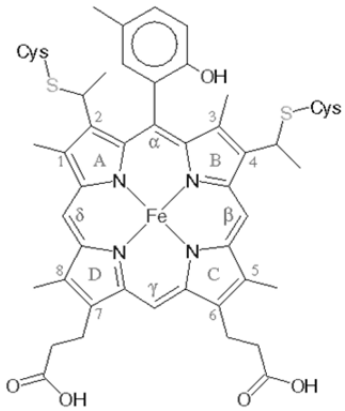
heme *d*
cytochromes *d*, catalase



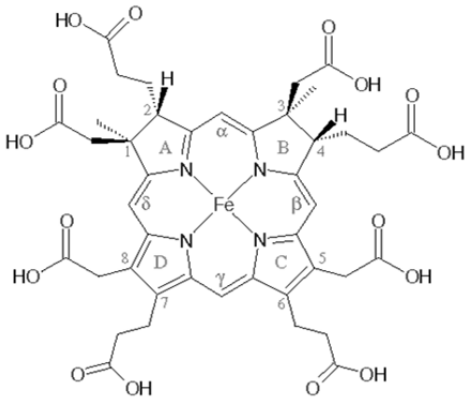
heme d_1
cytochromes cd_1 nitrit reductase



heme *o*
cytochromes *o* oxidase



heme P460
hydroxylamine oxidoreductase



siroheme
sulfite reductase

Figure 38. Heme types and examples of proteins in which they are found.[35]

5.3. Blue copper proteins

Copper-containing electron carriers have a mononuclear center, with ligands illustrated in Figure 39. With cysteinate a charge transfer from ligand to metal (LMCT) occurs in the UV-vis spectrum of the Cu(II) form. Incidentally, the wavelength of the LMCT band coincides with that of the band due to the d->d transitions of copper (II) and responsible for the blue color of many common Cu(II) compounds. However, copper-protein's LMCT transitions have much higher extinction coefficient than the d-> d transitions, and therefore the color of the "blue" copper proteins in oxidized form is far more intense than that of simple inorganic Cu(II) salts. Consistent with this relatively intense color, proteins in this class contain in their names the "cyanin" particle - such as plastocyanin, stellacyanin or amicyanin.

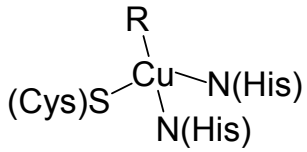


Figure 39. Active center of copper-based electron transport proteins; "R" can be methionine (examples - in azurin, plastocyanin or laccase), glutamate (in phytocyanine) or water (in ceruloplasmin).

5.3. Non-metallic electron transport

Electrons transport can also be achieved by purely organic units. Among these the most common are flavins (embedded in protein under FMN or FAD form), NAD(P)H and quinones (e.g. ubiquinone).

Chapter 6. Respiration

The main role of molecular oxygen in most known organisms is to contribute to the so-called "cellular respiration" by which nutrients in the diet (proteins, lipids, carbohydrates) are converted into CO_2 and H_2O. The energy released in this process is stored in the cell as ATP (adenosine triphosphate, the "universal energy currency" molecule in living organisms). Figure 40 presents an overview of the reaction pathways leading from nutrients (carbohydrates, lipids, proteins) to CO_2 and water. The "backbone" of this series of processes are glycolysis and the tricarboxylic acid cycle (TCA). Notably, these pathways do not directly require molecular oxygen, as the oxygen atoms in the product CO_2 derive from water molecules and not from O_2. The process consists in removing electrons (oxidation in redox terms) from the carbon atoms of carbohydrates/proteins/lipids; these electrons are taken over by specialized carriers - NADH, $FADH_2$ and some of the steps in this electron transfer from food molecules also have the privilege of producing energy directly in the form of nucleoside triphosphates (ATP, GTP). However, most of the energy brought by nutrients is, at the end of TCA, stored as electrons placed on NADH and $FADH_2$. The role of molecular oxygen (essential in fact) will be to facilitate the transformation of this energy into one stored as ATP.

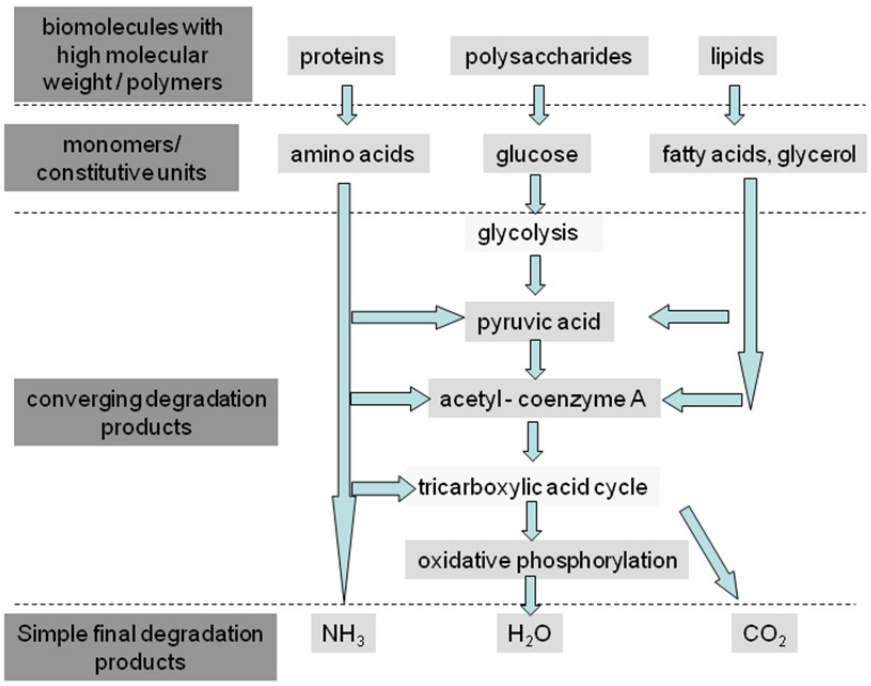


Figure 40. Metabolic pathways.

TCA, during which electrons are extracted from nutrients, takes place in cell organelles called mitochondria (see Figure 41). Mitochondria present two membranes (internal and external, respectively), bordering on the inside the "mitochondrial matrix" and between the two membranes the "intermembrane space."

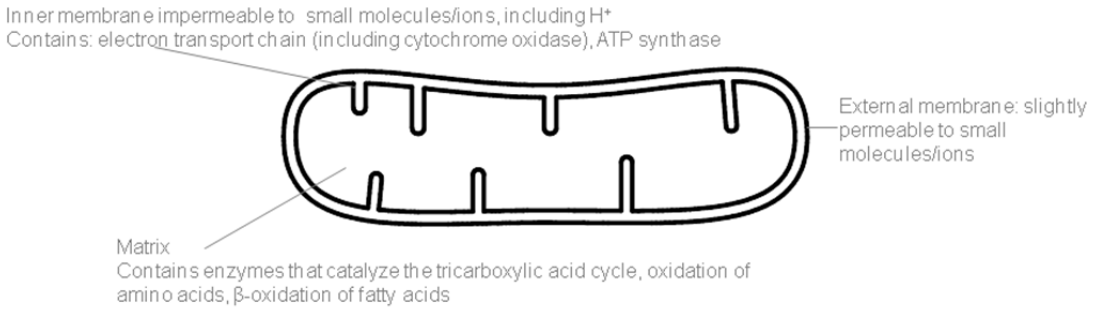


Figure 41. The mitochondrion

TCA takes place in the mitochondrial matrix; the electrons it produces are then transferred to a chain of electron transport proteins from the internal membrane called, because of its importance, the "electron transport chain". As shown in Figure 42, this chain consists of four protein complexes - Complex I, Complex II, Complex III and Complex IV. Depending on the exact source of the electrons in the TCA, they enter the electron transport chain either at Complex I or at Complex II, from where they are transferred to Complex III and then via cytochrome *c* to Complex IV, also called cytochrome *c* oxidase. The entire route of the electrons through the internal membrane involves their transfer in small steps between various redox active centers among categories discussed in Chapter 5: iron-sulfur centers, cytochromes, copper centers, quinones, flavins. Each such step leads to dissipation of part of electron's energy, which is intentionally used by the protein for movement of protons from the mitochondrial matrix to the intermembrane space. The net result, at the end of the electron path through Complexes I, II, III and IV, is an accumulation of protons in the intermembrane space, based on the energy of the electrons extracted from nutrients. The electrons themselves, having reached the end of the route in Complex IV, have already lost all of their biochemically-useful energy, as it was spent on the accumulation of protons in intermembrane space. Therefore the issue that rises is the disposal of these electrons from the system by placing them on a "trash-can" molecule. This molecule must satisfy two conditions: to be able to accept electrons without generating toxic products and to be found in abundance. In aerobic

organisms, including humans, this molecule is molecular oxygen and its reduction product is water. There are also organisms (especially bacteria) that use other molecules instead of oxygen - such as nitrate, sulfate, or insoluble inorganic salts ("stone"). The series of processes described here is generically called "respiration"; molecular oxygen (or other molecules which take its place in non-aerobic organisms) is called the electron "final acceptor", and the enzyme in which the last redox reaction takes place is generically called "terminal oxidase". If in humans the terminal oxidase is cytochrome *c* oxidase, in other organisms also exist related proteins that use other heme types and another electron donors (e.g., quinones instead cytochrome *c*).

On the other hand, the energy now stored as proton gradient (excess in intermembrane space compared to mitochondrial matrix) is then used by the last enzyme of this route, located also in the internal membrane, which, on account of this energy, synthesizes ATP as it allows protons to return to the matrix (operating in this way according to the principle of a hydropower plant). At this point, the energy extracted from nutrients was completely transformed into a form accessible to any other compartment of the organism - ATP.

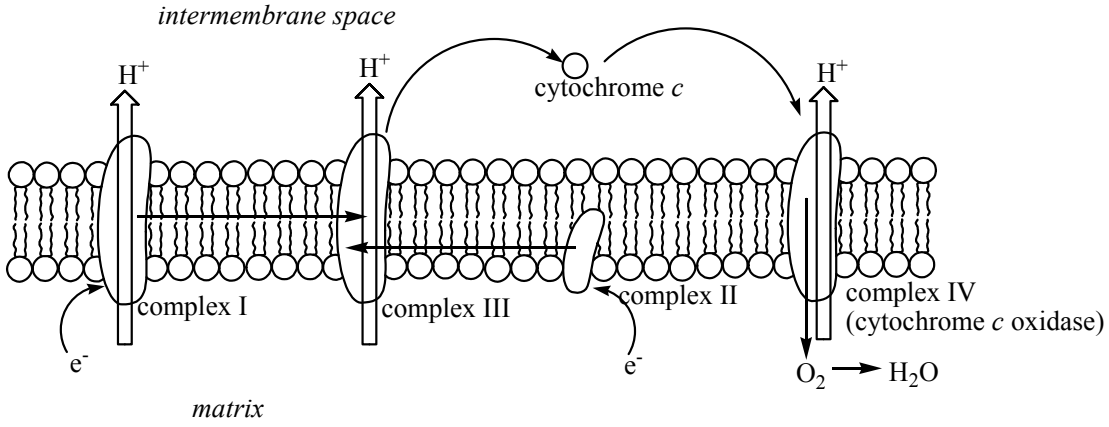


Figure 42. Electron transport chain in mitochondrial internal membrane.

The action mechanism of cytochrome oxidases is relatively complicated: they have to inject four electrons in the oxygen molecule almost simultaneously. A partial reduction of O_2 with only one, two or three electrons would mean the generation of extremely toxic species: O_2^-, O_2^{2-}, and OH•, respectively. Therefore it is not surprising that cytochrome *c* oxidase contains not less than four metal centers: a binuclear copper (the Cu_A), a heme type *a*, and a heme type a_3 placed in the immediate vicinity

of a copper center (Cu_B) - as shown in Figure 43. Also found in this protein are zinc and magnesium, with non-redox roles.

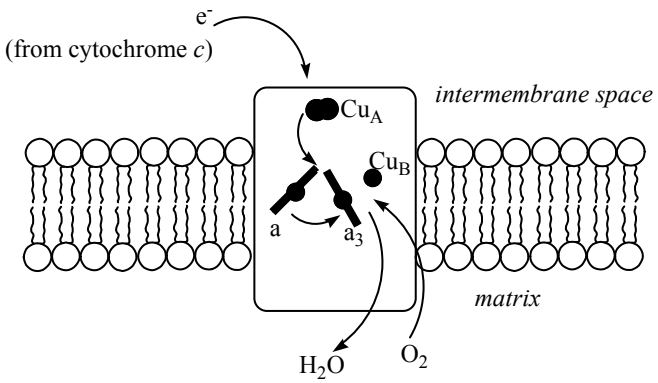


Figure 43. Cytochrome *c* oxidase metal centers.

Thus, it is possible to store all the electrons needed for O_2 reduction to water, even before O_2 is bound to protein, so that effective reduction directly to water could be achieved, avoiding the toxic partial reduction products mentioned above. A proposed mechanism for this is illustrated in Figure 44, with the remark that it is not yet fully elucidated - primarily because of the extremely high speed of the processes (some operating on micro and nano-second scale).

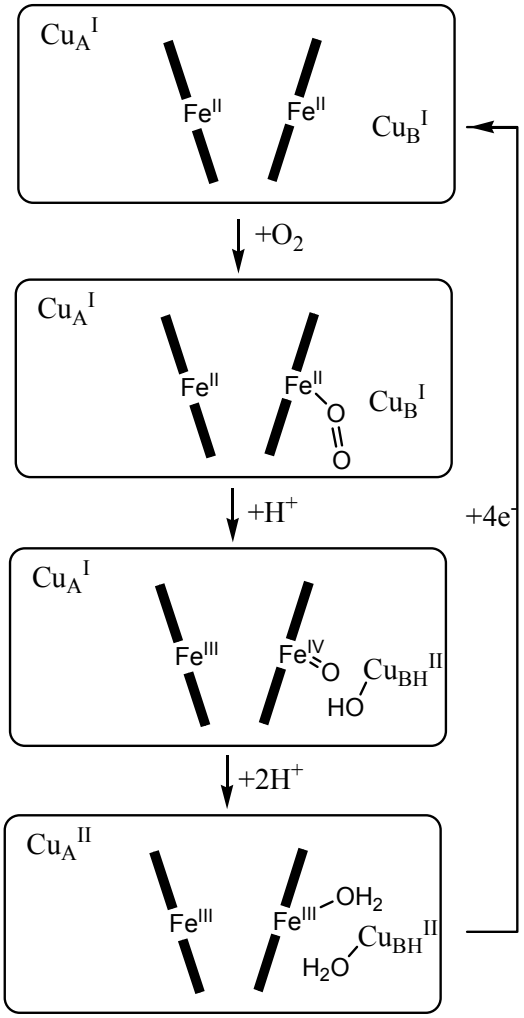


Figure 44. Proposed mechanism for cytochrome *c* oxidase.

Chapter 7. Tungsten, molybdenum: oxygenation without O_2

In most cases, molybdenum is found in proteins coordinated by the dithiolenic unit of a pterin, forming the so-called molybdopterin, as shown in Figure 45. The remaining coordination sphere is completed by oxo, hydroxo, sulfido, cysteinate, selenocysteinate ligands, or even by a second pterin unit. The metal mainly oscillates between two oxidation states - Mo(IV) and Mo(VI). The reactions catalyzed by molybdo-enzymes usually involve the transfer of an oxygen atom to or from the substrate - in principle involving one of the oxo/hydroxo ligands of molybdenum, which, notably, even though they are involved in oxygenation reactions, derive from water and not from molecular oxygen (as opposed to the oxygenases from Chapter 4). Examples are nitrate reductase, sulfite oxidase and arsenate reductase. Even if molybdoenzymes such as xanthine oxidase or various aldehyde oxidoreductases (Figure 45) require molecular oxygen to function, the oxygen atom which they insert in the substrate also comes from a water molecule, and not from O_2.

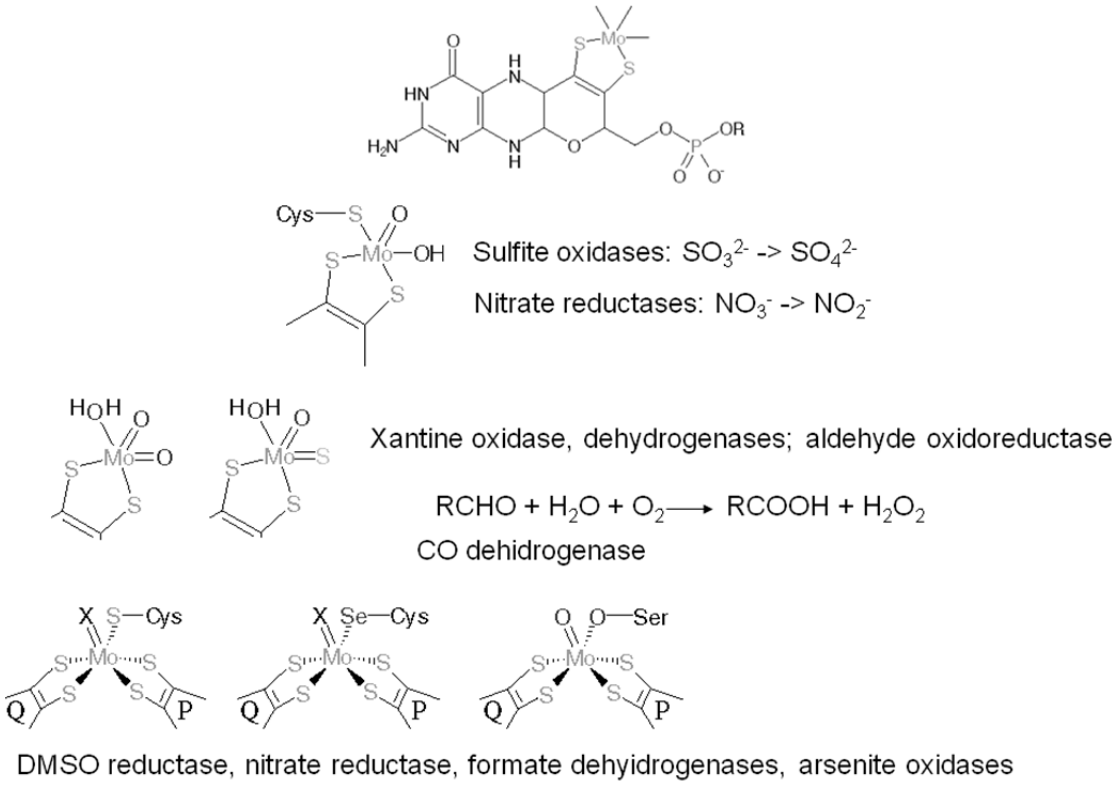


Figure 45. Coordination modes of molybdenum in proteins.[35]

Tungsten is found in coordination environments very similar to those of molybdenum. In fact, the two metals can replace each other in most proteins where they are present, without complete loss of enzymatic activity. However, there are also enzymes that accept only tungsten in the active site. These have two main features. First, are coordinated by two pterinic units. Secondly, they tend to be present in the strictly anaerobic organisms, some of them being extremophiles. We will define on this occasion in Table 1 several classes of extremophiles - organisms that best grow under conditions that are defined by human standards as extreme.

Table 1. Extremophiles.

Class	Favored environment	Examples
acidophiles	Acid	*Acetobacter aceti*, *Acidianus infernus*
alcalophiles	Basic	*Geoalkalibacter ferrihydriticus*http
halophiles	High concentrations of salts	*Halobacterium, Chromohalobacter beijerinckii*
hyperthermophiles	80-122ºC	*Pyrococcus furiosus, Geothermobacterium ferrireducens*
metallotolerant	High concentrations of heavy metals	*Ferroplasma, Cupriavidus metallidurans*

Class	Favored environment	Examples
osmophiles	High concentrations of carbohydrates	*Saccharomyces cerevisiae*
piezophiles/ barophiles	300-1000 atm	*Halomonas salaria*
Cryophiles/ psychrophiles	Under 15ºC	Some members of *Halomonas, Pseudomonas* families
radioresistent	Radioactivity	*Deinococcus radiodurans*
termophiles	45-80ºC	*Thermus aquaticus*
xerophiles	Dry environment	*Trichosporonoides nigrescens*

Chapter 8. The most abundant metals in living organisms - osmotic equilibrium, signaling, catalysis

The discussion so far on bioinorganic systems was focused on elements present in trace amounts in living cells. In much larger quantities because of their increased solubility (respectively to the ease with which changes their ligands) are alkali and alkaline-earth metals. Their kinetic lability does not generally recommend them as important components in catalytic sites of enzymes. However, they have other essential roles, such as charge balancing, signal transmission, or osmotic pressure. Thus, many biomolecules carrying negative electrical charges (nucleic acids being the most common examples due to the phosphate groups in their composition), need counterions to compensate these charges, sodium and potassium are excellent candidates for this purpose. Furthermore, sodium, potassium, calcium and other inorganic ions concentrations are maintained at constant levels, the osmotic pressure being generally kept constant inside a cell.

The mechanisms by which inorganic ion concentrations and osmotic pressure are kept constant involve transport, often against the gradient (and therefore energy consumption in the ATP form), of these ions through the cell membrane, using specialized membrane proteins (called, from case to case, canals, pumps, or transporters). Figure 46 illustrates several types of these proteins. A special case is that of sodium and potassium pump in Figure 46: it provides an asymmetry between the two sides of the cell membrane regarding electric charge.

Figure 47 illustrates how the sodium, potassium and calcium are involved in transmitting nerve impulses. An initial signal occurs at one end of the axon (upper side of Figure) by temporary opening of a channel-protein that allows entry of sodium ions in the cell. Local sodium afflux inside the membrane is detected by a channel-protein in the immediate vicinity downstream in the axon, and this second channel protein, in response, also opens briefly, thus propagating the signal. In this way the signal travels to the opposite end of the axon, to the junction with the next axon (synapse). Near the synapse calcium channel-proteins additionally open, which subsequently trigger the release of vesicles containing acetylcholine in the space

between the two nerve cells. Acetylcholine reaches the neighboring cell, where it initiates the transmission of nerve impulse through it, binding to a sodium channel-protein, which opens, further, in this second axon the cascade of signal propagation using sodium and potassium channel proteins.

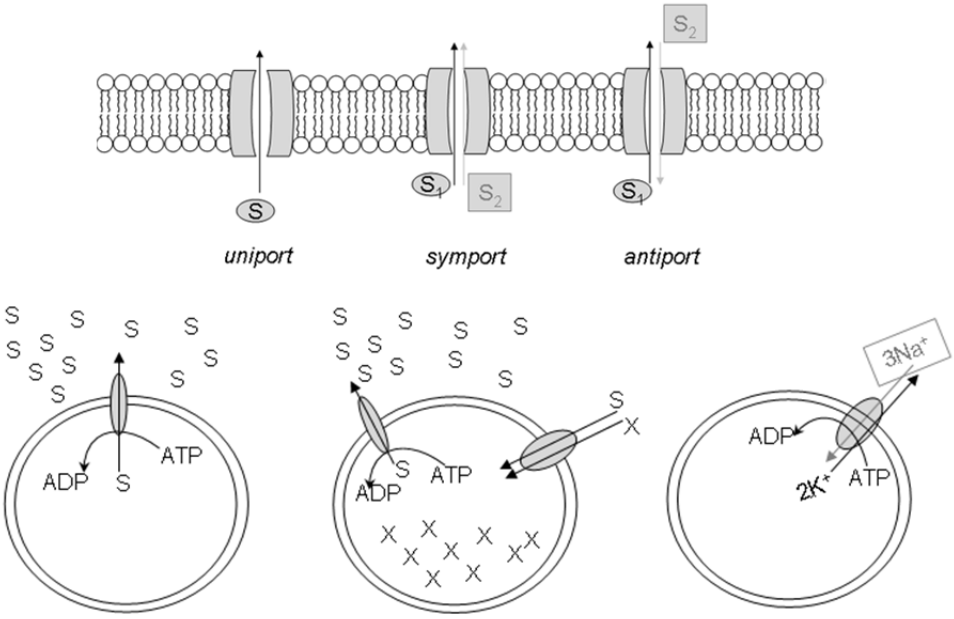


Figure 46. Pumping/transport ways of ions through membranes.

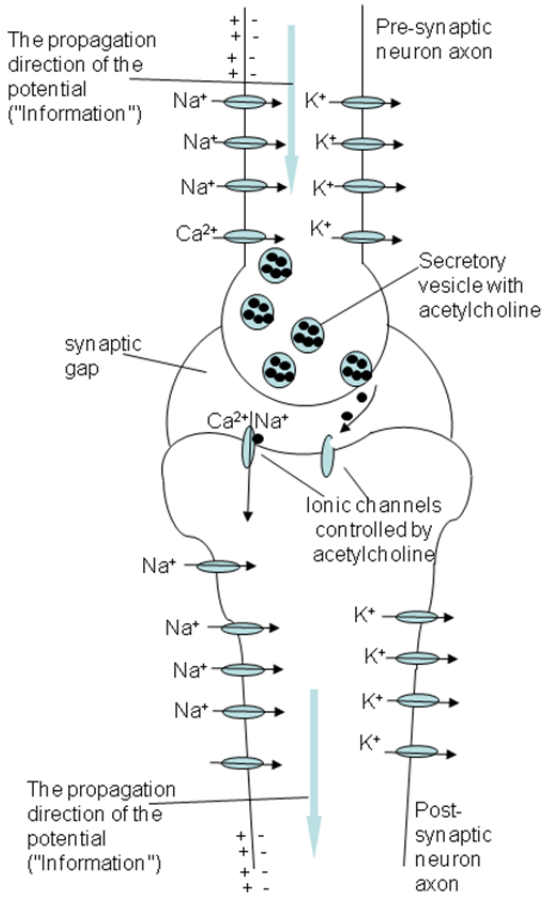


Figure 47. Signal transmission mode through nerve cells.

Besides nerve cells, calcium plays signaling roles in many other places. Often, the receptor that detects calcium presents a structural motif shown in Figure 48 for the protein called calmodulin, namely the "EF hand" - a calcium binding site at the junction between two portions of α helix which in calmodulin happen to be named helix E and F. This binding motif was taken to have some resemblance to a hand – hence its name.

Figure 48. „EF hand", a structural model of calcium binding in proteins.

Although, as noted above, alkali and alkaline earth metals tend not to be involved in catalytic sites, there are notable exceptions. One of them is the enzyme RuBisCO (1,5-bisphosphate ribulose carboxylase/oxygenase), whose active site contains a magnesium ion. Rubisco is found in plants and is responsible for the conversion of inorganic carbon, CO_2, in organic matter via the mechanism illustrated in Figure 49. The metal's role is only to fix the carbohydrate where carbon dioxide will attach. The particle "oxygenase" in the enzyme's name refers to the fact that occasionally the enzyme accepts O_2 instead of CO_2 as a substrate, catalyzing an oxygenation reaction instead of the desired carboxylation. Other examples of atypical catalytic sites are those based on calcium in some lipases or those based on potassium in some phosphatases.

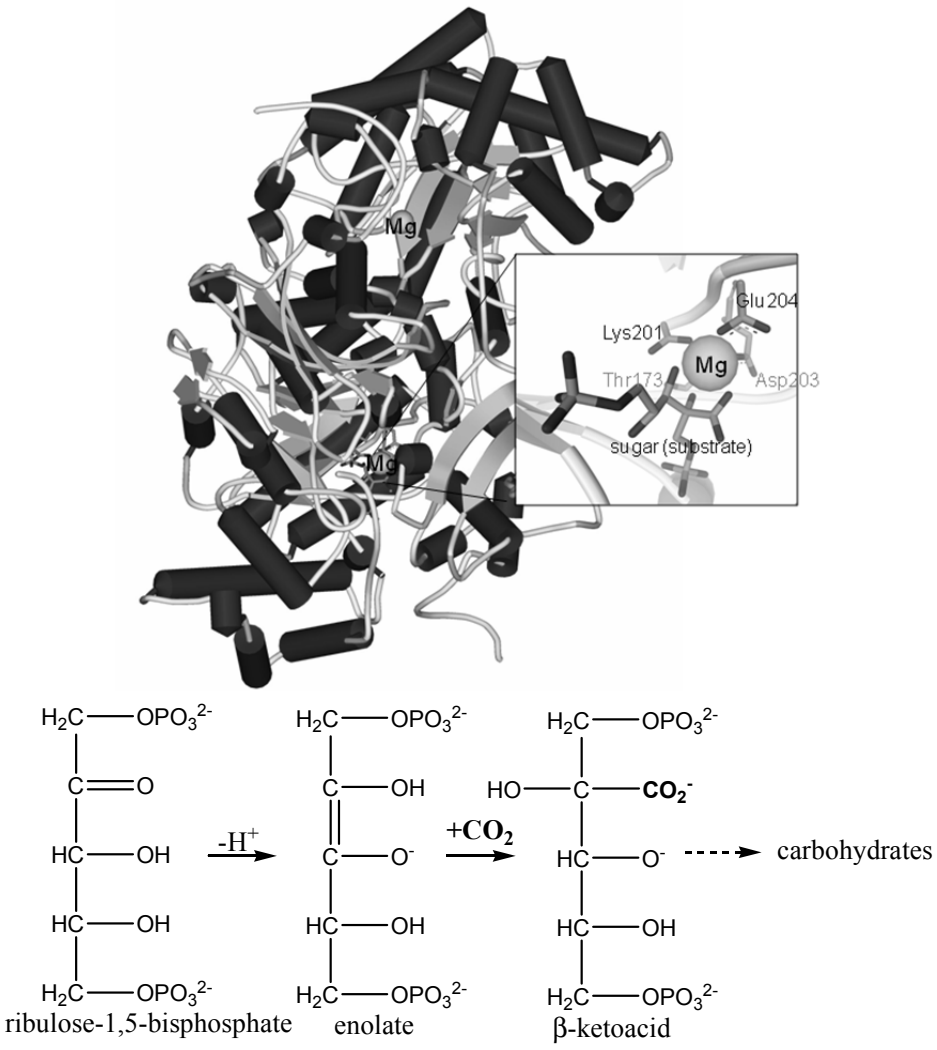


Figure 49. Active site and reaction catalyzed by RuBisCO.

With some of the more abundant metals in nature living systems occasionally synthesize minerals (thus called "biominerals", and the process called "biomineralization"), with different roles, as illustrated in Table 2.

Table 2. Types of minerals synthesized by living organisms and their roles.

Substance	**Function/examples**
Calcium carbonate	Coral exoskeleton, egg shell, mollusk shell, C
Calcium phosphates	Endoskeleton (humans, other vertebrates)
Calcium oxalate	Ca storage, defense mechanisms in plants
Ca, Ba, or Sr sulfates	Gravity sensors, exoskeletons
Amorphous silicates	Diatom valves, defense mechanisms in plants
Iron oxides	Magnetic sensors, iron storage, teeth in some mollusks

Chapter 9. Proteins involved in the nitrogen cycle

Figure 50 presents the nitrogen cycle in nature; most of these processes are characteristic to bacteria. All enzymes that catalyze these reactions are metalloproteins. The purposes for which these reactions are performed can be diverse. For example, some organisms produce ammonia in order to use it in organic compounds; others use nitrogen oxyanions for respiration; for others, nitrogen oxides and oxyanions (especially NO and nitrite, but also species such as NO^+, NO^-, N_2O_3, derived by secondary reactions from the species shown in Figure 50) constitute a form of stress (called, by analogy with oxidative stress, "nitrosative stress").

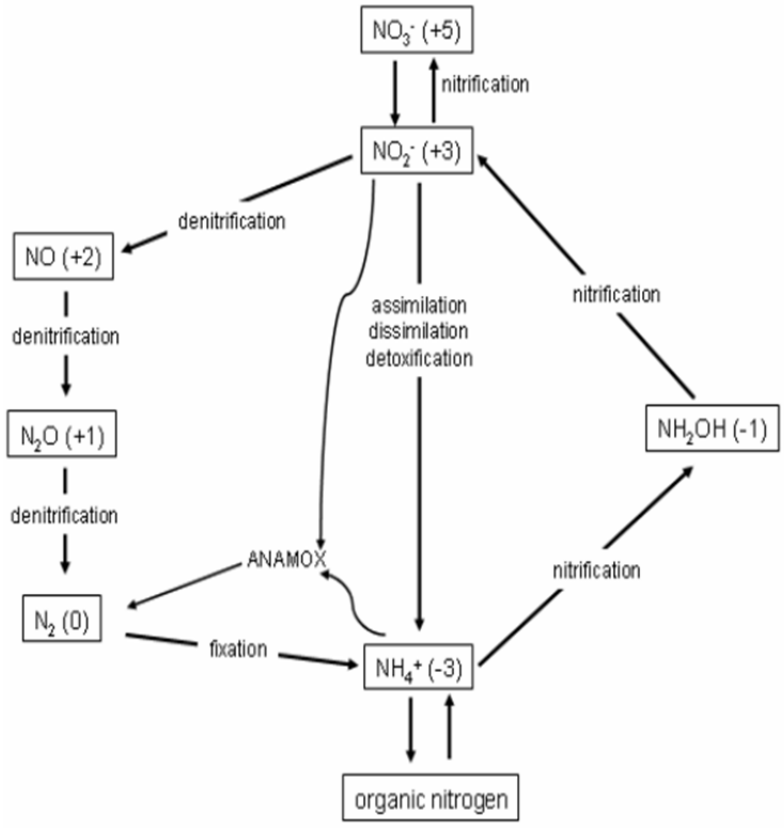


Figure 50. Nitrogen cycle in living organisms. In parentheses are shown the formal oxidation states of nitrogen in each compound.

Nitrate reductases are molybdenum based enzymes present in plants and bacteria, and were discussed in Chapter 7; their reaction product is nitrite. The latter is further reduced by nitrite reductases.

Nitrite reductases are divided into two classes according to the reaction product - ammonia or nitric monoxide. Some of them reduce nitrite with metabolic purpose others with protective purpose.

Copper-based nitrite reductases operate by the mechanism illustrated in Figure 51, producing nitric oxide. The same product is provided by cytochrome cd_1 nitrite reductases, which have at the active site a d_1 type heme and operate by the mechanism illustrated in Figure 52. On the other side, cytochrome *c* nitrite reductase, although with a catalytic center very similar to the cytochrome cd_1 nitrite reductase, center, produces ammonia not nitric oxide.[36]

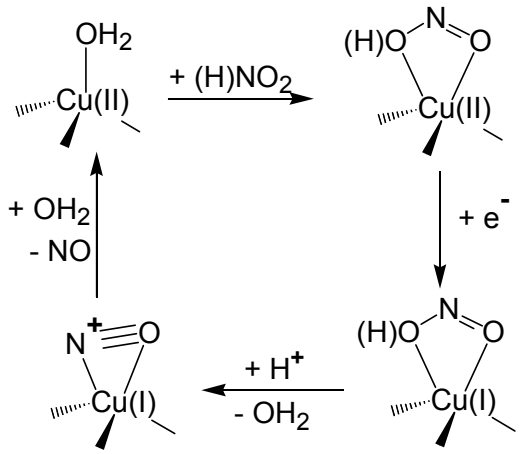


Figure 51. Catalytic cycle of copper-based nitrite reductases.
The metal is coordinated by three protein histidines.[37]

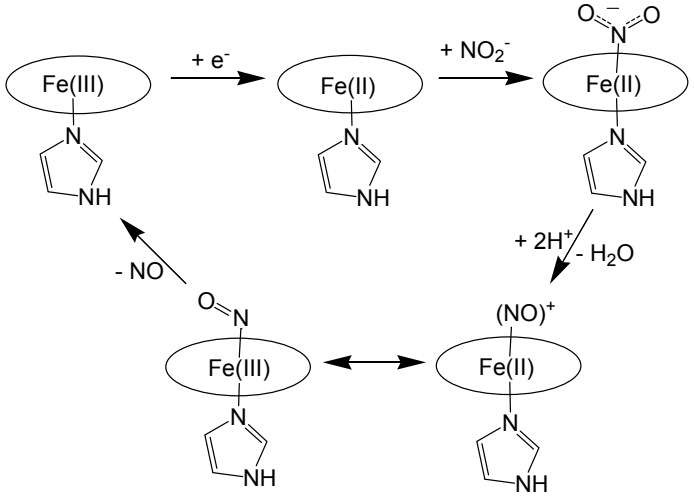


Figure 52. Catalytic cycle of cytochrome cd_1 nitrite reductase.
The heme is symbolically represented as an oval.[38]

NO reductases reduce nitric oxide to N_2O. Their catalytic sites are classified into two main categories. Cytochrome P450 NO-reductase (P450NOR, Figure 53) has a catalytic site similar to cytochromes P450 - a ferric heme coordinated axially by

cysteinate Unlike the P450 type monooxygenases in P450NOR the active site is very accessible from the exterior of the protein, so that after binding NO to Fe(III) binding of a NAD(P)H molecule occurs; subsequently, a hydride ion is transferred from NAD(P)H to the iron-coordinated nitric oxide, yielding an Fe-N(H)-O adduct. The latter reacts with a second NO molecule, probably yielding an intermediate species in the form of hyponitrous acid, HO-N-N-OH, which is unstable and decomposes in N_2O and H_2O.

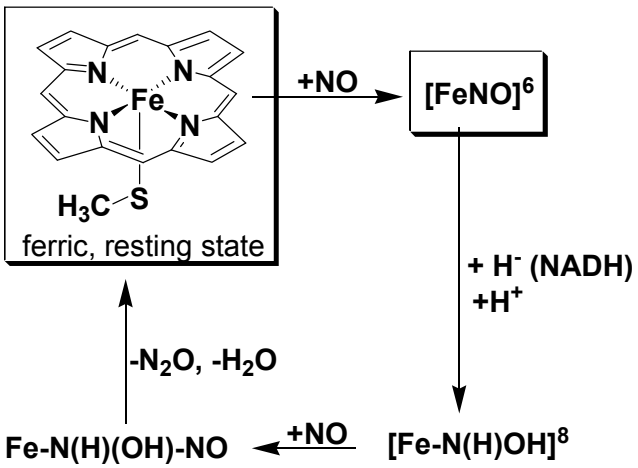


Figure 53. Catalytic cycle of P450NOR. Species enclosed in boxes are stable and the rest - feedstock with very short life.[36]

A second class of NO-reductases contains a binuclear active site consisting of one heme and one non-heme iron. The action mechanism proposed for these enzymes is illustrated in Figure 54. More recently discovered is a class of enzymes that functions both as NO reductases and oxidases (reducing molecular oxygen to water), and whose active site contains a non-heme bimetallic iron center similar to that of methane monooxygenases.[39-41]

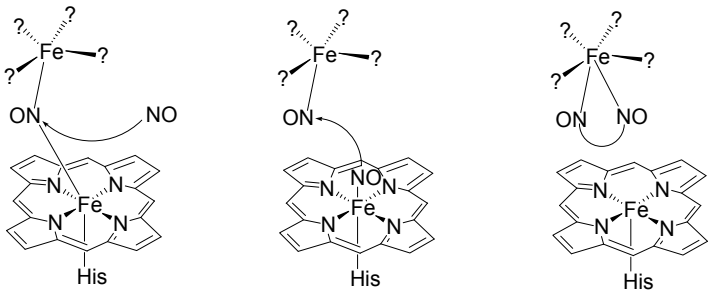


Figure 54. Catalytic site of NO-reductases with binuclear mixed site, heme + non-heme, illustrating also the possible reaction intermediates of catalytic cycle.

N_2O is a particularly unreactive molecule, which usually does not function as ligand to metals. Therefore it is not surprising that N_2O reductase, which reduces the gas to N_2, presents a particular active site, copper multinuclear, called Cu_Z in Figure 34. Even so, the most recent structural data suggests that N_2O does not coordinate to this cluster.

The enzyme that reduce N_2 to ammonia, nitrogenases, are among the best known metalloproteins, both because of the remarkable reaction they catalyze and due to their importance in ecosystems, fixing inorganic nitrogen from the air and thereby allowing its transformation in organic matter. The active site of nitrogenases is extremely complex in comparison with all other metalloenzymes, as illustrated in Figure 55. The action mechanism is not clear yet.

The enzyme that acts on hydroxylamine (hydroxylamine oxidoreductase) is a hemoprotein, somewhat similar to some of the nitrite reductases.

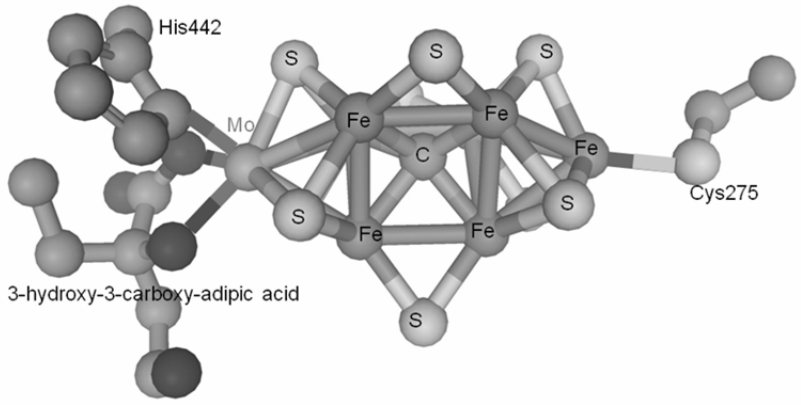


Figure 55. Active site of molybdenum nitrogenases. It is important to note that also exist nitrogenases versions using vanadium instead of molybdenum and apparently, some using only iron.

Chapter 10. Hydrogenases, methanogenesis

Hydrogenases catalyze the reversible reduction of protons to molecular hydrogen. They contain three types of active centers, of which two are illustrated in Figure 56: with Fe and Ni, and respectively with Fe only. A third class contains one iron, with a ligation sphere similar to the iron in the other two classes. All hydrogenases whose structures are known to date feature some the atypical ligands, CO and CN^- type (as shown in Figure 56). This most likely allows for enriching the metal ions in electrons, in the context in which they must be capable to coordinate extremely atypical ligands: H^+ H_2. Mechanisms proposed so far for these enzymes imply in fact unusual valence states for these metals - Ni(I), Fe(I).

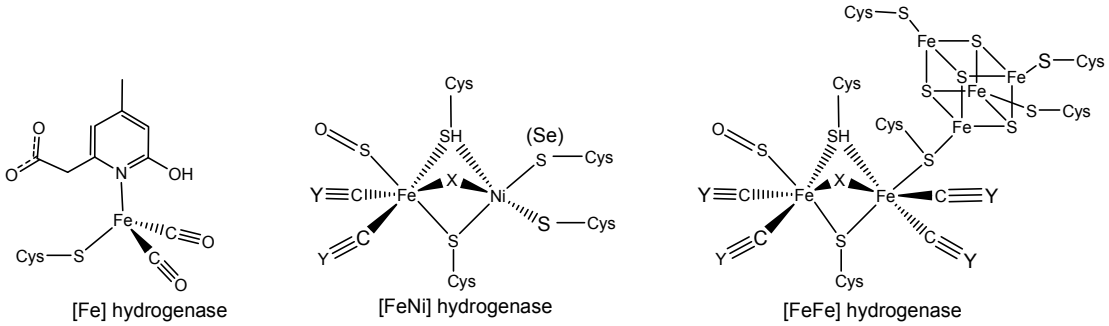


Figure 56. Hydrogenases active sites. Y can be nitrogen, oxygen or sulfur; X is most likely sulfur.[35]

In terms of cellular location, hydrogenases can be found in the cytoplasm or attached to the membrane. Membrane-bound hydrogenases have the capacity to be involved in generation of proton gradients, therefore with a role in energy transformation (similar to the phenomena discussed in Chapter 6.

Some organisms have the ability to produce methane (methanogens). The final stage in the chain of reactions leading to methane raises an interesting bioinorganic chemistry issue. It involves a metal center called coenzyme F_{430}, whose structure is illustrated in Figure 57.

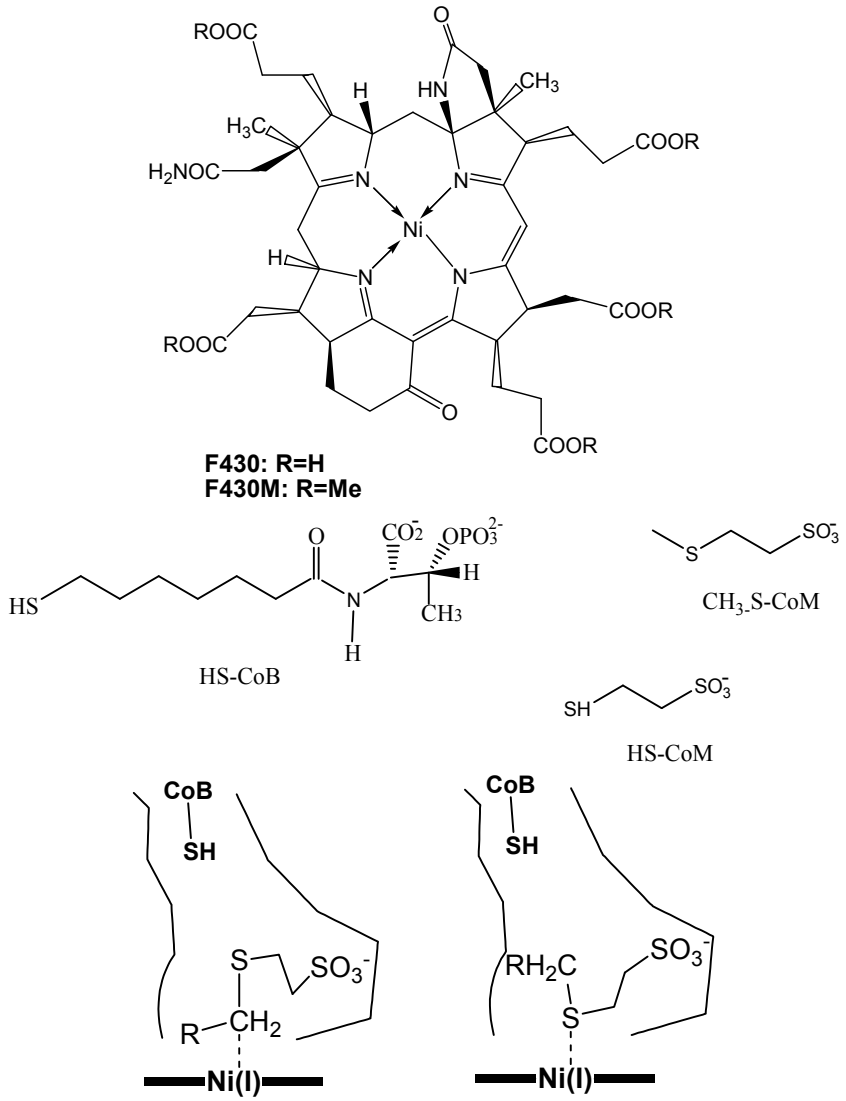


Figure 57. Coenzyme F_{430}, accessories coenzymes, and the two possible reaction intermediates for the reaction where it catalyzes the last step in the bacterial synthesis of methane.

A methyl group is brought to the nickel ion of F_{430} by a specialized transporter - coenzyme M. Two reaction mechanisms are proposed below, both involving the Ni(I) form of the metal. One is based on Ni-C bond formation between the methyl group and F_{430}, followed by sulfur-methyl bond breaking and subsequent protonation of methyl group; the other mechanism proposes the formation of a Ni-S bond between F_{430} and methyl-coenzyme M, which leads to weakening the sulfur-methyl bond and facilitates the transfer of a methyl proton. In both mechanisms an important role is played by coenzyme B (Figure 57), which provides the proton needed by the methyl group in order to turn into methane, and at the same time couples with coenzyme M, forming an –S-S- bond with it after (or concurrent with) the release of methane.

Chapter 11. Metal transport and storage

Most metals are, in certain concentrations, toxic to living organisms, although many of them are useful or even essential at lower concentrations. In this context, there are proteins and peptides specifically developed to control metal trafficking. Thus, siderophores (Figure 58) are peptides secreted by some bacteria and sent outside the cell in order to recruit iron, once iron is bound, the siderophore is detected by membrane proteins that recognize selectively the metal-coordinated form and carry it inside the cell.

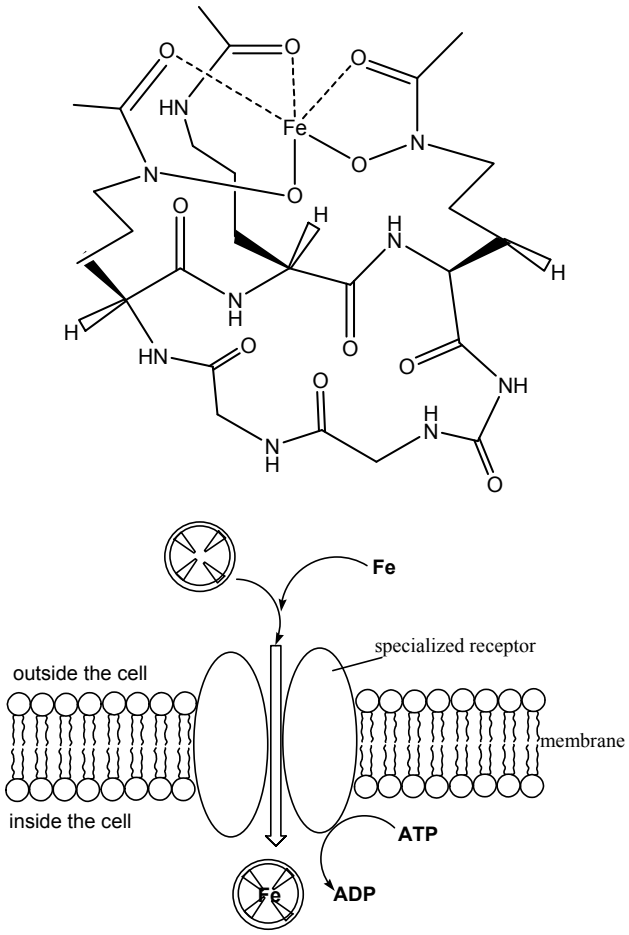


Figure 58. Structure of a siderophore example, and their operating principle.

Transport and storage of metals are not the exclusive attribute of small peptides: in most cases these functions are performed by proteins. Probably the best known in this sense is ferritin, whose role is iron storage. As previous chapters can

illustrate, iron presents very diverse and potentially dangerous reactivity, so that it cannot be allowed to circulate inside the cell in uncontrolled manner. Ferritin is a multimeric protein, formed by 24 monomers which have their tertiary and primary structure very similar to hemerythrin (see Chapter 3). As shown in Figure 59, these 24 protein units form a spherical structure; in the center cavity of this sphere the iron is stored as a mineral - iron oxide. Iron is brought to ferritin in the Fe(II) form – more soluble than Fe(III) - and oxidized by ferritin with a di-ferrous catalytic site similar to that of hemerythrins.

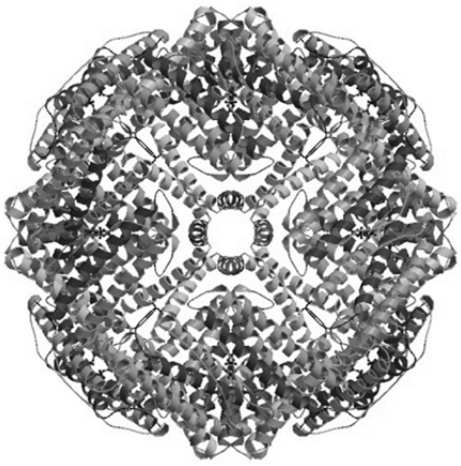


Figure 59. Ferritin structure.

Although the ferritin diiron-binding site is similar to the binuclear one in hemerythrin, they differ to such an extent that ferritin does not form *stable* adducts with iron at this site. Instead, the iron passing through the binding site is immediately oxidized to Fe(III) by molecular oxygen, after which it migrates to the spherical cavity enclosed by the 24 monomers, where it associates as iron oxide and carbonate, in a structure similar to rust. From here, it may be released if necessary after a reductive process. A protein related to ferritin, mini-ferritin has a lower molecular weight than ferritin, and uses as oxidant for Fe(II) no O_2, but H_2O_2. Our bodies also contain transferrin, a monomeric protein capable of transporting two iron ions.

Other metals also use carrier or storage proteins. Thus, the cysteine-rich protein metallothionein is specialized in binding soft metals (mercury, copper). There are also proteins specialized in inserting metals into a newly synthesized proteins (chaperones); the structure of such a chaperone is illustrated in Figure 60.

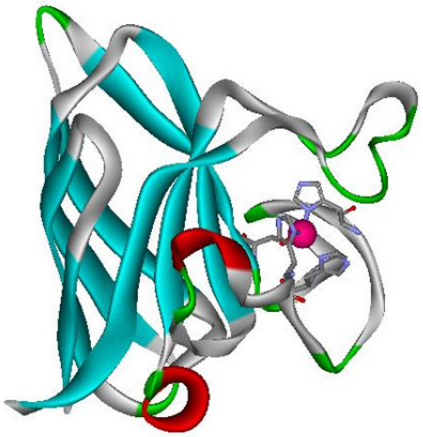


Figure 60. A protein used to insert a metal in the active site that it was designed for (chaperone). The metal coordination sphere is not completely saturated by the protein, so that in the presence of the target protein it can be transferred through mechanisms that usually involve binding the copper simultaneously to the target protein and to the donor protein.

Chapter 12. Hydrolases, hydratases [4]

When using metals, the hydration or hydrolysis reactions, not being redox, tend to require at the active site of the enzyme metals which are not active redox. Zinc is the ideal candidate for this position, although, depending on the ligand system, even a few redox-active metals can be used in non-redox reactions.

12.1. Enzymes/proteins with zinc

Figure 61 presents the main types of zinc-based active sites known so far, mentioning the names of some representative enzymes.

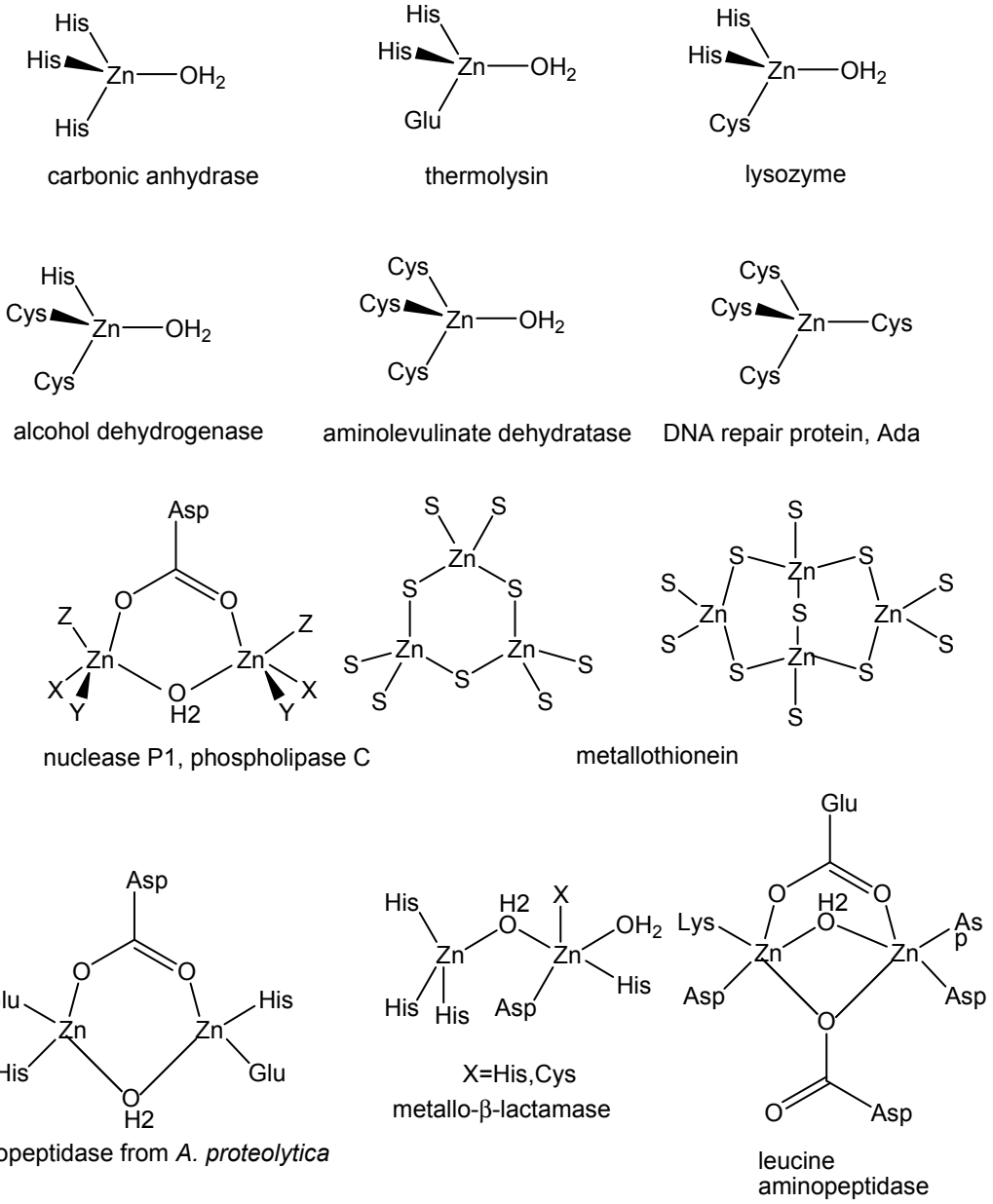


Figure 61. Zinc binding sites in proteins.

As shown in Figure 62, there are three main strategies that can be adopted by a hydrolytic enzyme: simple binding of water to activate it in order to become a better nucleophile, binding and (with help from the protein matrix) deprotonation of water to exacerbate its nucleophilic character by transforming it into hydroxide, or, on the contrary, binding the substrate to the metal, so that it can be effectively attacked by water. Figures 63-65 illustrate the action modes of several enzymes that use these strategies.

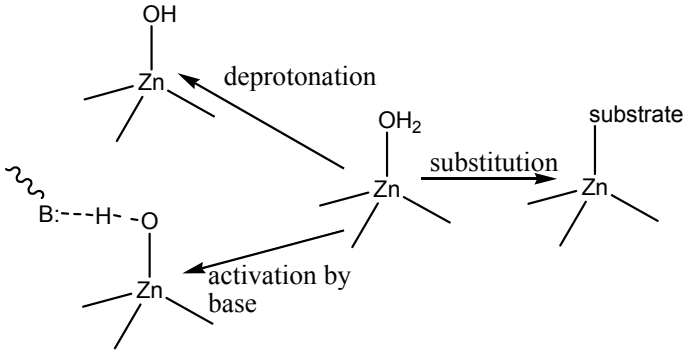


Figure 62. Strategies that can be adopted by a zinc-based hydrolytic enzyme.

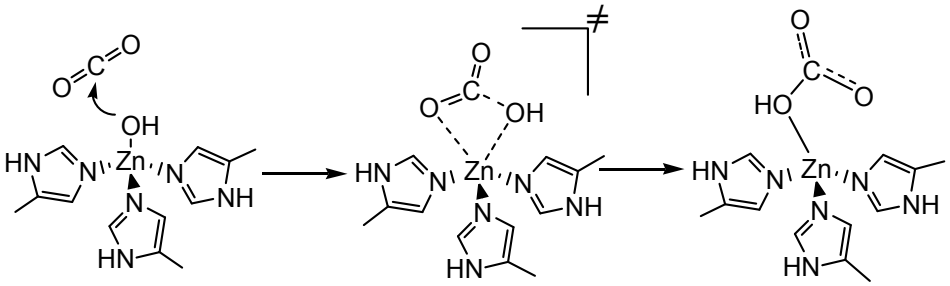


Figure 63. The mechanism of carbonic anhydrase.[42]

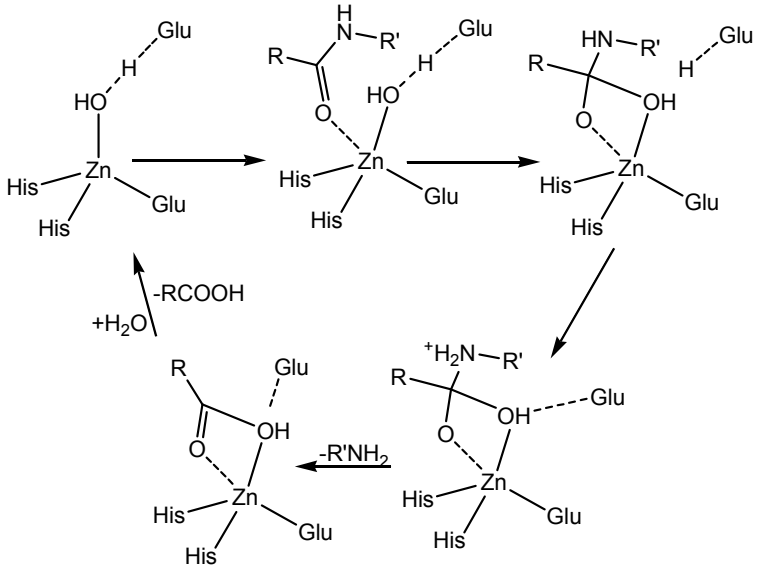


Figure 64. Thermolysine mechanism.

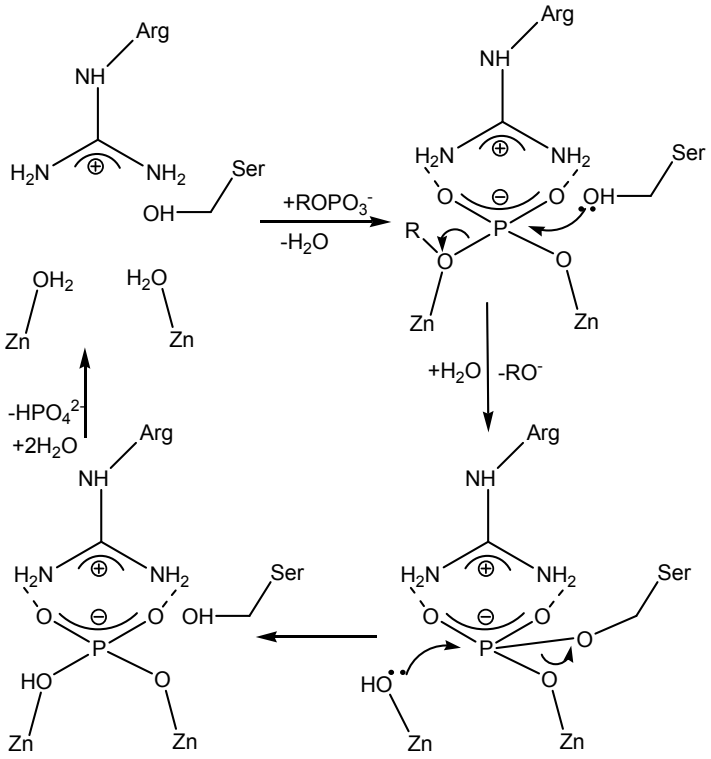


Figure 65. The action mechanism of a zinc phosphatase.

Although non-hydrolytic in his nature, the principle used in Figure 66 is somewhat similar to those used in hydrolytic reactions and therefore is discussed here: sulfur coordinated to zinc is activated for an electrophilic reaction, thus functioning as a carrier of methyl groups - in DNA repair protein Ada or to methionine synthetase.

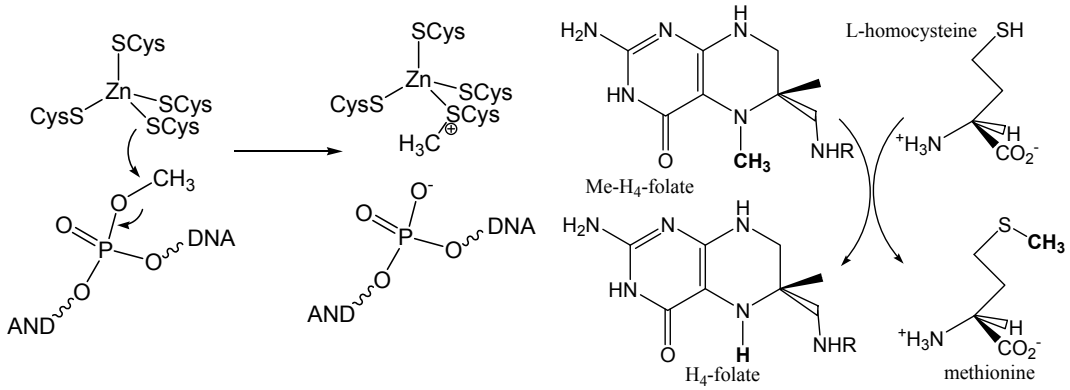


Figure 66. The mechanism of DNA repair protein, Ada, and the reaction catalyzed towards a similar principle by methionine synthetase.

12.2. Hydrolases and hydratases with other metals than zinc

Figure 67 illustrates the active site of the cobalt-containing nitrile hydratase; although cobalt is basically a redox-active metal, the ligand system provided by nitrile hydratase, carrying five negative charges on five ligands, moves the redox potential of

the metal out of the cell's accessible domain, keeping the metal in the Co(III) form, and practically rendering it redox-inactive. Notable in this respect is the presence of two amide ligands coming from deprotonated peptide bonds (-CO-NH-deprotonated at nitrogen) - a unique structural feature of this class of enzymes; also unique is the coordination mode of the two of the cysteines, which have been oxidized to sulfenic acid and sulfinic acid, respectively, as shown in Figure 67. A virtually identical site (except for the metal) is seen in iron nitrile hydratases. However, these latter iron enzymes are produced by the cell in a non-reactive form, with the iron blocked as Fe(III)-NO adduct; the catalytic site is activated by light, which causes the release of NO from the metal. Figure 67 shows the proposed mechanisms for nitrile hydratase.

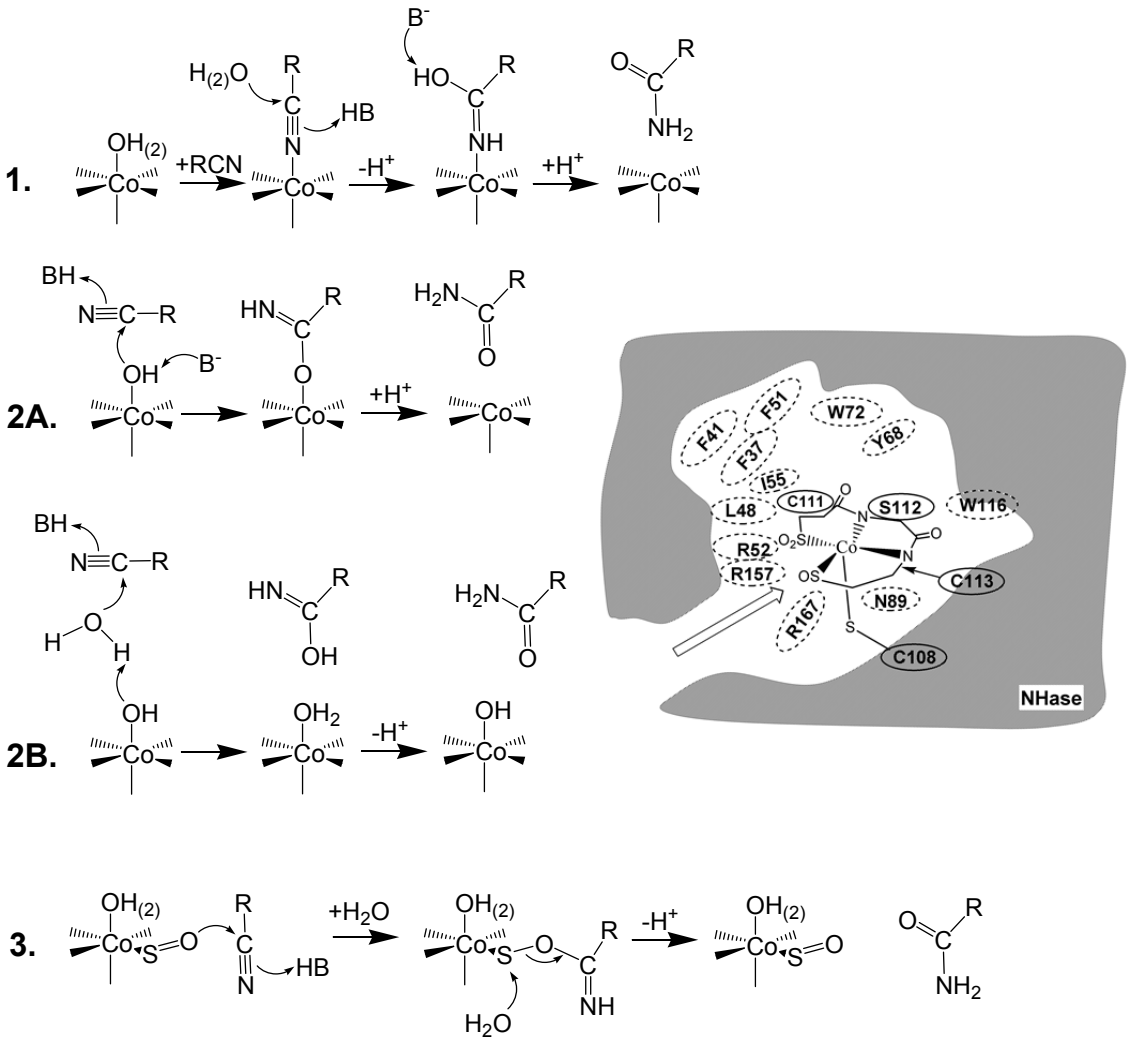


Figure 67. Active site structure and mechanisms proposed for cobalt nitrile hydratase.[43]

Another example of cobalt-based hydrolase is the aminopeptidase, cf., Figure 68.

One of the best known metalloproteins is urease. It catalyzes urea hydrolysis to ammonia and CO_2, and is found in organisms such as the bacterium *Helicobacter pylori*, which colonizes the extremely acid environment in the stomach and is a causative factor of disease. The role of urease is to generate ammonia, increasing the local pH and allowing bacteria to survive more efficiently. The active site of the urease contains a binuclear nickel center, cf. Figure 68.

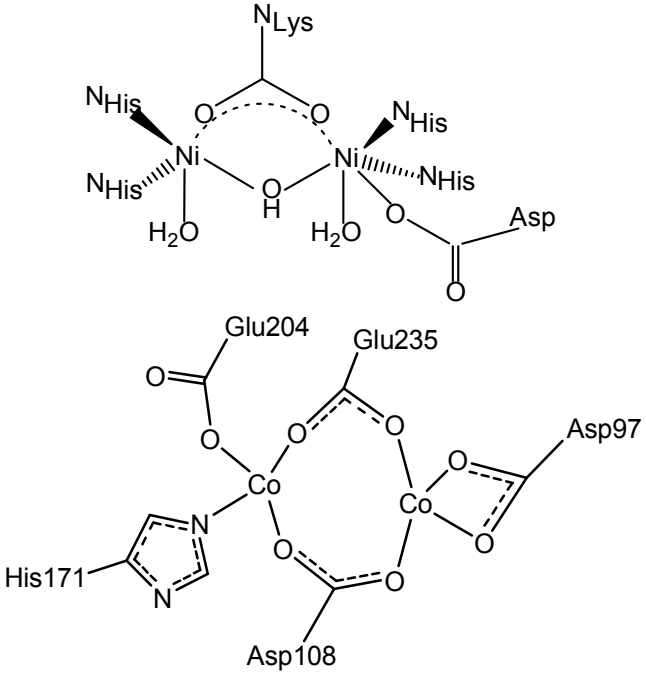


Figure 68. Active sites of cobalt aminopeptidase and urease.

Chapter 13. Bioorganometallic chemistry. Vitamin B_{12}

Vitamin B_{12} or cobalamin (Figure 69) is the only vitamin that contains a metal. Its unique property is to access a very low formal oxidation state at the metal, Co(I), which is able to form cobalt-carbon bonds. Metal-carbon bonds are in principle the attribute of so-called organometallic chemistry, and are usually destroyed by water and oxygen; cobalamin is a notable exception in this respect, being the basis of defining a separate field in science - bioorganometallic chemistry.

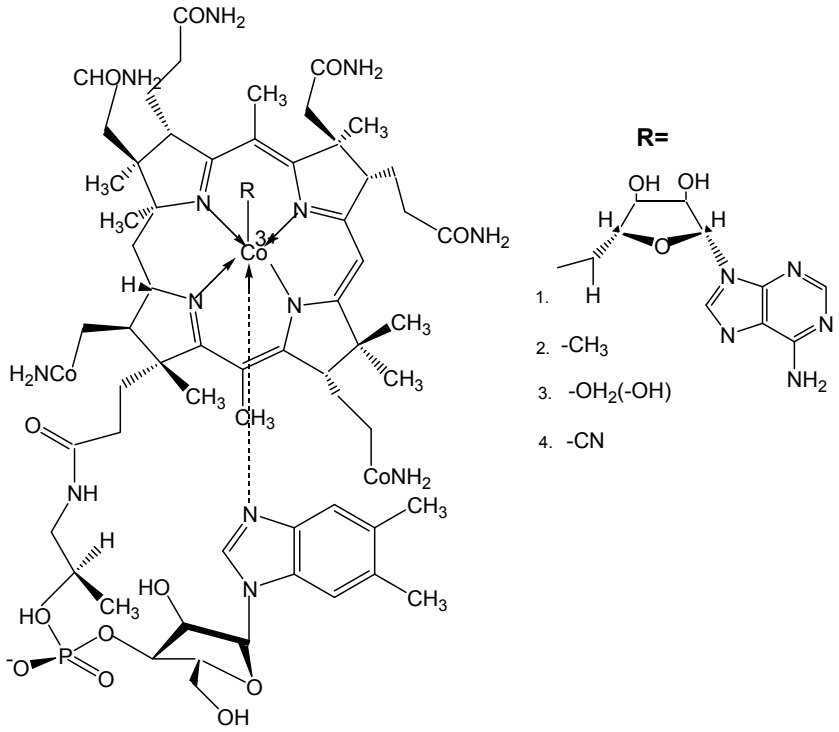


Figure 69. Cobalamin structure. Among the potential ligands ("R"), cyanide is not biologically relevant, appearing only as impurity resulted on purification.

The reactions catalyzed by vitamin B_{12} revolve around two manners for cleaving the cobalt-carbon bond: heterolysis (employed in B12-dependent methyl transferases) or homolysis (employed in B_{12}-dependent isomerases). The two reaction mechanisms are illustrated in Figures 70 and 71.

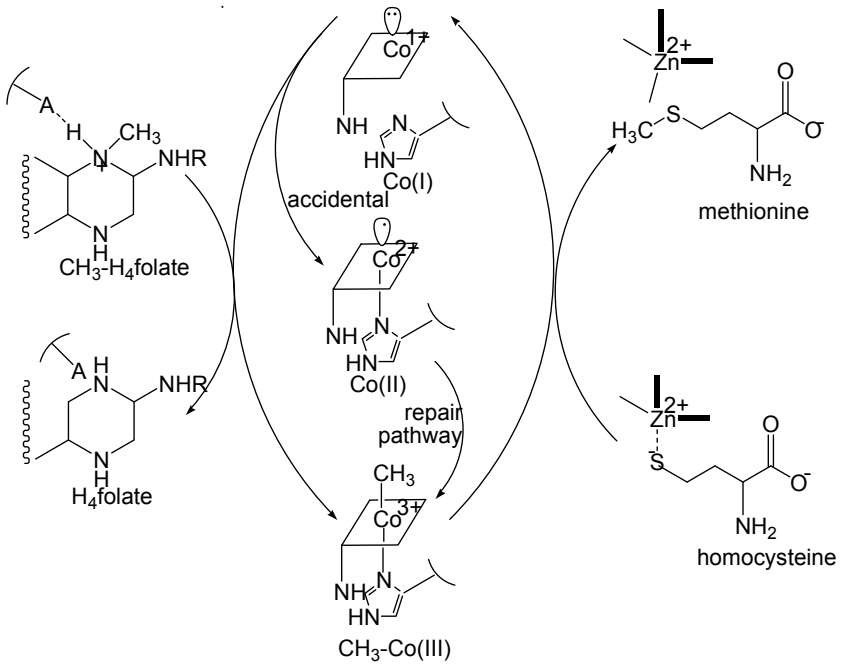


Figure 71. The mechanism of methionine synthase, an example of B_{12}-dependent enzyme involving carbon-cobalt bond heterolysis. It involves only Co(I) and Co(III) forms; Co(II) form can appear accidentally, and Figure illustrates that exist enzymatic mechanisms to return the metal in a oxidation state of the catalytic cycle.

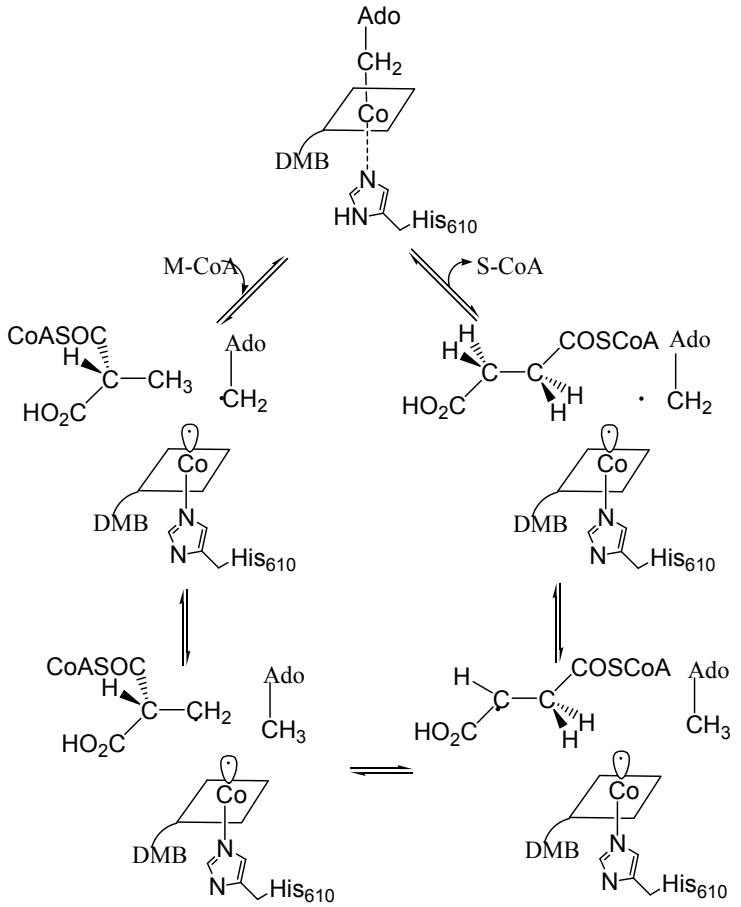


Figure 72. Methylmalonyl coenzyme A mutase mechanism, an example where the heterolytic cleavage of carbon-cobalt bond essential.

Although "bioorganometallic" chemistry is largely restricted to cobalamin, there are other types of metal-carbon compounds. Figure 72 illustrates an example - a Fe-phenyl adduct of cytochrome P450. Nitrogenase, discussed in previous chapters, has also recently emerged as an organometallic center.

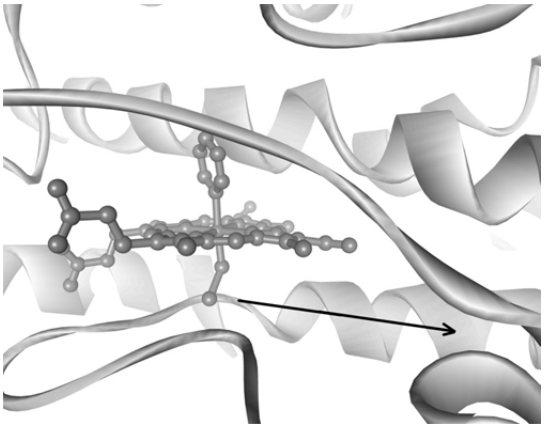


Figure 72. A bioorganometallic adduct not involving vitamin B_{12}: cytochrome P450 complexed with phenyl.[44]

Chapter 14. Proteins involved in photosynthesis

Photosynthesis serves to synthesize organic matter using CO_2 as a raw material; the process being endergonic, energy from light is needed in order to sustain it. As illustrated in Figure 73, the reactions in photosynthesis are divided into two segments separated in space and time. The first part involves the capture of photons and the transformation of their energy into a form accessible to other compartments of the cell - NADPH, ATP. These are reactions that require light directly. A second series of processes may also be conducted in the dark and involve the reaction of CO_2 with a carbohydrate, and its transformation in organic matter.

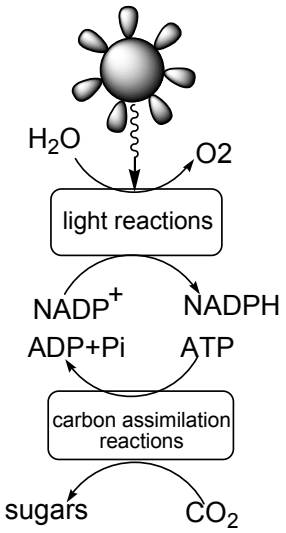
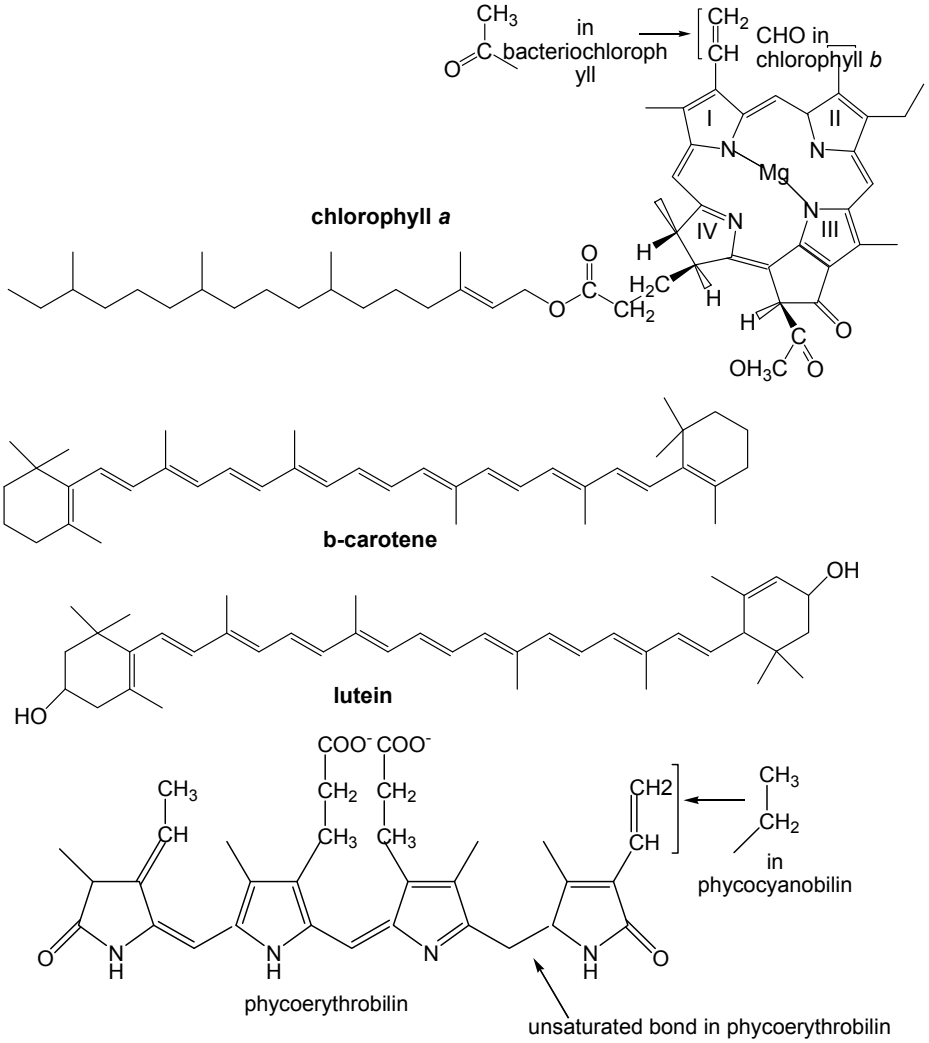


Figure 73. The series of processes involved in photosynthesis.

The capture of light energy (photons) takes place in chloroplasts - cellular organelles similar to mitochondria by the presence of two membranes (external and internal) and bu the presence of numerous membrane proteins (see Chapter 5 for a discussion of the importance of this type of structure). In fact, the proteins responsible for capturing photons are also membrane proteins. They contain non-protein groups capable of photon absorption (see Figure 74), characterized by the presence of conjugated double bonds which allow a low-energy for the LUMO orbital (lowest unoccupied molecular orbital) therefore needing lower-energy photons to promote an electron from an occupied orbital to LUMO. Due to the conjugated double bonds all the systems in Figure 74 are colored; the most commonly known of them are

undoubtedly the chlorophylls, which give the green color to most plant leaves. However, "antenna" molecules such as those in Figure 74 have evolved to cover the entire visible light spectrum (~400-800 nm, as shown in Table 3), thus allowing, through combined utilization of more of these pigments, the usage of photons of any energy in this domain.



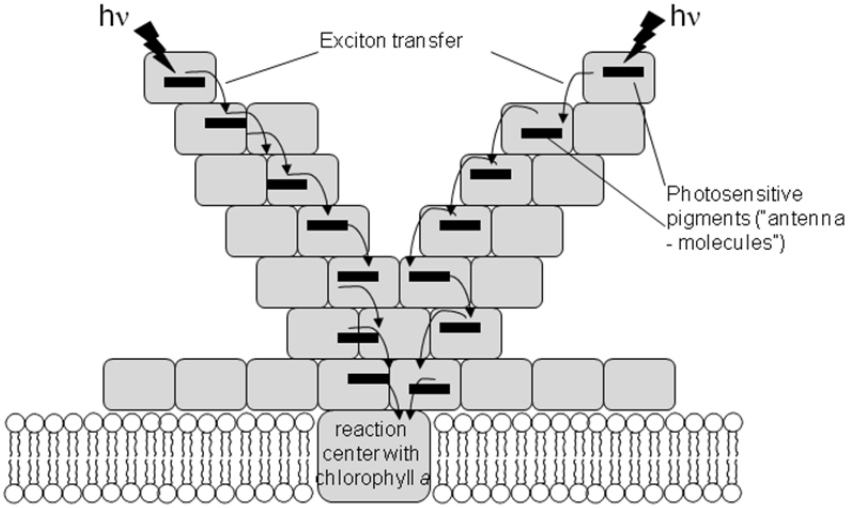


Figure74. Specialized molecules in light capture in chloroplasts; a schematic illustration of the route followed by photon and exciton through the reaction center.

Table 3. Regions with absorption maxima for various pigments used in photosynthesis

Compound	Optimum absorption domain (nm)
chlorophyll a	400-450, 650-700
chlorophyll b	430-500, 630-680
lutein	400-500
phycocyanin	500-700
phycoerythrin	450-600
β-carotene	400-500

Photon are absorbed by one of the antenna molecules of the membrane protein complex suggestively called the "reaction center". This phenomenon will lead to the promotion of an electron of the pigment, from an occupied orbital to an unoccupied one, creating an excited state in terms of electronic structure. This state will quickly return to its original state (fundamental), releasing energy; this energy will be taken over by a pigment similar to the first one and found in its immediate neighborhood, which will suffer the same phenomenon of transition to an excited state. Further, this second pigment will return to its ground state, passing on the energy to a third pigment, the chain continuing in the same manner. The phenomenon is described as "exciton transfer" - because, apparently, what travels from one pigment to another is the excited state (“the exciton”). The exciton is passed on the route discussed and

illustrated also in Figure 74, until it reaches a so-called "special pair" - a site that contains two chlorophylls located very close to each other. The excited state of this special pair has the particular property of having a redox potential more negative than the ground state so that it easily loses an electron to a redox partner outside the protein. The next steps can be glimpsed by comparison to the phenomena described in Chapter 5 (Respiration): the electron's energy is converted into a form usable in other parts of the cell - preferable ATP. Figure 75 presents the partners involved in the photosynthetic processes in two types of bacteria. In one type (left in Figure 75)), the electron is transmitted through a chain of proteins and transporters (similar as concept to the electron transport chain in mitochondria), which, importantly, contains a cytochrome capable of generating a proton gradient on account of the energy brought by the electron originating from the "special pair" (thus transferring, as Complexes I - IV did, protons from one side to another of the internal membrane). The excess of protons generated in this way is then used by an ATP synthase placed on the same membrane. The electron derived from the "special pair", once used in producing the proton gradient, is returned to the starting point, allowing the resumption of the reaction cycle with a new photon. On the other hand, other types of bacteria use a second strategy type, as illustrated on the right side of Figure 75. Here the electron can be irreversibly removed from the reaction center, being stored on the carrier molecule, NADH. In compensation, the reaction center is forced to find a convenient and efficient source of electrons to regenerate the reaction center; in principle, there are no restrictions on the nature of the electron donor at this point, bacteria in Figure 75 using H_2S, but other organisms can use other sources, such as arsenite.

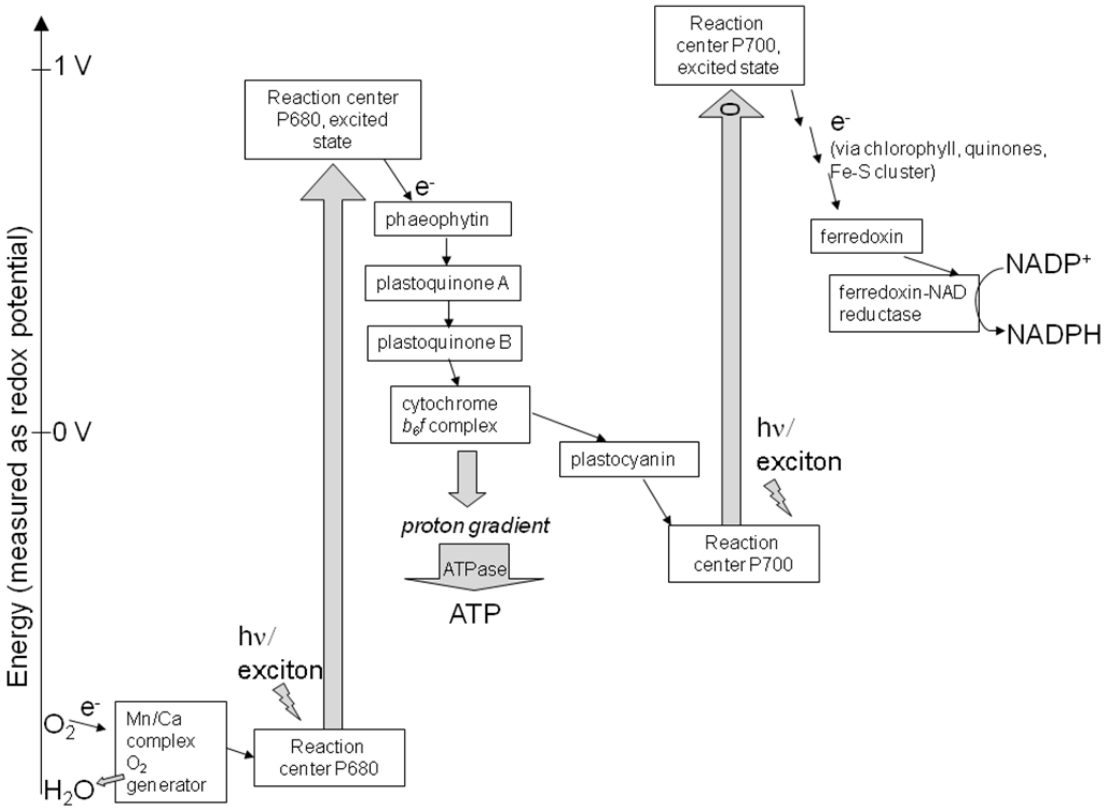


Figure 75. Types of photosynthetic systems in bacteria.

Plants use both of the strategies illustrated above for bacterial organisms - as shown in Figure 76. Thus, plants have two photosystems, that allow both generation of proton gradient and therefore of ATP, and NADPH generation. The electron needed to regenerate the reaction center is then provided by water molecules. In turn, water is oxidized during this process to molecular oxygen. The protein responsible for this latter process uses a manganese and calcium cluster, whose structure (illustrated in Figure 77) contains an incomplete cube with manganese and oxygen atoms placed alternatively on the corners, and with a calcium atom located slightly outside of one of the corners instead of a manganese atom; a fourth manganese atom is located outside the cube. The metals are connected to the protein matrix by oxygen rich amino acids (aspartate, etc.). The reaction itself (water oxidation to O_2) is, as in the case of the cytochrome *c* oxidase (see Chapter 5), difficult to accomplish, due to the risk of releasing highly toxic intermediates - hydroxyl radical, superoxide or hydrogen peroxide.

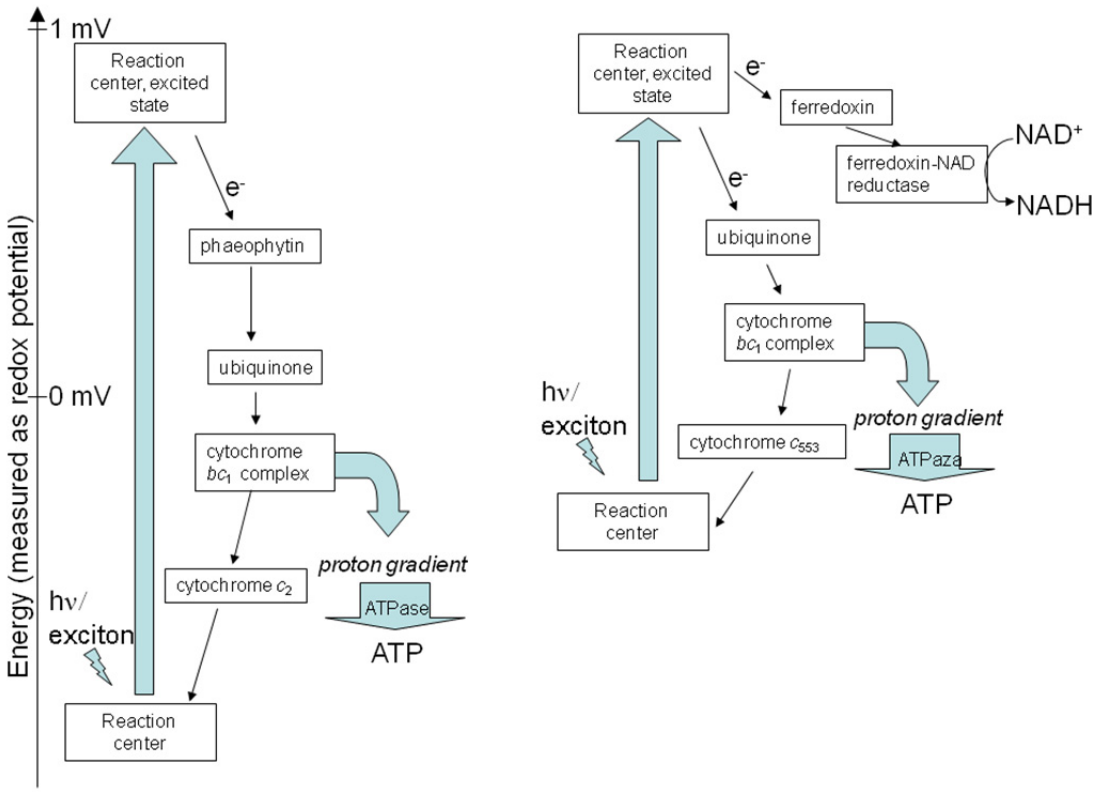


Figure 76. Photosynthetic system in plants.

The reactions in the second part of photosynthesis (those held "in the dark") require only one important metalloenzyme, which was already discussed in Chapter 8 - RuBisCO. Figure 78 presents the reaction chain that leads to the incorporation of CO_2 into carbohydrate metabolism, and which form the subject of classical biochemistry texts.[1]

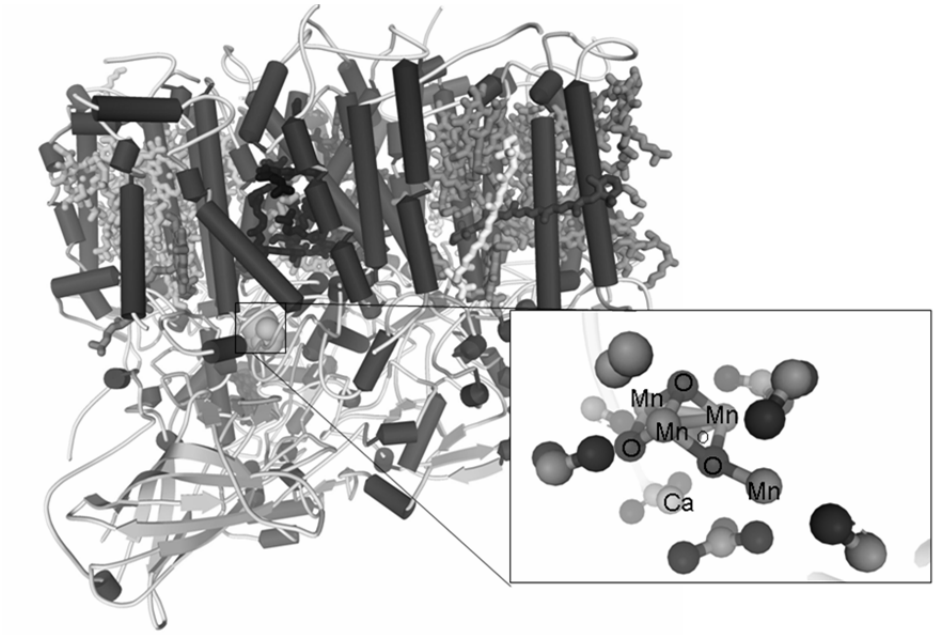


Figure 77. The reaction center where water oxidation to molecular oxygen occurs in photosynthesis. Represented in "stick" model are the numerous redox active prosthetic groups other than Mn-Ca cluster - chlorophylls, heme, etc. - present in the same protein.

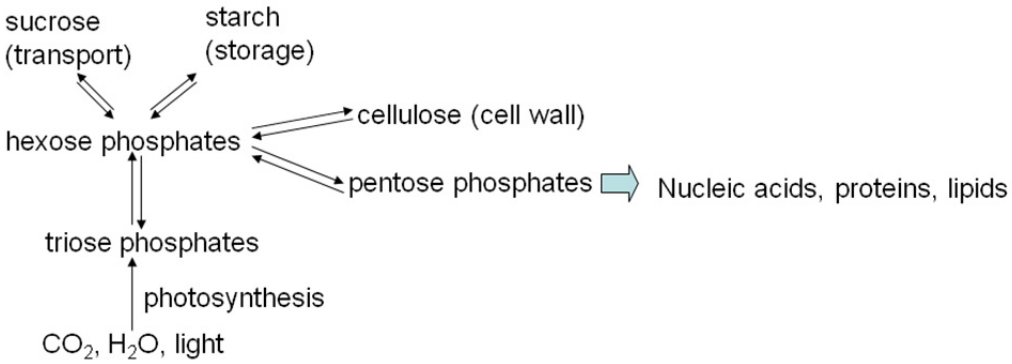


Figure 78. Chain of reactions whereby the carbon from CO_2 is assimilated in plants using the energy extracted from light in the previous steps discussed in this chapter.

Chapter 15. Elements of medicinal bioinorganic chemistry [4]

Among the oldest metals known to have medical use are gold and silver, used as early as thousands of years ago, at first in combination with magical/mystical uses. The coincidence makes that ~ 200 years ago, the first metal-based drug with a well-defined chemical structure was also a gold complex - $K[Au(CN)_2]$ with antibacterial properties. Today, gold complexes are used among other things for rheumatoid arthritis (such as those in Figure 79).

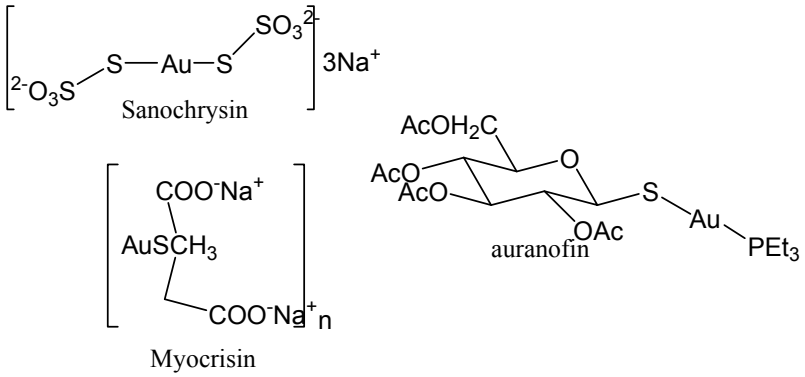


Figure 79. Gold-based drugs. As shown, in some cases the chemical composition is not known in all details.

Another metal with a long history in medicine is iron; the need for using added iron in the diet to combat anemia was recognized as early as ~ 1,200 years ago. On the other hand, diseases caused by metal excess (iron or others, such as copper or mercury) are now well known, and commercially available compounds that are used in medical treatment of these diseases exist (Figure 80); these compounds serve to bind the metals and thus block at least part of their reactivity – although some chelates also have protective antioxidant properties by themselves.

Another chelating agent for iron (which works similarly with copper) is the peptide called bleomycin (Blm). Blm has the ability to non-covalently attach to the DNA, thus bringing the metal in the vicinity of the nucleic acid. What follows is an outbreak of oxidative activity of these metals, in similar ways to those presented in Chapter 4, where the ingredients are Fe(II), an electron source (such as ascorbic acid) and molecular oxygen (all three are universally present in a living cell). The chemical

system formed from these three basic ingrediants is called "Fenton reaction", and involves, after the models in Chapter 4, generation of free radicals and high-valent states of the iron, all with destructive potential and therefore, able to degrade biomolecules such as DNA. Bleomycin is used, for this reason, as anti-cancer drug.

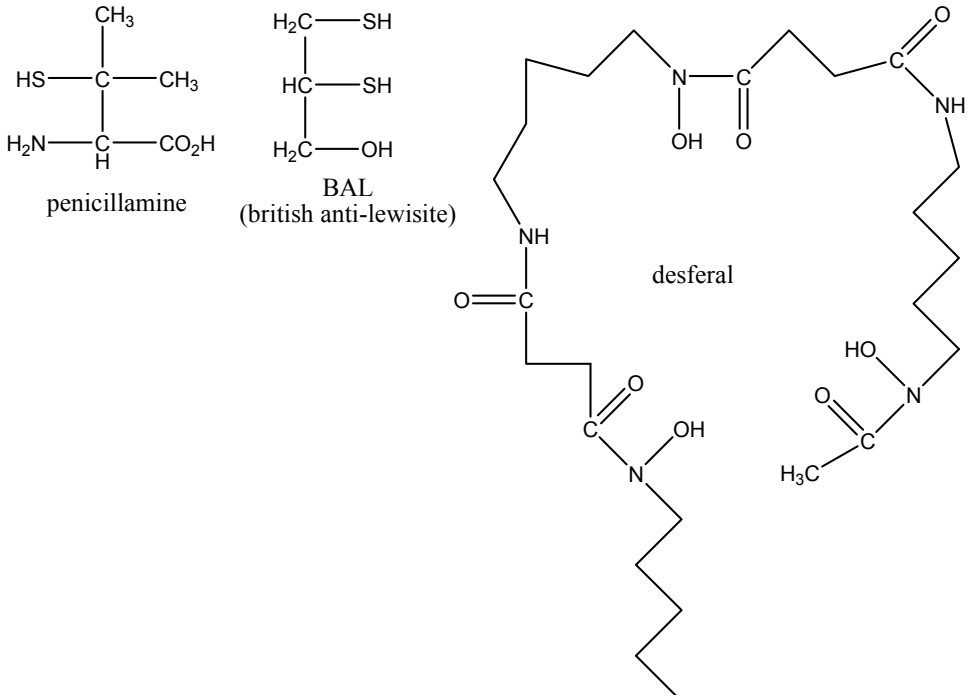


Figure 80. Chelating agents with medical use against excces iron (desferioxamin) or copper (penicilamin).

Although by itself is a non-metal, arsenic forms highly toxic inorganic compounds (such as arsenic trioxide); their use as poisons throughout history is known and probably was largely popularized by the Italian family, Borgia, in the Middle Ages. The primary target of arsenic are thiol groups, to which it has a special affinity. Independent of this, organisms have developed arsenic detoxification mechanisms, illustrated in Figure 81. On the other hand, there are medical uses of arsenic; salvarsan, discovered by Ehrlich about 100 years ago as a medicine against syphilis (Figure 82), entered into history as the first drug created from a systematic study that correlates chemical structure and biological activity (today a separate domain in science - SAR, Structure-Activity Relationships).

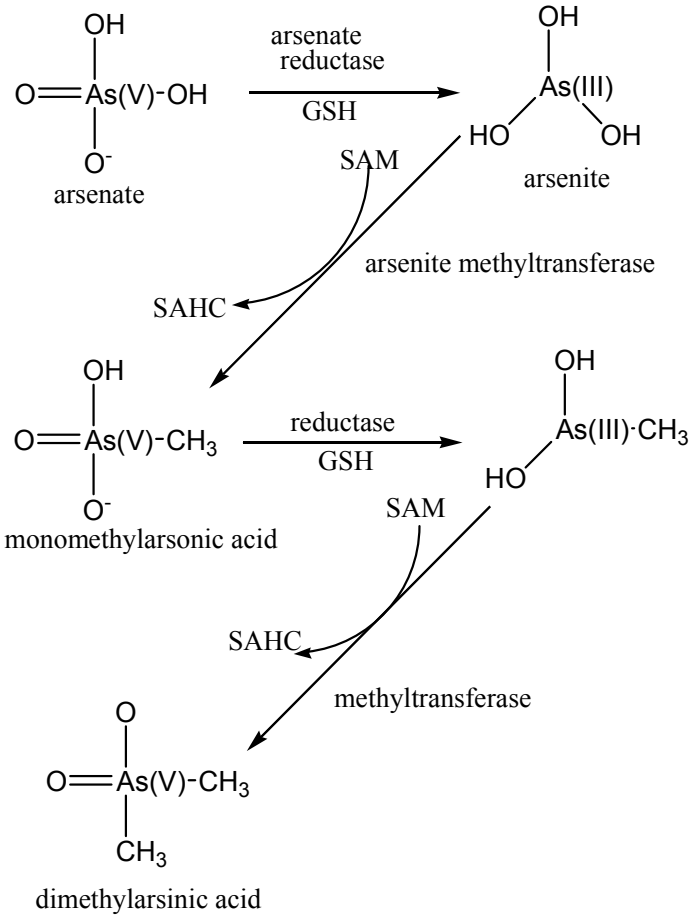


Figure 81. Enzymatic mechanisms of arsenic toxicity reduction. GSH = reduced form of glutathione (a cysteine-based peptide, acting as reducing agent via its SH groups), SAM = S-adenosyl methionine.

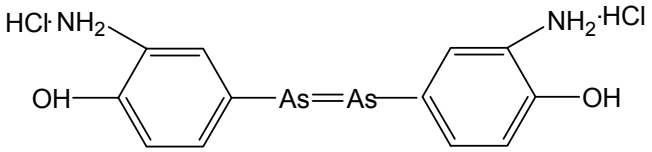


Figure 82. Arsfenamine originally proposed structure.

Another element with historical importance is bismuth; used even today to treat digestive problems, such bismuth-based preparations were used in the first clinical trial conducted by doctors to test a bioinorganic drug, about 300 years ago.

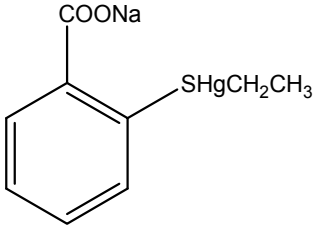


Figure 83. Thimerosal, a mercury derivative used in medicine.

Mercury is one of the best known elements for having toxic effects, which it manifests especially in the form of organometallic combinations. One of the most dangerous sources of mercury is ocean fish food, because mercury accumulates in fish. Under normal conditions this accumulation would be negligible, but it can become extremely dangerous when it comes from fish that exposed to industrial contaminated water. The disaster from Minamata, Japan, remains, due to the large number of human victims, a reference point for this kind of pollution. On the other hand, other mercury-based compounds have much lower toxicity. Starting from 1920-1930, thimerosal (Figure 83) has been used as preservative agent in vaccines. After 2000, the substance was in the center of a controversy about its possible involvement in increasing the risk for autism. As arsenic, mercury‘s main target are thiol groups (both partners being soft centers). On the same principle, another element that in excess becomes toxic, selenium, at the same time has high affinity for arsenic or mercury - so high that, by binding to them, they block each other and mutually reduce their toxicity.

One of the metals best known for their applications in medicine is platinum. Cisplatin (Figure 84) was found to have significant potential as anti-cancer drug (a potential discovered by chance, many decades after the actual discovery of cisplatin). Together with other similar derivatives, cisplatin has reduced mortality rate for some forms of cancer from 95% to only 5%. Action mechanisms of this class of drugs are apparently numerous, but probably the most important is the fact that inside the cells the cisplatin platinum loses its two chloride ions, and replaces them with the nitrogen atoms of nitrogen DNA bases. This binding phenomenon induces a change in the local tertiary structure of DNA (Figure 84), so that information from it cannot be read accurately by specialized enzymes, which affects not the functionality of the cell, including its ability to multiply.

One of the disadvantages of cisplatin is its lack of specificity, which leads to damage throughout the entire organism, not only on the tumor cells. Therefore, one of the current research directions is focused on trying to selectively target the drugs to specific tissues or organs. An example in this sense is the derivative of Figure 85, which, due to the nature of the ligand, is directed mainly to bones.

A special role is played by radioactive elements. Some of them are used for therapeutic purposes (such as that discussed above in Figure 85), in fact by taking advantage of their toxicity, to the extent that it can be controlled. Others can be used

for diagnostic purposes, such as derivative in Figure 86. There are of course also non-radioactive metals with diagnostic utility. Barium (as sulfate) is known for its usefulness in endoscopy, because of its ability to absorb x-rays. Gadolinium or europium are used as visualization agents in MRI examination, their strong paramagnetic character allowing better visualization of water-rich areas in the body (for example the cardiovascular system).

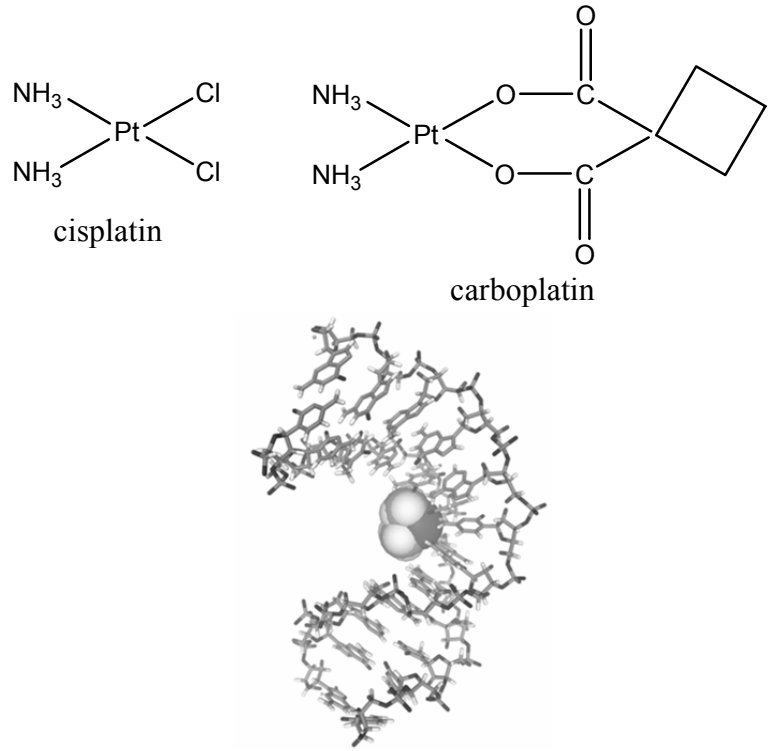


Figure 84. The structure of cisplatin and a related derivative, carboplatin, and the effect it has on their binding to DNA structure.

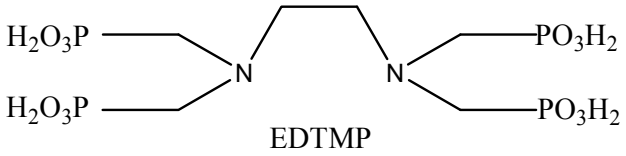


Figure 85. EDTMP, a ligand used for ^{153}Sm for a radioactive drug with specificity for bones.

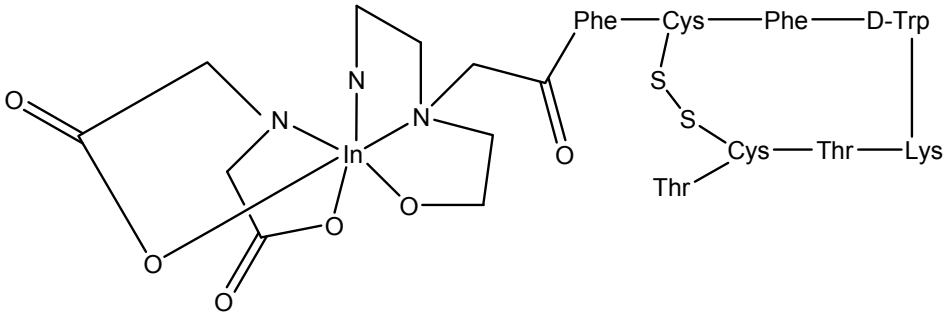


Figure 86. A derivative of ^{111}In, whose peptide group directs it selectively to the tumor tissues, which are thus marked radioactively.

The vanadium compound in Figure 87 has the property of functioning in vivo as an analogue to insulin, with the advantage of permitting oral administration; the reason why it is effective is still unknown, the structure being completely different from the purely organic, polypeptide, structure of insulin.

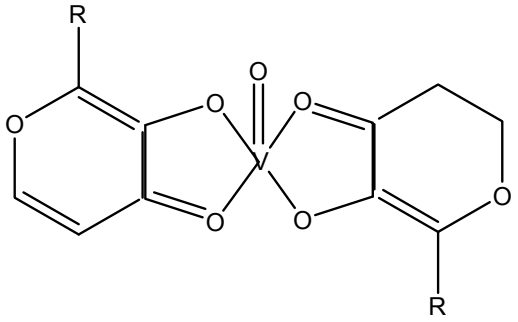


Figure 87. A functional analog of insulin.

Various other metals have potential applications in medicine, and can be found in commercially available food supplements, although it is important to note that there does not exist a firm scientific basis for their use in this form. Examples could be lithium (assumed to be important for the nervous system), aluminum, chromium or silicon.

Chapter 16. References

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