

Introduction to Bioinorganic Chemistry

University of Lund, May/June 2008

Lecture notes

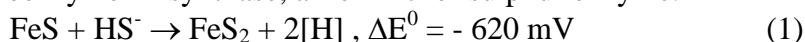
Dieter Rehder

1. Scope and Introduction

“Bioinorganic Chemistry“ is at the gate-way of inorganic chemistry and biochemistry, i.e. it describes the mutual relationship between these two sub-disciplines, with focus upon the function of inorganic “substances“ in living systems, including the transport, speciation and, eventually, mineralisation of inorganic materials, and including the use of inorganics in medicinal therapy and diagnosis. These “substances” can be metal ions (such as K^+ , ferrous and ferric), composite ions (e.g. molybdate), coordination compounds¹ (like cisplatin and carbonyltechnetium), or inorganic molecules such as CO, NO, O₃. Medicinal inorganic chemistry on the one hand, and biomineralisation on the other hand, are important integral parts.

Inorganic reactions have possibly played an important role in the formation and development of organic “life molecules” in the prebiotic area (terrestrial and/or extraterrestrial), and from the very beginning of life on Earth. Inorganic chemistry is involved in structure and function of all life forms present nowadays on Earth, belonging to one of the three main branches, viz. bacteria, archaea and eucarya (Fig. 1). Life started ca 3.5 billion years ago with LUCA, the first uniform (and unknown) common ancestor. At that time, our planet was already covered by oceans. The overall situation was, however, completely different from that of today: The primordial atmosphere (also referred to as “primordial broth”) contained CO₂, N₂ and H₂O as the main components, and trace amounts of gases like H₂, CO, COS, H₂S, NH₃ and CH₄ from volcanic exhalations, and trace amounts of oxygen from the decomposition of water by electric discharges, cosmic rays and radioactivity. The Earth’s crust was essentially unstable due to wide-spread volcanism and bombardment by debris (meteorites), remainders from the constitution of the solar system some 4.5 billion years ago.

A key reaction at that time was the conversion of ferrous sulfide to ferrous disulfide (pyrite, FeS₂) (eqn. 1), accompanied by a reduction potential of -620 mV, enough to enable reductive carbon fixation, including reductive C-C coupling, and thus to allow entrance into the world of organic compounds. Eqns. (2) (formation of thiomethanol as a key compound) and (3) (formation of thioacetic acid) are examples. Of particular interest is the formation of “active acetic acid methylester“ (eqn. 3b), which is an essential constituent of acetyl-coenzyme-A, a focal product in biological carbon cycling, the synthesis of which is catalysed by an acetylcoenzyme-M synthase, a iron-nickel-sulphur enzyme.



¹ For the definition and further aspects of coordination compounds see insets on pp. 5 and 7.

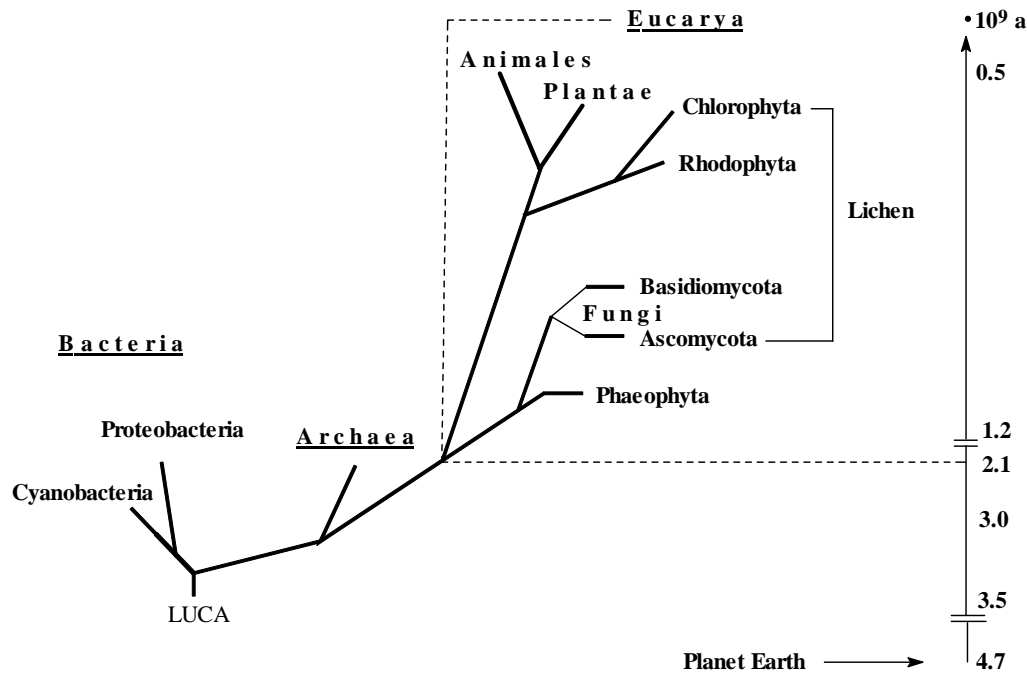


Figure 1. Phylogenetic tree. Time scale in billion years. LUCA = last uniform common ancestor.

“Active acetic acid” readily reacts with amino acids (formed in the primordial broth by electric discharge; and/or in interstellar clouds by irradiation and carried to Earth confined in the ice cores of comets) to form peptides, which chiral selection and further polymerise on chiral matrices provided by certain clays and quartz minerals. Concomitantly, nucleobases can form under primordial and interstellar conditions, and polymerise to RNA, unique molecules which not only store information and transform this information into proteins, but also can act – like proteins – as enzymes (so-called ribozymes). The first life forms, primitive cellular organisms capable of self-sustenance and self-replication, are actually believed to have been members of an “RNA world”, which later has been replaced by our DNA world.

Fig. 2 classifies the bio-elements: Along with the “organic elements” (C, H, O, N, S), building up bio-mass, many “inorganic elements” play an important role in the physiological context. Some of these inorganic elements, such as Fe, Cu and Zn, are present in (practically) all organisms, others are important for a restricted number of organisms only. An additional group of elements are used for diagnostic or therapeutic applications.

H																			
Li											B	C	N	O	F				
Na	Mg											Si	P	S	Cl				
K	Ca			V		Mn	Fe	Co	Ni	Cu	Zn			As	Se	Br			
						Mo	Tc							Sn	Sb		I		
	Ba	Gd								Pt	Au				Bi				

Figure 2. Periodic Table of the bio-elements: **elements building up bio-mass**, **additional essential elements**, **essential for some groups of organisms**, **medicinally important elements**.

Significance of biologically important elements (selection)

Na^+ and K^+ : Most important „free“ intra- and extracellular cations. Regulation of the osmotic pressure, membrane potentials, enzyme activity, signalling.

Mg^{2+} : Chlorophyll; anaerobic energy metabolism ($\text{ATP} \rightarrow \text{ATP}$).

Ca^{2+} : Signalling, muscle contraction, enzyme regulation. Main inorganic part of the endoskeletons (bones, teeth, enamel: hydroxyapatite; $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$). Exoskeletons of mussels, shells, corals, sea urchins etc: aragonite or calcite; CaCO_3 .

$\text{V}^{\text{IV/V}}$, $\text{Mo}^{\text{IV/VI}}$, $\text{W}^{\text{IV/VI}}$, $\text{Mn}^{\text{II/III/IV}}$, $\text{Fe}^{\text{II/III}}$, $\text{Ni}^{\text{I/II/III}}$, $\text{Cu}^{\text{I/II}}$: active centres in electron-transport (redox) enzymes, oxygenases, dismutases.

Fe and Cu: Transport of oxygen.

Fe^{III} : Iron-storage proteins (ferritins).

$\text{Fe}^{\text{II}} + \text{Fe}^{\text{III}}$ in magnetite (Fe_3O_4): orientation of magnetobacteria, pigeons, bees in Earth's magnetic field.

Co: Synthases and isomerases (cobalamines, e.g. vitamin- B_{12}); methylation of inorganics.

Zn^{2+} : In the active centre of hydrolases, carboanhydrase, alcohol dehydrogenase, synthases; genetic transcription (zinc fingers), stabilisation of tertiary and quaternary structures of proteins; repair enzymes.

Si^{IV} („silicate“): Involved in the built-up of bones. In the form of SiO_2 /silica-gels as support in monocotyledonous plants (like grass) and the shells of diatoms.

P^{V} (phosphate): Constituent in hydroxi- and fluorapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH/F})$); energy metabolism (ATP), NADPH, activation of organic substrate; phospholipids in cell membranes; phosphate esters (DNA, RNA, ...).

Se^{II} : Selenocystein in special enzymes (e.g. glutathionperoxidase)

F^- : Fluorapatite ($\text{Ca}_5(\text{PO}_4)_3\text{F}$) in dental enamel

Cl^- : Along with hydrogencarbonate the most important free anion.

I: Constituent of thyroid hormones (such as thyroxine).

Medicinal relevant elements (selection):

Li^+ : Treatment of bipolar disorder (maniac depression) and hypertension.

Gd^{3+} : Contrast agent in magnetic resonance tomography of soft tissues.

BaSO_4 : Contrast agent for X-ray tomography. Sun protection.

$^{99\text{m}}\text{Tc}$ (a metastable γ -emitter; $t_{1/2} = 6 \text{ h}$): Radio diagnostics (bone cancer, infarct risk, ...)

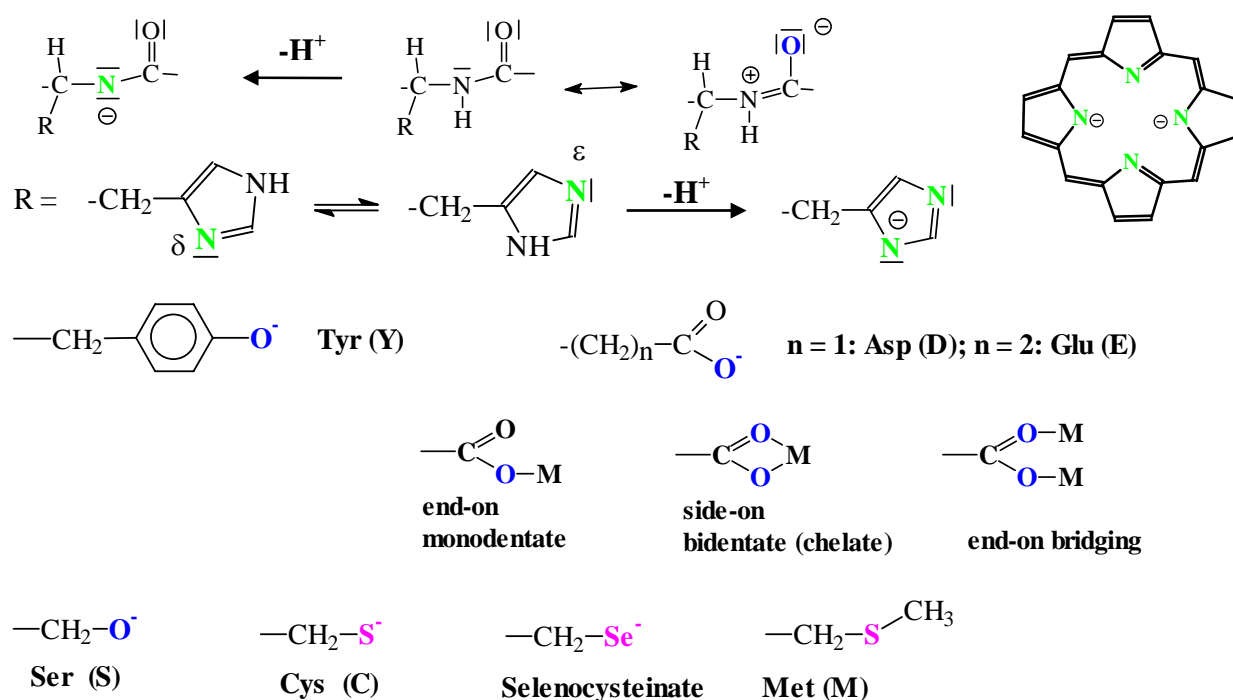
Pt^{II}: Chemotherapy (e.g. with cisplatin *cis*-[Pt(NH₃)₂Cl₂]) of cancer (ovaria, testes)

Au^I: Therapy of rheumatic arthritis.

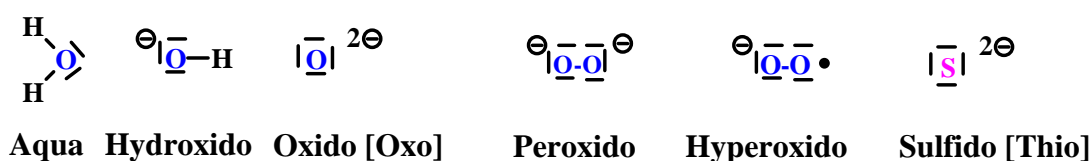
Sb^{III}: Treatment of inflammatory skin pimples like acne.

Bi^{III}: Treatment of gastritis.

Transition metal ions commonly are not present in a free form, but rather coordinated (complexed) to ligands. In particular, this applies to metal ions in the active centres of enzymes or otherwise integrated into peptides and proteins. Examples for the respective ligands are listed below (**N**-functional: peptide moiety, porphinogenes, histidine; **O**-functional: tyrosinate, serinate, glutamate and aspartate; **S**-functional: cysteinate and methionine):



Additional inorganic ligands:



2. Iron

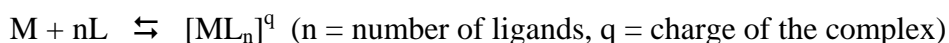
Iron takes over a central role in biological events (see also its role in the primordial synthesis of organic compounds; ch. 1). On the one hand, this is due to its general availability (iron is abundant and ubiquitous in the geo- and biospheres), on the other hand, iron has specific and “biologically suitable” properties otherwise not (or less) available with other transition metals:

- (1) Ease of change between the oxidation states +II and +III (and disposability also of the oxidation states +IV and +V);
- (2) Formation of hexaaqua cations in water; these hexaaqua cations are Brønsted acids;

- (3) Tendency to form oligo- and polymers by condensation;
- (4) Easy change between high- and low-spin states in ligand fields of medium strength (spin cross-over; see inset on p. 7, upper part);
- (5) Flexibility with respect to the nature of the donating ligand function (see inset on p. 7, lower part, for ligand classification), the coordination number and coordination geometry.

Tutorial: Coordination compounds (1): Definition

Coordination compounds, or complexes, are integral molecular or ionic units consisting of a central metal ion (or atom), bonded to a defined number of ligands in a defined geometrical arrangement. The ligands can be ions or (induced) dipolar molecules. Each ligand provides a free electron pair, i.e. the ligands are Lewis bases, while the metal in the coordination centre is the Lewis acid. The bonding can thus be described in terms of Lewis acid/Lewis base interaction. Other descriptions of the bonding situation are: (i) donor bond; (ii) coordinative covalent bond, often denoted by $L \rightarrow M$, where L = ligand and M = metal. Complexes tend to be stable when the overall electron configuration at the metal centre (the sum of metal valence electrons plus electron pairs provided by the ligands) is 18 (or 16 for the late transition metals).

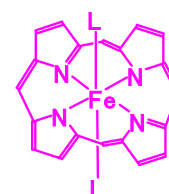


$$\frac{c(ML_n)}{c(M) \cdot c^n(L)} = K$$

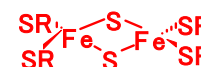
K is the *stability constant* or *complex formation constant* ($pK = -\log K$); its inverse, K^{-1} , is termed *dissociation constant*.

The average amount of iron in the human body (70 kg) is ca. 5 g; iron is thus the most abundant transition metal in our organism. About 70% of this amount is used for oxygen transport and storage (haemoglobin, myoglobin), almost 30% are stored in ferritins (iron storage proteins), and about 1% is bound to the transport protein transferrin and to various iron-dependent enzymes; cf. the rough classification to the right.

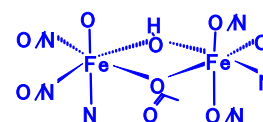
Haeme-type
(e.g. cytochromes, haemoglobin)



Iron-sulfur proteins
(e.g. ferredoxins, Rieske proteins)

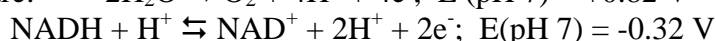
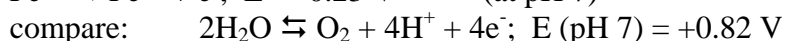
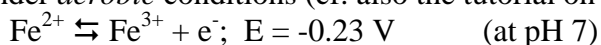


Two-iron centres
(e.g. ribonucleotidreductase)



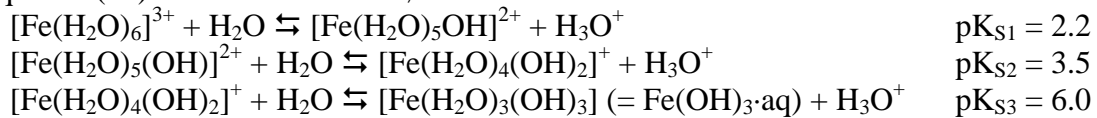
Aqueous iron chemistry

The redox potential for the pair Fe^{2+}/Fe^{3+} at $pH = 7$ demonstrates that Fe^{II} is easily oxidised to Fe^{III} under *aerobic* conditions (cf. also the tutorial on oxidation and reduction on p 16):

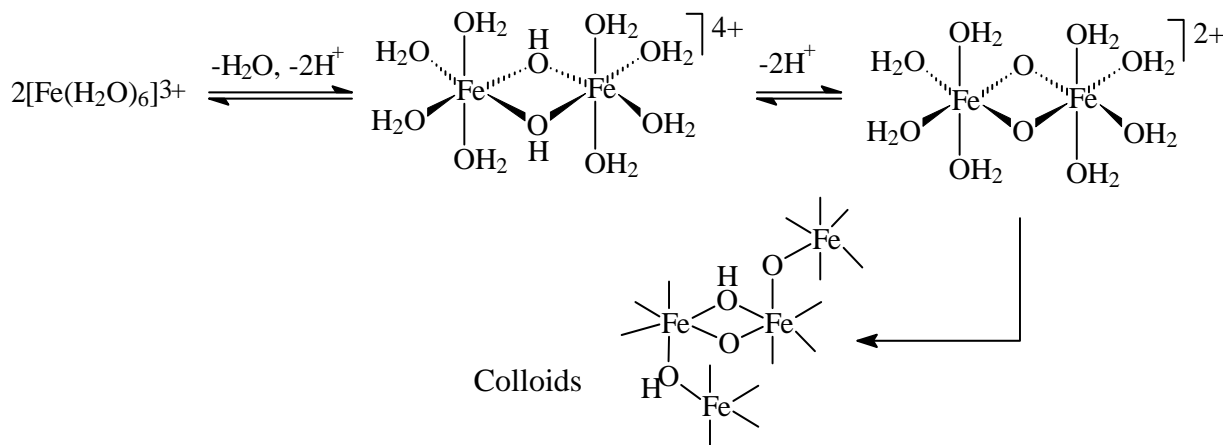


(NADH = nicotine-adenine-dinucleotide in its reduced form)

Hexaaquairon(III) ions are cationic Brønsted acids:



The formation of ferric hydroxide $\text{Fe}(\text{OH})_3 \cdot \text{aq}$ hence already starts in weakly acidic media. The protolytic reactions are accompanied by condensation reactions, leading to hydroxido- and oxido-bridged aggregates and finally to colloids and hardly soluble ferric oxide hydrates. The colloids can be spheroids of molecular mass M ca. $1.5 \cdot 10^5$ Da and ca. 70 Å diameter, or needles/rods ($M = 1.9 \cdot 10^6$, length up to 500 Å). The composition of these ironoxide hydrates correspond to that of the minerals $\text{FeO}(\text{OH})$ (goethite) and $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ (ferrihydrite).



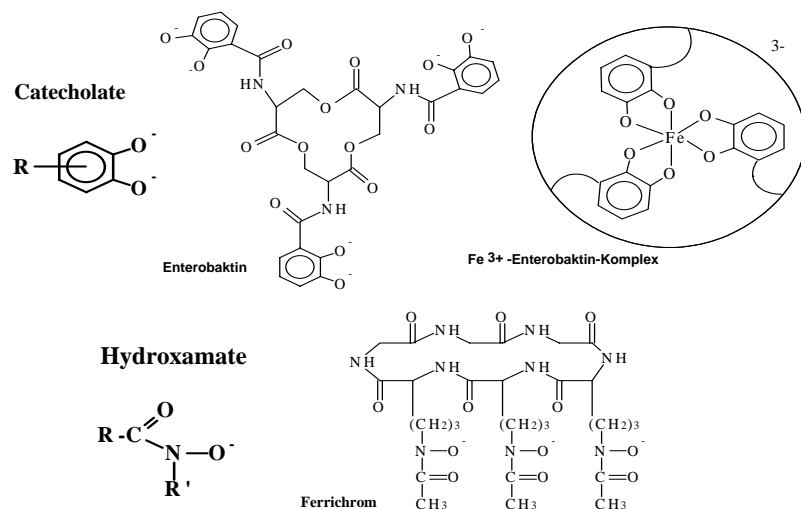
Mobilisation of Fe^{3+} by siderophores

The extremely low solubility of $\text{Fe}(\text{OH})_3$ [solubility product $L = 2 \cdot 10^{-39}$, solubility (pH 7) $l = 10^{-18} \text{ mol} \cdot \text{l}^{-1}$], and thus the unavailability of iron in aqueous media under oxic conditions, has forced many groups of organisms to develop suitable systems for the mobilisation of iron. These systems, so-called siderophores (Greek for iron transporter), excreted by the organisms, are multidentate anionic ligands which form extremely stable complexes with Fe^{3+} (complex formation constants up to 10^{50} M^{-1}). The functional groups of these ligands are, in many cases,

catecholates (*o*-hydroxyquinolates), as in the case of enterobactin, or hydroxamates

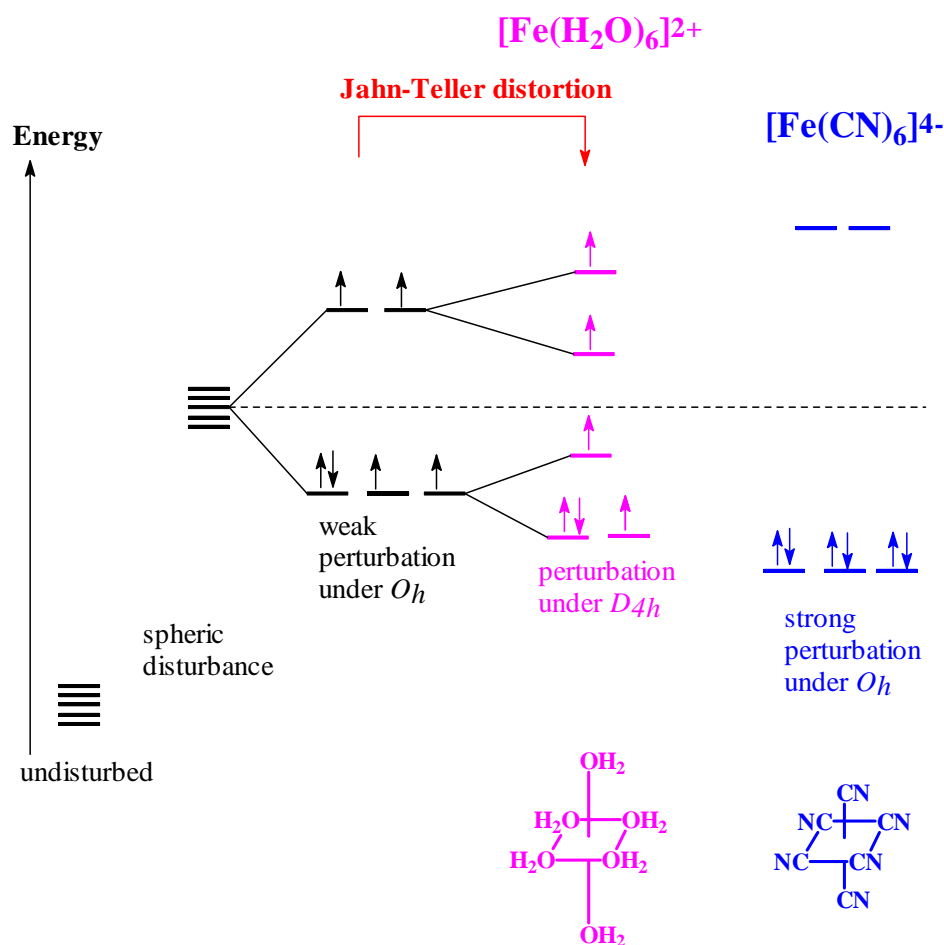
(ferrioxamines and ferriochromes). The complexes are more or less globular, with the outer sphere furnished with hydrophilic groups (amide and ester groups), allowing for the water solubility and easy transport in the aquatic medium. Internalisation of the iron-loaded siderophore by the organism typically

takes place by endocytosis; the cytosolic remobilisation of the iron either by reduction of Fe^{3+} to Fe^{2+} and recycling of the siderophore, or by oxidative destruction of the siderophore.



Tutorial: Coordination compounds (2): Ligand-field splitting and spin state

Example: Fe^{II} (d^6): In an octahedral (O_h) field, the degeneracy of the five d -orbitals is lifted. Depending on the strength of the ligand field, the ligand field stabilisation energy (i.e. the energy *set free* as all of the electrons are accommodated in the orbitals of lower energy) can be (i) less and (ii) more than the energy *needed* for electron pairing. In the case of (i), i.e. aqua ligands, a high-spin complex is formed; in the case of (ii), i.e. cyanido ligands, a low-spin complex is formed. Asymmetrically occupied orbital sets, as in the case of $[\text{Fe}(\text{H}_2\text{O})_6]^{2+}$, result in further stabilisation through symmetry lowering: Jahn-Teller distortion.



Tutorial: Coordination compounds (3): Classification of ligands; and the chelate effect

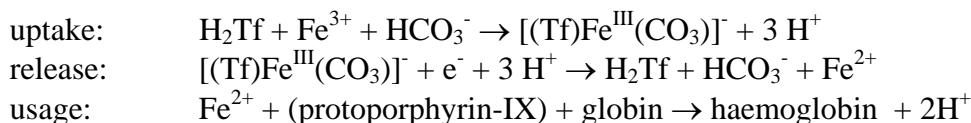
Series of ligand strengths: Halides $\approx \{\text{S}\} < \{\text{O}\} < \{\text{N}\} < \text{CN}^- < \text{NO}^+ \approx \text{CO}$

Pearson classification (soft and hard): hard metal centres (usually early and transient transition metals in high oxidation states, e.g. Mo^{6+} and Fe^{3+}) prefer hard ligand (i.e. more electronegative ones, such as oxygen-based donors), soft metal centres (late transition metals, e.g. Cu^+) prefer soft ligands (such as cysteine). There are *many* exceptions from this “rule”.

Chelate effect: Stabilisation of a complex by multidentate ligands. The chelate effect is an entropic effect (high entropy = high disorder [increase of particle number]). Example: The complex formed between the siderophore enterobactin (ent^{6-} , a hexadentate ligand) and Fe^{3+} is particularly stable: $[\text{Fe}(\text{H}_2\text{O})_6]^{3+} + \text{ent}^{6-} \rightarrow [\text{Fe}(\text{ent})]^{3-} + 6\text{H}_2\text{O}$.

Uptake, transport and storage of iron

Iron, when taken up with the food and processed in the mouth (chewing, admixture of saliva) is mostly present in its ferric (Fe^{3+}) form and thus gets into the gastro-intestinal tract as Fe^{3+} . In case of an intact milieu in the small intestines, ferric iron is reduced to its ferrous form (Fe^{2+}). Only in this oxidation state can iron be absorbed by the epithelium cells of the mucosa. For transfer to the blood serum, reoxidation to Fe^{3+} is necessary. The oxidation $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ in the mucosa is catalysed by a copper enzyme (ceruloplasmin, containing 7 copper centres: $\text{Cu}^+ \rightarrow \text{Cu}^{2+}$). The Fe^{3+} ions are then taken up by apotransferrin (H_2Tf); simultaneously, carbonate is coordinated to iron. Fe^{3+} -Tf is the transport form for iron. The iron-loaded transferrin, Fig. 3) delivers iron to sites of potential use (e.g. incorporation into protoporphyrin IX and generation of haemoglobin), or stored in iron storage proteins (ferritins). The delivery of iron affords reduction from the ferric to the ferrous state; a reductant employed here is ascorbate (vitamin C):



The daily absorption rate of iron supplied by food amounts to ca. 1 mg. Within our organism, about 40 mg of Fe are mobilised and transported by Tf into the spinal marrow for the haemoglobin synthesis, and about 6 mg are stored within or mobilised from the ferritins (vide infra). Transferrin is a glycoprotein of molecular weight 80 kDa (containing ca. 6% carbohydrate), having available two almost equivalent binding sites for iron(III), in the C- and N-terminal lobes, respectively. The pK (K = stability constant; see inset on p. 5) at pH 7.4 (the pH of blood) is -20.2. Transferrin is also an effective transporter for other tri- and divalent metal cations, and even for anions (e.g. vanadate). Since its loading capacity for iron commonly is only ca. 40%, other ions can be transported simultaneously.

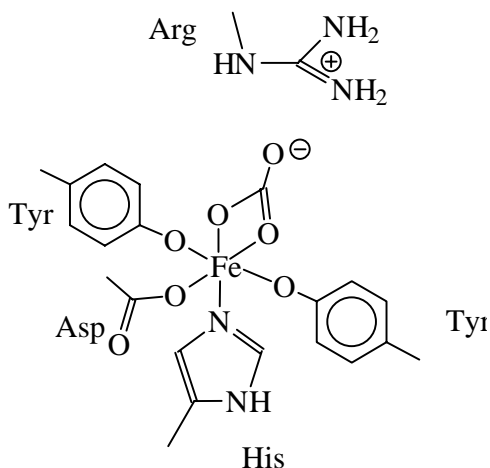


Figure 3: The Fe^{3+} -carbonate-transferrin complex. Coordination of carbonate(2-) is supported by salt interaction with an arginine residue in the protein pocket.

Ferritins (Fig. 4) are iron storage proteins, built up of a hollow protein sphere (apo-ferritin, $M = 450$ kDa, 24 subunits of 163 amino acids each) with an outer diameter of 130 and an inner diameter of 70 Å. The inner surface of this capsule is lined with carboxylate functions, which can coordinate Fe^{3+} . Up to 4500 Fe^{3+} can be taken up. The various iron centres are connected by bridging oxido and hydroxido groups very much as in the colloidal form of ferric hydroxide (see above) or the mineral goethite. The overall composition of the iron nucleus is $8\text{FeO}(\text{OH}) \cdot \text{FeO}(\text{H}_2\text{PO}_4)$. Channels of threefold symmetry and a width of 10 Å allow for an exchange of Fe^{3+} between the interior and exterior. For the primary uptake process, iron has to be in the oxidation state +II. Its transport along the channels and built-in into the core is accompanied by oxidation to the +III state:

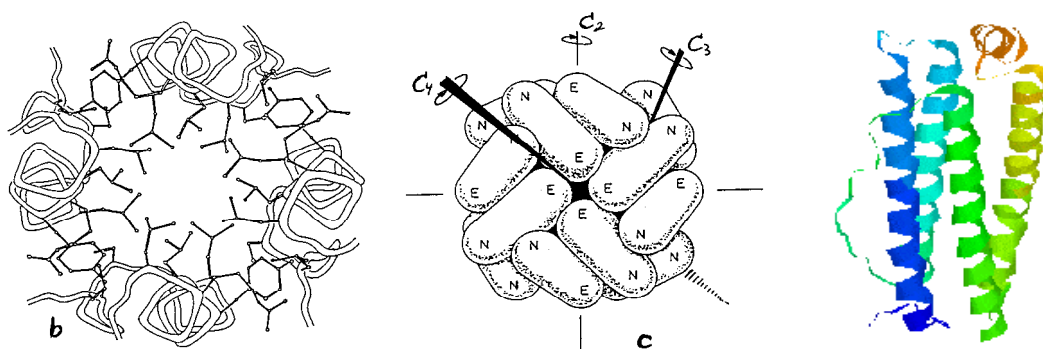
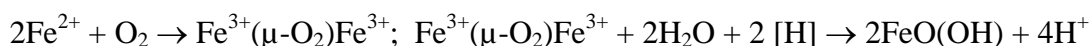


Figure 4. The iron storage protein ferritin. Left: Apoferritin (the inside of the hollow sphere is lined with carboxylates); centre: subunit structure and channels of C₂, C₃ (for iron exchange with the surroundings) and C₄ symmetry; right: one of the subunits.

Ferritins – like many other proteins – exhibit high symmetry. High symmetry (also found with higher organised forms of life such as viruses, bacteria and even proteins in plants and animals) makes less reactive – as a consequence of minimised overall polarity – and thus has “a protective function”. For some basic considerations on symmetry, also of relevance in the context of the electronic configuration of metal ions in coordination compounds (and thus for oxygen binding by haeme; next chapter), see the inset on page 10.

3. Oxygen transport

Dry air contains 20.96 vol.-% of O₂; 1 L of water at 20 °C can dissolve 31 ml of O₂; with increasing temperature, the solubility decreases (23 ml at 40 °C). In the pulmonary alveoli, O₂ is taken up by haemoglobin (Hb, *M* = 65 kDa; Fig. 5); at saturation, 1 L of blood can dissolve ca. 200 ml of oxygen. Simultaneously, hydrogencarbonate is converted to carbonic acid, which in turn is catalytically degraded into CO₂ and H₂O (by the zinc enzyme carbonic anhydrase):

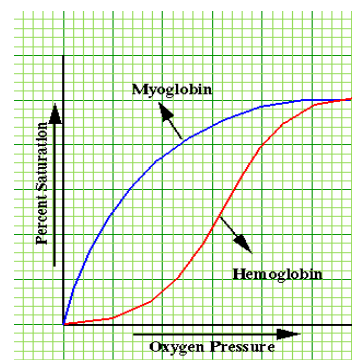
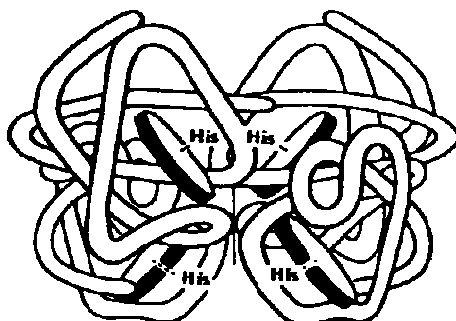
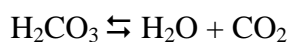
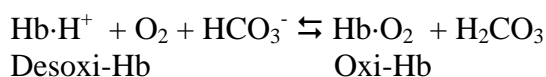
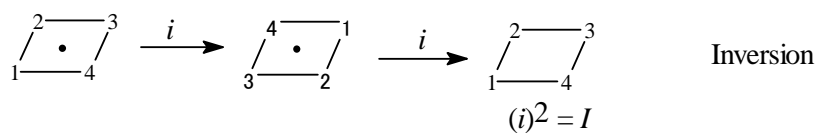
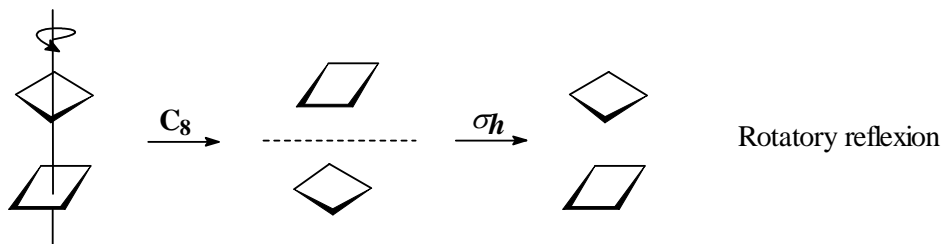
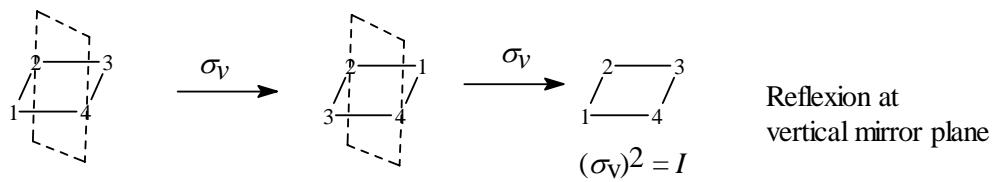
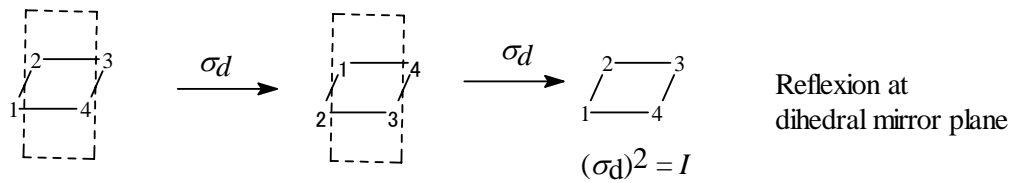
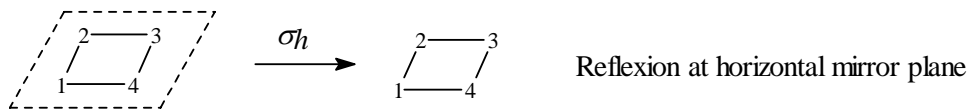
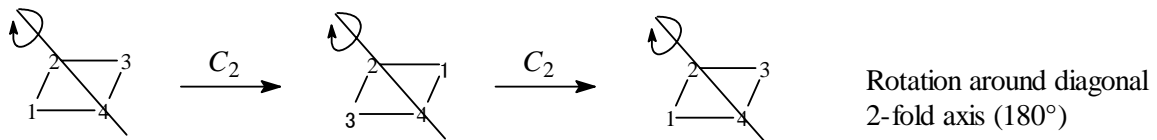
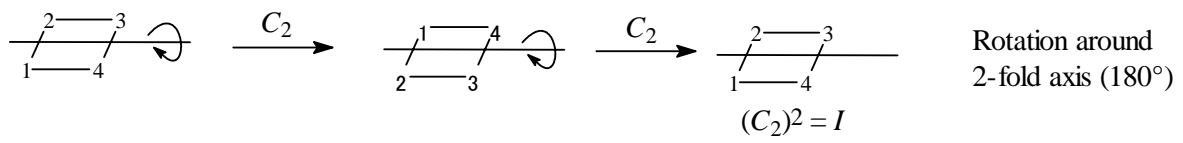
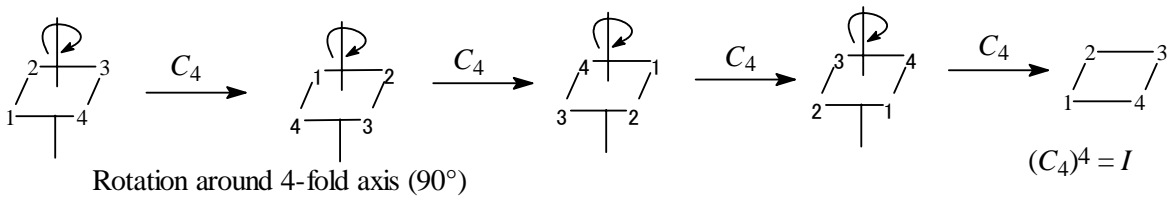
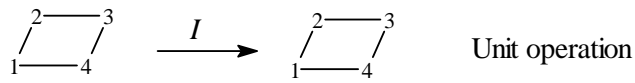


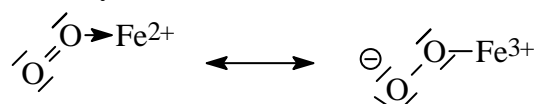
Figure 5. Left: Schematic view of haemoglobin (a tetramer, mainly α₂β₂ in adults; there is one haeme group per subunit). Myoglobin is a monomer. Right: Affinity of haemoglobin and myoglobin to oxygen. The O₂ partial pressure at saturation (100%) is ca. 100 Torr (ca. 0.13 bar = 13 kPa). The graphs apply to the normal blood pH of 7.35 and temperature of 37 °C. Decreasing the pH and increasing the temperature decreases the affinity for O₂.

Digression: Symmetry operations



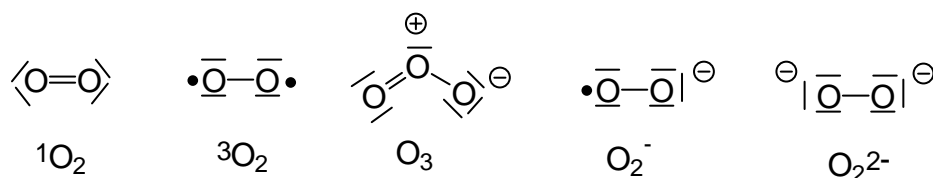
After transport of O₂ by haemoglobin in the blood stream, the oxygen is transferred to tissue myoglobin (Mb). As shown in Fig. 5, Mb has a higher affinity to O₂ than Hb.

In the desoxy form of Hb, Fe²⁺ is in its high-spin state (cf. the inset on p. 7, top) and thus exhibits a paramagnetism corresponding to four unpaired electrons. The diameter of high-spin Fe²⁺ is 92 pm; the Fe²⁺ ion thus is too large to fit into the space left by the four N-functions of the protoporphyrin. Actually, Fe²⁺ is displaced from the plane spanned by the porphyrin by 40 pm towards the proximal His; cf. Fig. 6; resulting in a watchglass bulge of the porphyrin, i.e. a tensed situation. Consequently, desoxy-Hb is termed T (for tensed) form. On uptake of oxygen, the iron spin state converts to low-spin, resulting in a reduction of its diameter to 75 pm (Fe²⁺, no unpaired electrons) or 69 pm (Fe³⁺, 1 unpaired electron), respectively. The iron ion now moves into the plane of the porphyrin (R form; R = relaxed). Oxi-Hb is diamagnetic. If iron remains in its ferrous state, overall diamagnetism can only be achieved in case the coordinated oxygen converts from the paramagnet triplet state (in free O₂) to the diamagnetic singlet state (in Oxi-Hb) (cf. also box below). Alternatively, the uptake of O₂ can occur in the sense of an oxidative addition, i.e. Fe²⁺ + O₂ → Fe³⁺-O₂⁻. In that case, the unpaired electron of the ferric ion and the unpaired electron of superoxide have to couple in order to provide the overall diamagnetism. The overall situation is conveniently described in terms of a resonance hybrid:



Tutorial: Oxygen

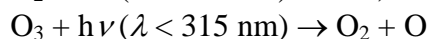
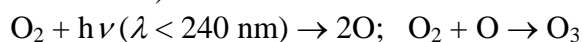
One commonly distinguishes three oxygen modifications: Singlet-O₂ (¹O₂; high energy content, unstable, diamagnetic), triplet-O₂ (³O₂, stable, biradical and hence paramagnetic), and ozone (O₃; toxic; very reactive [strong oxidant]). [A high pressure modification, (O₂)₄, is also known]



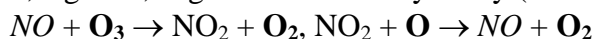
Formation of ozone in the troposphere (ozone smog): NO + O₂ → NO₂ + O; O₂ + O → O₃;

NO₂ + hν → NO + O

Stratospheric ozone: Stratospheric ozone is an effective filter for “hard“ UV (responsible for cancers of the skin):



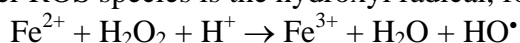
Radicals, e.g. NO, degrade ozone catalytically (“ozone whole“):



Other radicals can do the same job, e.g. Cl atoms, which are liberated from chloro fluoro alkanes (CFC) under stratospheric conditions

Reduction of O₂ produces superoxide (O₂^{•-}) or peroxide (O₂²⁻), both of which are strong oxidants and physiologically harmful (reactive oxygen species, ROS). To cope with these oxidants, the body holds ready catalases (H₂O₂ → H₂O + O₂) and superoxidedismutases (2O₂^{•-} + 2H⁺ → H₂O₂ + O₂).

Another ROS species is the hydroxyl radical, formed, e.g. by the Fenton reaction:



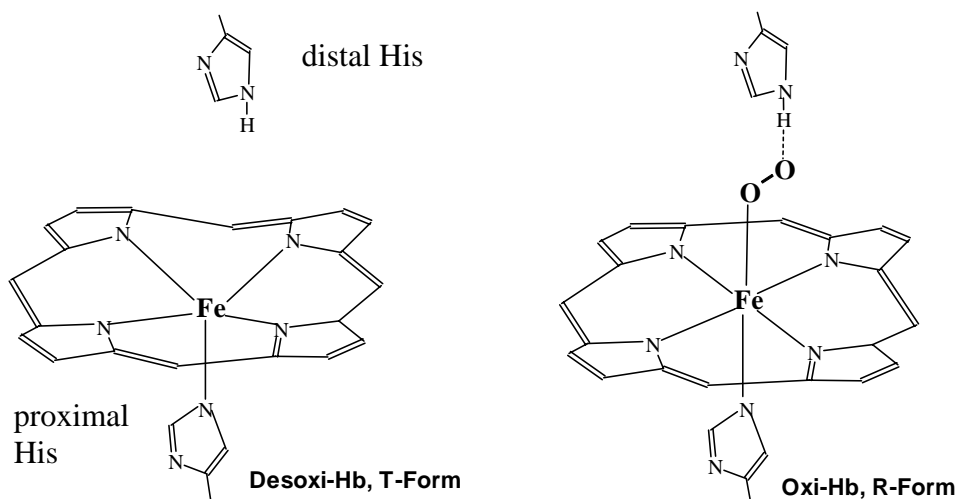


Figure 6. Desoxy and oxy forms of haemoglobin/myoglobin. The central ligand system, protoporphyrin IX, is shown here without the characteristic ring substituents.

Transport, formation and degradation of hydrogencarbonate

Oxygen is finally reduced to water in the mitochondrial respiratory chain (ch. 4). The reduction equivalents come from organic compounds (such as glucose), which are degraded to CO_2 . CO_2 is converted enzymatically to hydrogencarbonate according to $\text{CO}_2 + \text{OH}^- \rightarrow \text{HCO}_3^-$, most of which is extruded out of the erythrocytes (concomitantly, chloride is taken up) and transported, via the blood plasma, to the pulmonary aveoli, where carbonic acid is formed through the reaction with $\text{Hb} \cdot \text{H}^+$, coupled with binding of O_2 to haemoglobin. Carbonic acid finally is enzymatically split into CO_2 and H_2O . The enzyme catalysing the formation and degradation of hydrogencarbonate/carbonic acid is called *carbonic anhydrase* (CA). CA has a molecular weight of 29.7 kDa and contains Zn^{2+} in its active centre. Zn^{2+} is coordinated to three histidine residues plus an aqua ligand (in its resting state) or a hydroxido ligand (in its active state). A histidine close to the active centre participates in the proton shuttle. For the catalytically conducted mechanism see Fig. 7.

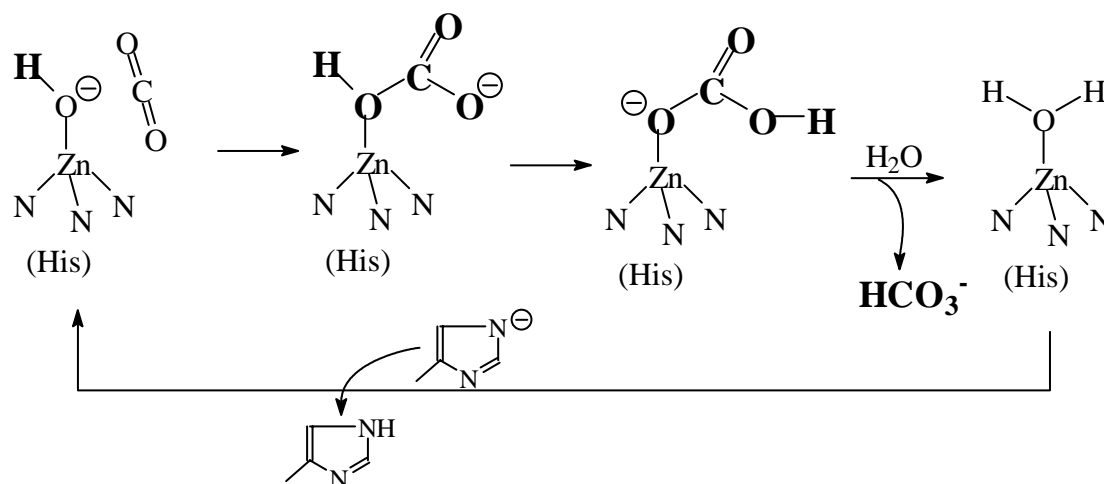


Figure 7. Mechanism of the formation of hydrogencarbonate catalysed by carbonic anhydrase. The reverse reaction (formation of CO_2 from carbonic acid) is also catalysed by this enzyme.

Inactivation of haemoglobin

Oxygen binding to haemoglobin can only occur if the Fe^{2+} site directed towards the distal His is accessible. There are specific mutations where this is not the case, such as in the so-called Boston-Hb, where the distal His is replaced by Tyr, the tyrosinate-oxygen of which tightly coordinates to the iron site and thus blocks off access of O_2 . Carbon monoxide exerts a comparable effect, which is responsible for the toxicity of CO. CO is bound 220 times more strongly to Fe^{2+} than O_2 : 0.25% of CO in air, i.e. the CO contents of inhaled cigarette smoke of 20 cigarettes per day, block off about 25% of the oxygen binding capacity of Hb. NO (formed by reduction of nitrite) has a comparable effect

The naturally occurring mutant Glu6Ala (glutamate at position 6 exchanged for alanine) causes sickle cell anaemia, a deformation of the red blood cells by polymerisation of the globin subunits of Hb. The blood of people suffering from sickle cell anaemia has restricted O_2 capacity. These people are, however, immune against malaria, which has led, by selection, to a high percentage of anaemic individuals amongst the populations of some tropical African regions.

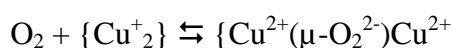
A certain amount of haemoglobin is consistently oxidised to methaemoglobin (MetHb) by oxidants such as peroxide, hyperoxide and OH radicals:



Met-Hb, in which the second axial position is blocked by OH^- is, however, consistently "repaired" by methaemoglobin-reductase (containing NADH as cofactor).

Oxygen transport by haemocyanins and haemerythrin

Hemocyanins are oxygen transport proteins occurring in arthropods (spiders, crabs, lobsters, ...) and molluscs (snails, squids, ...). They contain a dinuclear copper centre per subunit. Molecular weights vary from 450 kDa (arthropods, subunits of 75 kDa) to 9 MDa (molluscs, subunits of 50-55 kDa). The oxygen is reversibly taken up by oxidative addition:



The peroxide thus formed coordinates in the unusual side-on bridging mode, $\mu\text{-}\eta^2\text{:}\eta^2$; Fig. 8).

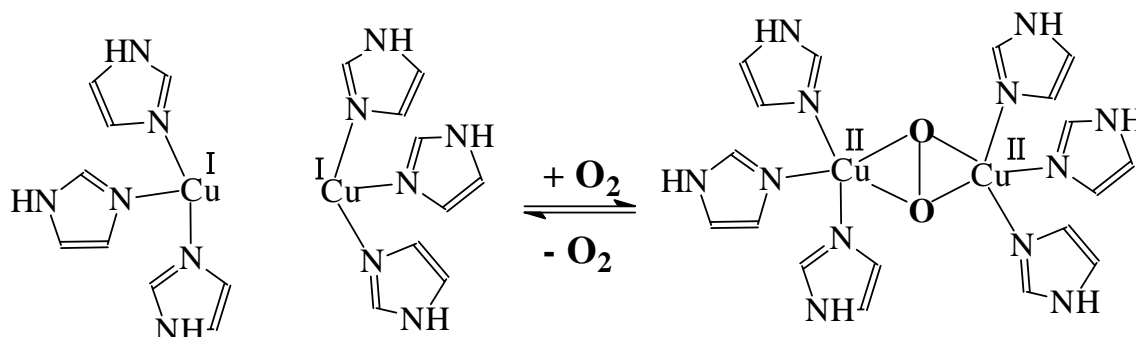


Figure 8. Reversible uptake and release of oxygen by haemocyanins.

An oxygen transport protein occurring in certain non-segmented worms (the *sipunculid* family) is haemerythrin, consisting of eight subunits (overall molecular weight 108 kDa), each of which containing a dinuclear iron centre; Fig. 9. In the desoxy form, these are ferrous centres, bridged by OH^- , an aspartate and a glutamate. One of the iron centres is additionally

coordinated to three histidines, the other one to two His, leaving one of its coordination sites vacant for the access of oxygen. As in the case of haemocyanins, oxygen is coordinated in the sense of an oxidative addition, i.e. the ferrous centres become ferric centres, and the oxygen is converted to peroxide. Concomitantly, the bridging hydroxide converts to a μ -oxido group by protonating the peroxide to hydroperoxide, HO_2^- .

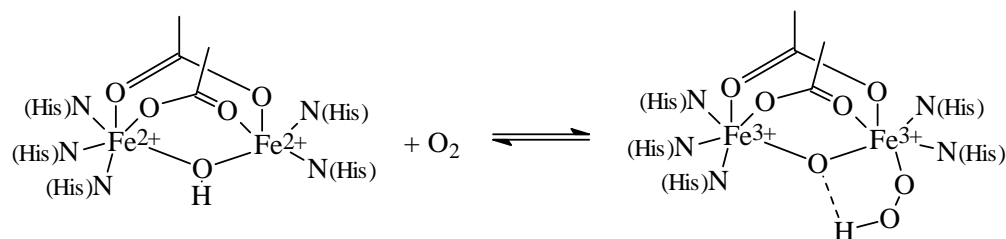
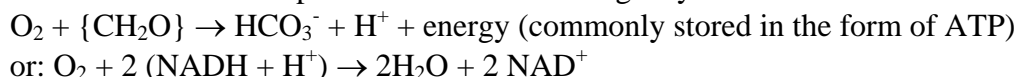


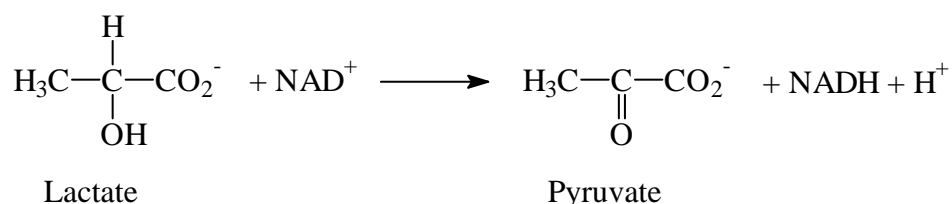
Figure 9. Desoxy form (left) and oxy form (right) of haemerythrin.

4. The mitochondrial respiratory chain

The overall reaction can be represented in the following way:



The *free* enthalpy of reaction (ΔG) of this reaction amounts to -217 kJ/mol , the redox potential to 1.14 V . The reduction equivalents are delivered by, e.g., products formed in the course of the degradation of glucose, such as lactate:



The reduction of O_2 to H_2O takes place step by step in order to prevent damage to cellular constituents by the burst of energy liberated in a single step. The reaction cascade is termed respiratory chain, which takes place in the mitochondria, and serves the generation of energy. For the overall process, cf. Fig. 10. For some general remarks on oxidation and reduction, see inset on p. 16.

Step 1: Primary acceptor for two reduction equivalents delivered by NADH is an iron-sulphur protein belonging to the [4Fe,4S] ferredoxin family (for a systematic treatment of ferredoxins see below). Such an iron-sulphur cluster can accept and deliver just one electron per cluster. The charge is delocalised over the complete cluster system; the mean oxidation state of iron is 2.5 in the oxidised and 2.25 in the reduced form.

Step 2: Electron acceptor for the ferredoxin is a quinone (so-called ubiquinone, containing a polyisoprene side-chain in position 2), a two-electron acceptor which becomes reduced to the hydroquinone.

Step 3: Another iron-sulphur protein, the Rieske protein (or Rieske centre) then takes over. Rieske proteins are two-centre iron proteins with one of the irons carrying two His (the other one is coordinated to 2 Cys⁻ and two bridging S^{2-}). In the oxidised form, both iron ions are in

the +III state, in the reduced form, the ferric iron coordinated to four sulphur functions turns to the ferrous state.

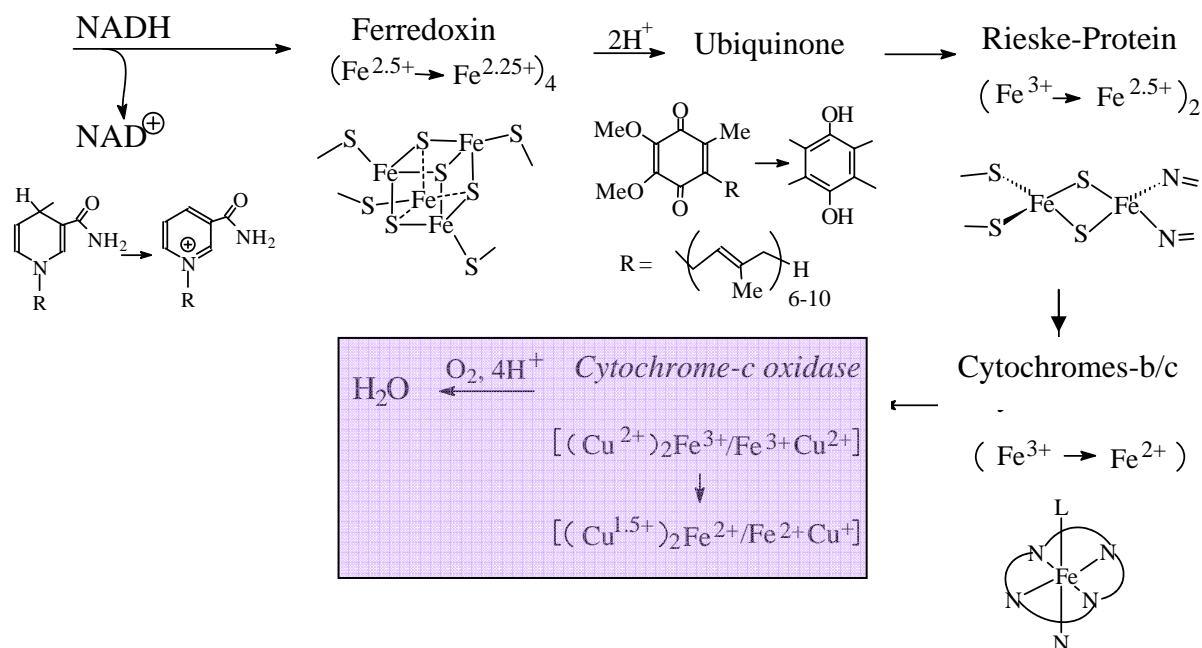
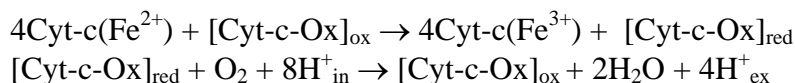


Figure 10. Reaction cascade in the mitochondrial respiratory chain (shortened).

Step 4: The reduction equivalents are now transferred to cytochrome-b (Cyt-b) and further to cytochrome-c (Cyt-c). In these haeme-type electron transporters (for details see below), iron switches between the ferric and ferrous state.

Step 5: The reduced (Fe^{II}) form of Cyt-c is re-oxidised by cytochrome-c oxidase, an enzyme that contains 5 redox active centres: 2 haeme type Fe^{II/III} (Cyt-a and Cyt-a₃), a dinuclear copper centre {Cu^{II}₂/Cu^{1.5}₂} = Cu^A and a mononuclear Cu^{I/II} = Cu^B centre. In addition, there are two structural metal centres (Zn²⁺ und Mg²⁺).

Step 6: Cytochrome-c oxidase (Cyt-c-Ox) can take up 4 electrons from 4 Cyt-c. These electrons are employed for the reduction of O₂ to H₂O. 8 protons are handled in this process: 4 of the protons are needed to form water; 4 additional protons are translocated across the membrane (from the intra- to the extra-mitochondrial space); i.e. Cyt-c-Ox also works as a proton pump. Activation and reduction of the oxygen (via peroxide) occurs between the adjacent Cyt-a₃ and Cu^B centres For the organisation of Cyt-c-Ox see Fig. 13.



The iron-sulphur proteins

The more important (in the sense that they are more generally used) members of this family are collated in Fig. 11. (1) Rubredoxins contain one iron centre tetrahedrally coordinated to four cysteinates. (2) [2Fe,2S] ferredoxines, with two iron centres, constitute two edge-bridged FeS₄ tetrahedra. The bridging sulphur functions are inorganic sulphide S²⁻, the remaining ligands are cysteinate. (3) [4Fe,4S] ferredoxins have a cubane structure. The four trebly bridging functions are again sulphide, also termed labile sulphur because they can be

converted to volatile H_2S with acids. The mean oxidation state in the reduced form is 2.25, in the oxidised form 2.5, the redox potential is typically around -200 mV. (4) HiPIPs (High Potential Iron Proteins) are identical to the [4Fe,4S] ferredoxins in as far as the core structure is concerned. However, the mean oxidation state in the reduced form is 2.5, in the oxidised form 2.75, and the redox potential is typically around +300 mV. Along with these “classical” iron-sulphur clusters, others are known, in which one iron centre is missing ([3Fe,4S] ferredoxins), or where two [4Fe,4S] ferredoxins form double-cubanes, or where a fifth ligand (Ser or His) is coordinated to one of the iron centres. The Rieske proteins have already been mentioned above; the angle N-Fe-N is ca. 90° , i.e. there is strong distortion from tetrahedral symmetry for this specific iron.

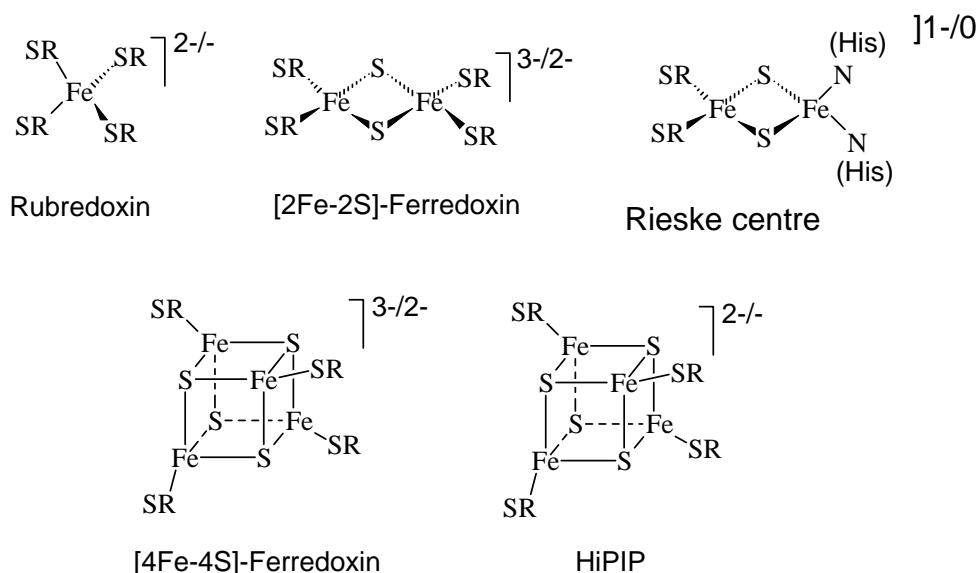


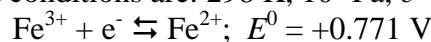
Figure 11. The iron centres of the classical (and more frequently used) iron-sulphur proteins. SR = cysteinate(1-).

Tutorial: Oxidation and reduction

An oxidation corresponds to a removal of electrons (increase of the oxidation number), reduction correspondingly to a transfer of electrons to a substrate (decrease of the oxidation number). Oxidation and reduction are coupled; an example is the oxidation of ferrous to ferric iron, coupled with the reduction of oxygen to water:



In principal, all redox reactions are equilibrium reactions. The direction is determined by the redox potentials of the two pairs of underlying electron transfer processes. Standard redox potentials E^0 are tabulated; standard conditions are: 298 K, 10^5 Pa, $c = 1$ mol/l:



Recalculation of the potential for real concentrations, E^c , is achieved with the Nernst equation:

$$E^c = E^0 + (0.059/n)\log(c_{\text{Ox}}/c_{\text{Red}})$$

where n is the number of transferred electrons; c_{Ox} and c_{Red} the concentrations of the oxidised and reduced forms, respectively. In particular, the pH dependence has to be taken into account: At pH 7, ($c(\text{H}^+) = 10^{-7}$), E^c for the pair H_2/H^+ is -0.414 V ($E^0 = 0$), for $\text{H}_2\text{O}/\text{O}_2$ +0.815 V.

Cytochromes and cytochrome-c oxidase

Cyt-b und Cyt-c, and the cytochromes-a and -a₃ of the cytochrome-c oxidase contain haeme type iron centres. They are distinct by their substitution pattern at the porphyrin ring and the axial ligands; see Fig. 12. They transfer electrons, moving between the iron oxidation states +II and +III. Cytochromes may also attain other than simple electron transfer functions. An example is cytochrome P₄₅₀, an oxygenase in which, during turn-over, iron passes through the oxidation state +IV (and, perhaps, +V).

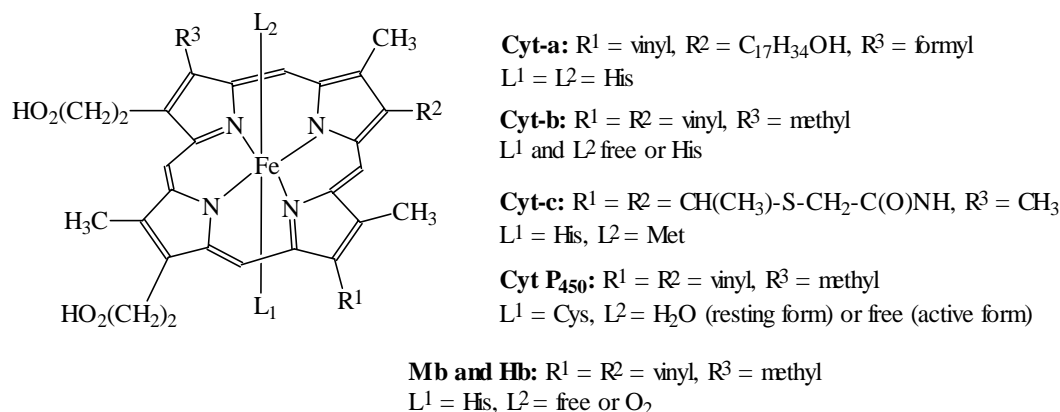


Figure 12. The active centres of selected haeme-type proteins. For Cyt-P₄₅₀, see the chapter on oxygenases.

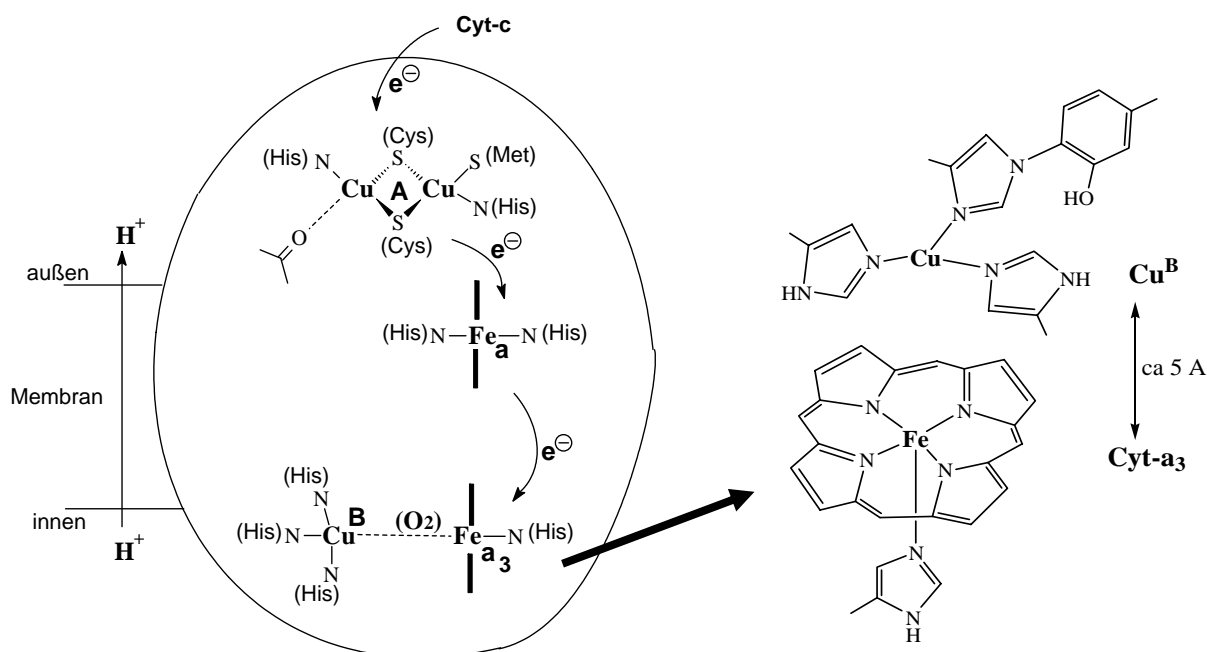
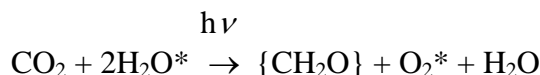


Figure 13. Organisation of the redox-active centres of cytochrome-c oxidase (left). Oxygen activation and reduction occurs at the dinuclear Cu^B...Cyt-a₃ pair (see expansion to the right).

5. Photosynthesis

Photosynthesis (assimilation) and respiration (dissimilation) are complementary processes. Photosynthesis results in reductive carbon fixation and production of oxygen, energy driven by light energy, $h\nu$.



Carbon dioxide is reduced, in a $4e^-$ reduction, to $\{\text{CH}_2\text{O}\}$ (symbolising a carbohydrate such as 1/6 of glucose). Reducing agent is water, which is oxidised to O_2 . Instead of resorting to light as an energy source, chemical energy (energy liberated in the course of a chemical reaction) can be employed, and sources for carbon other than CO_2 , e.g. CO or acetate, can be used.

Depending on the energy and the carbon source, one distinguishes the following categories:

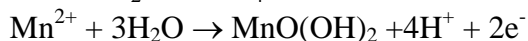
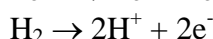
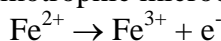
Light energy: phototrophic

Chemical energy: chemotrophic

CO_2 as C-source: autotrophic

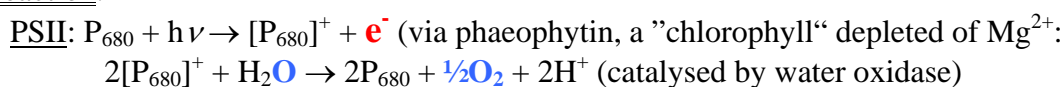
Other C-sources: heterotrophic

Green plants, cyanobacteria and other photosynthetically active bacteria, and protozoa containing chlorophyll produce bio-mass photo-autotrophically. A 100 year old beech tree produces about 1000 l of O_2 and 12 kg of carbohydrates per day (this corresponds to 100 ml of O_2 and 1.2 g of glucose per 1 m^2 of foliage). The major part of bio-mass is, however, produced by chemotrophic microorganisms. Examples for chemical processes supplying energy are:

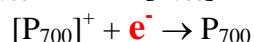
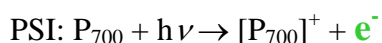


In the photosynthetic process carried out by plants, one distinguishes between the light reaction and the light-independent (or dark) reaction on the one hand and, within the light reaction, between photosystems I and II (PSI and PSII, also referred to as light harvesting complexes LHC) on the other hand:

Light reaction:



e^- transfer chain from PSII to PSI (Fig. 14)



Dark reaction: $2(\text{NADPH} + \text{H}^+) + \text{CO}_2 \rightarrow \{\text{CH}_2\text{O}\} + 2\text{NADP}^+ + \text{H}_2\text{O}$ (energy driven by ATP)

The photosystems are collectives of pigment molecules (ca. 200), mainly chlorophyll-a and -b, carotenoids, anthocyanes and xanthophylls. These pigments act as collectors over the complete spectrum of the (visible) sun light. The energy thus collected is transferred to the reaction centres, which represent specific molecules of chlorophyll-a, termed P_{680} in PSII, and P_{700} in PSI; see Fig. 15.

The **water oxidase** (oxygen evolving centre, OEC), which catalyses the oxidation of water via P_{680} , contains 5 metal centres at its active site. Four of these metal ions (3 $Mn^{3+/4+}$ and one Ca^{2+}) form, together with 4 O^{2-} , a cubane-like cluster (Fig. 16). In Fig. 16, the (assumed) catalytic process is also shown. During turn-over, the four manganese centres change between the oxidation states +IV to +III step by step in the 4-electron oxidation of 2 molecules of water.

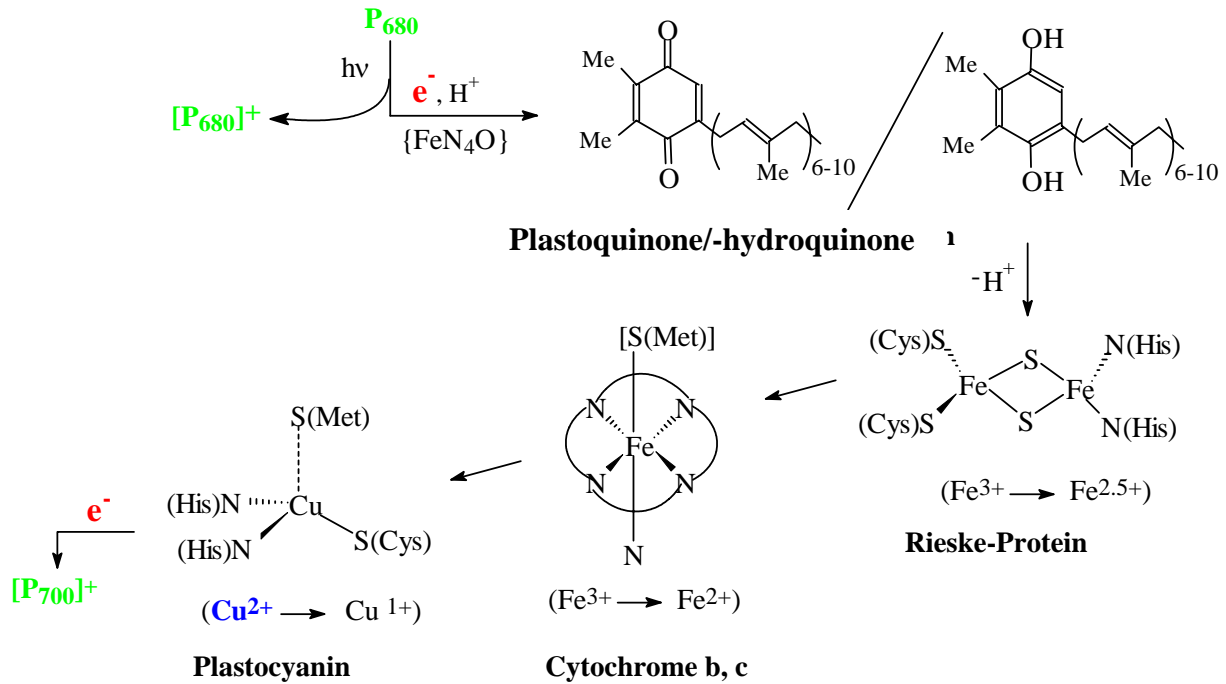


Figure 14. Simplified representation of the electron transport chain between PSII and PSI.

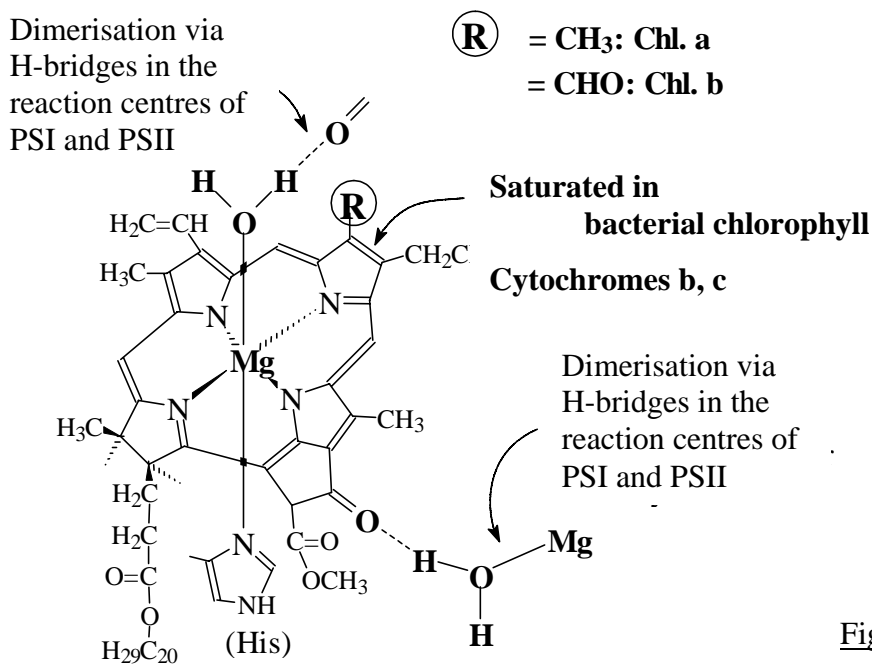


Figure 15: Chlorophylls

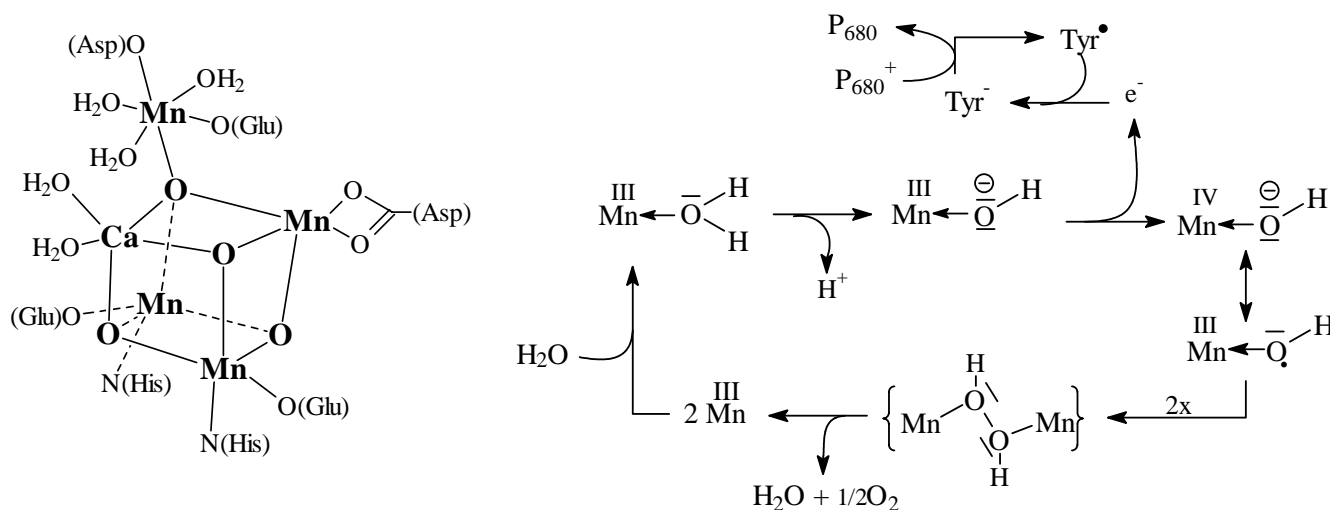


Figure 16. Organisation of the metal cluster in the oxygen evolving centre (left), and the presumed catalytic process of water oxidation (right). Tyr[•] = tyrosyl radical, Tyr⁻ = tyrosinate.

Plastocyanin, at the end of the electron transfer chain between PSII and PSI belongs to the category of 'blue copper proteins', or type I Cu proteins. In these Cu proteins, Cu^{1+/2+} is coordinated in a flat trigonal-bipyramidal fashion by two Cys⁻ and one His, and – in the axial position at a rather long distance of 2.9 Å – methionine. The intense blue colour of the oxidised (Cu²⁺, d⁹) form comes about by a ligand-to-metal charge transfer (LMCT), i.e. transfer of electron density from non-bonding orbitals of the coordinated Cys-S into the 3d 'hole' of Cu²⁺. While charge transfer within the d-d system of a metal ion is parity (Laport) forbidden, and the corresponding absorption bands hence are weak in intensity (see, e.g., [Cu(H₂O)₆]²⁺), LMCT transitions are allowed and thus very intense.

Disgression: Systematics of copper proteins

Type I (Blue Cu-proteins): trigonal coordination geometry; ligands: 2 Cys(1-), 1 His, 1 weakly bonded Met. Strong LMCT at 600 nm; small EPR-spectroscopic hyperfine coupling constant ($A = 5 \cdot 10^{-4} \text{ cm}^{-1}$). Function: e⁻ transfer; Example: Plastocyanin in the e⁻ transfer chain PSII→PSI.

Type II: Tetragonal coordination geometry; ligands: His, Tyr(1-), H₂O, no Cys. "Normal" optical behaviour (d-d transitions); normal EPR patterns ($A = 18 \cdot 10^{-4} \text{ cm}^{-1}$). Function: Redox reactions; Examples: Galactoseoxidase (RCH₂OH → RCHO + 2H⁺ + 2e⁻), CuZn-superoxidedismutase (2O₂⁻ + 2H⁺ → O₂ + H₂O₂).

Type III: Contain 2 cooperating Cu centres; trigonal coordination geometry; ligands: 3 His per Cu. Intensely blue in the oxidised form (O₂²⁻→Cu²⁺ LMCT); no EPR signal (antiferromagnetically coupled). Function: Transport and transfer (to a substrate) of oxygen; examples: haemocyanin, Fig. 8; tyrosinase (Tyr + 1/2O₂ + 2e⁻ → DOPA).

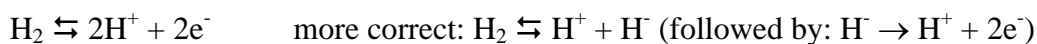
Ceruloplasmin, important for the absorption of iron, is a Cu protein containing 7 Cu centres representing types I, II and III. **Nitritoreductase** contains type II (substrate activation) and type I Cu centres (e⁻ transfer)

Others: e.g. Cu^A und Cu^B in cytochrom-c oxidase; Fig. 13.

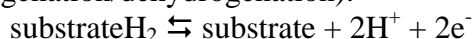
6. Hydrogenases, oxigenases, oxidoreductases, peroxidases and dismutases

Overview

Hydrogenases (often associated with the cofactors NADH or FADH₂)

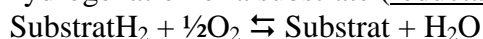


May be coupled with the transfer/abstraction of hydrogen to/from a substrate (hydrogenation/dehydrogenation):

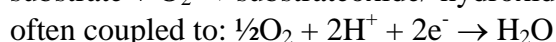


Oxidoreductases generally catalyse oxidations (electron abstraction) and/or reductions (electron delivery), such as the iron-sulphur proteins or the cytochromes.

Some oxidoreductases use oxygen for the dehydrogenation of a substrate (oxidases) or water for the hydrogenation of a substrate (reductases):

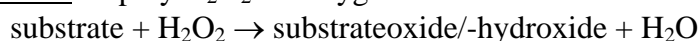


Oxigenases transfer/insert, usually starting from oxygen O₂, oxo groups (O²⁻) to/into a substrate:

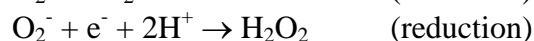
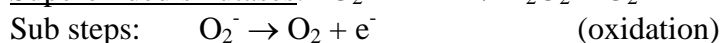
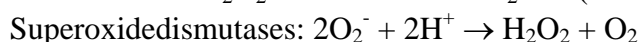
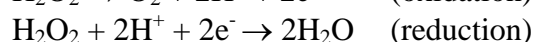
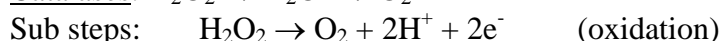
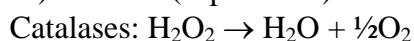


The reverse process, i.e. the removal of O²⁻ from a substrate, is catalysed by deoxygenases. Substrates can be organic in nature (RH → ROH; (CH₃)₂S → (CH₃)₂S=O), or inorganic (NO₃⁻ → NO₂⁻).

Peroxidasen employ H₂O₂ for oxygenation:

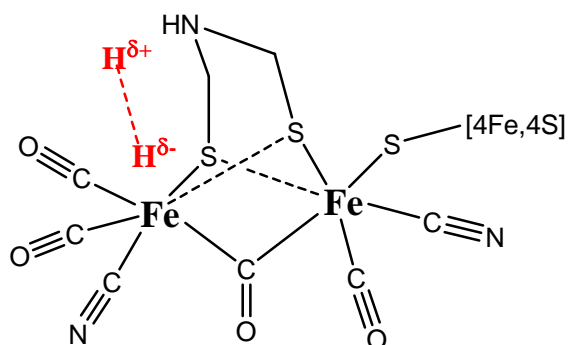


Dismutases disproportionate oxygen species with the oxygen in the oxidation states -I (peroxide) and -1/2 (superoxide):



Iron-only hydrogenase

This enzyme catalyses the charge separation in the H₂ molecule via polarisation between the NH function of the bridging aminedithiolate and one of the iron centres, and finally the bond cleavage to form a hydridic Fe-H⁻ and a protic R₂NH₂⁺ intermediate. Electrons from the H⁻ are then transferred off via a [4Fe,4S] ferredoxins in direct contact with the hydrogenase.



Nitritoreductase

This enzyme catalyses the deoxygenation of nitrite NO_2^- to nitrogenmonoxide NO via a one-electron reduction, one of the focal steps in dinitrification (see ch. 7). The enzyme is built up of three identical subunits. Each of these subunits contains a catalytic type-II Cu centre (for the activation of nitrite) and a type-I Cu centre for electron transfer (reduction of nitrite) [see inset on p. 20 for the classification of copper enzymes). Reaction steps (cf. Fig. 17):

- (1) Exchange of water for nitrite/nitrite activation (at {Cu-II})
- (2) Formation of nitrosyl: $\text{NO}_2^- + 2\text{H}^+ \rightarrow \text{NO}^+ + \text{H}_2\text{O}$
- (3) $\text{NO}^+ + \text{e}^- \rightarrow \text{NO}$ (by {Cu-I})
- (4) Reestablishment of the starting situation by exchange of NO for H_2O

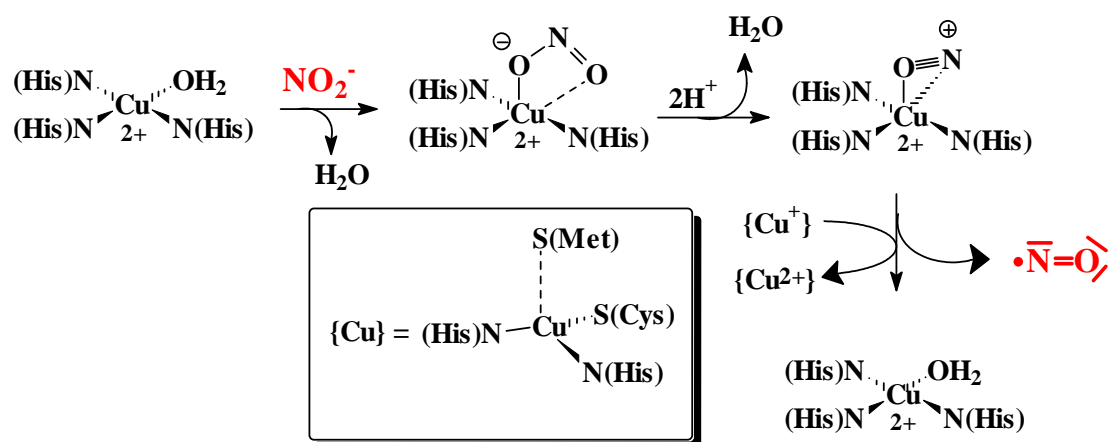
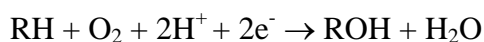


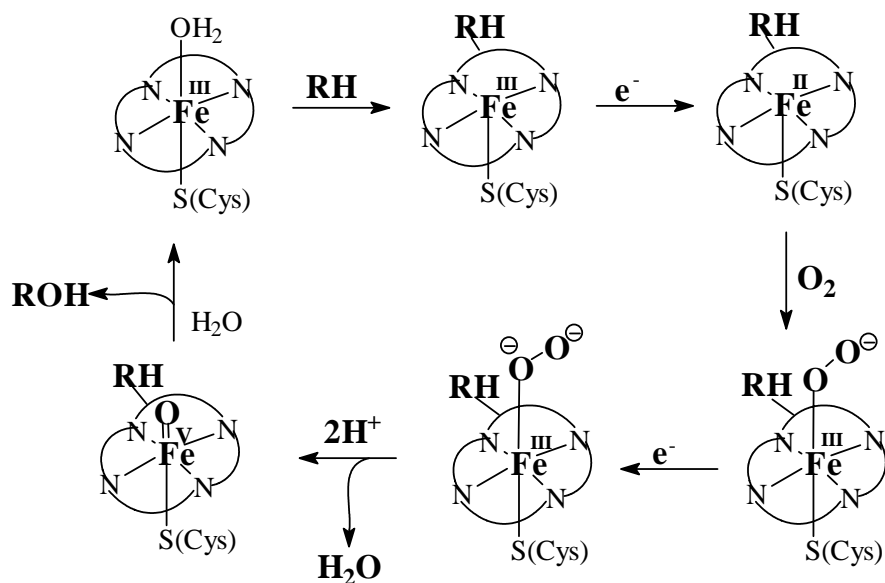
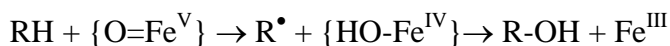
Figure 17. The course of reaction catalysed by nitritoreductase.

Oxygenases

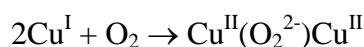
Cytochrome P₄₅₀ is an oxygenase belonging to the haeme-type proteins. Axial ligands are a cysteinate and – in the resting state – water. In the active state, the position occupied by water is free. Cyt-P₄₅₀ detoxifies hydrophobic substrates (such as benzene) in the liver by conversion to hydrophilic compounds (such as phenol) which are then secreted. The overall reaction can be formulated as shown:



The substrate RH is bonded by hydrophobic interaction into the protein pocket close to the active centre of the active form of the enzyme. The course of reaction is illustrated in Fig. 18. In the first step, Fe^{III} is reduced to Fe^{II} , followed by oxidative addition of O_2 ($\text{Fe}^{\text{II}} + \text{O}_2 \rightarrow \text{Fe}^{\text{III}}-\text{O}_2^-$), i.e. O_2 is reduced to superoxide, and further – by an external e^- source – to peroxide: $\text{Fe}^{\text{III}}-\text{O}_2^- + \text{e}^- \rightarrow \text{Fe}^{\text{III}}-\text{O}_2^{2-}$. In the succeeding step, Fe^{III} transfers 2 electrons to the peroxo ligand. One of the oxo groups is released and trapped by two protons to form water. The other oxo group remains coordinated to iron. The intermediate thus formed can be formulated with Fe^{V} or Fe^{IV} : $\{\text{O}=\text{Fe}^{\text{V}}\} \leftrightarrow \{\text{O}=\text{Fe}^{\text{IV}}\}^{*+}$, in the latter formulation (with Fe^{IV}) as a radical cation, with the radical character being dislocated over the oxygen and part of the protein matrix. In the final step, a hydrogen atom of the substrate is transferred to the ferryl oxygen, and the $\{\text{OH}\}$ transferred back to the substrate radical:



Tyrosinase and catecholoxidase: These two closely related enzymes contain type III copper centres (see inset on p. 20) and thus resemble the haemocyanins (ch. 3). The homology of the amino acid sequence is, however, restricted to the direct surroundings of the copper centres. Activation of oxygen by tyrosinase and catecholoxidase compares to that of haemocyanin, except that is irreversible:



One of the histidine ligands on one of the Cu centres in tyrosinase can be weakened to allow for attachment of the substrate tyrosine. Tyrosinase catalyses the oxygenation of tyrosine to dopa (*o*-**dihydroxyphenylalanine**; precursor for the neurotransmitter dopamine, and for adrenaline), catecholoxidase further oxidises dopa to the respective quinone (Fig. 18). These reactions are followed by further dehydrogenation to form indolquinone and finally melanin. Melanin, a very complex compound, is the dark pigment formed as freshly broken fruits (like apples or bananas) or vegetable (like potatoes) are exposed to air. Melanin is also the dark pigment responsible for the suntan, or present in the brown beauty patches and in melanomas.

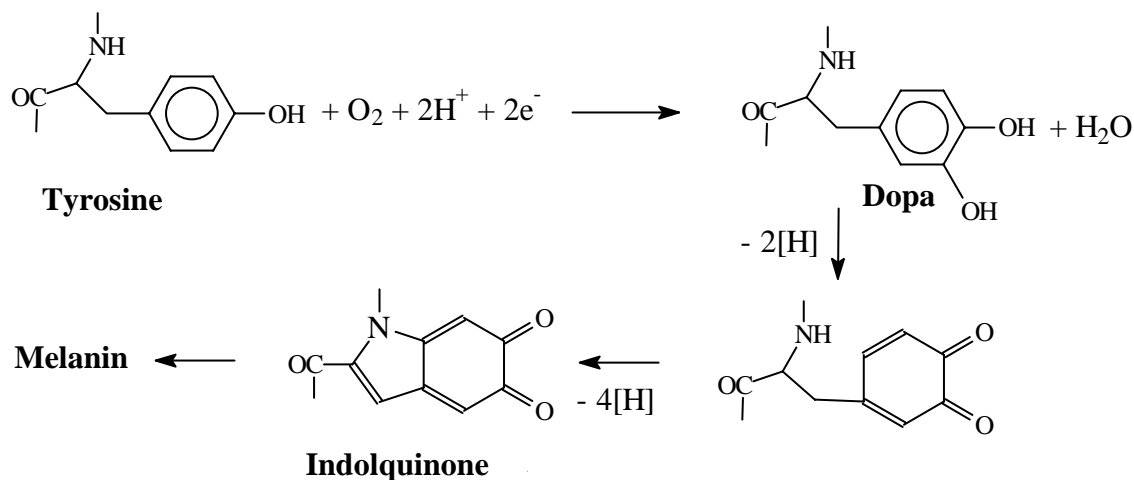


Figure 18. Reactions which are catalysed by tyrosinase/catecholase.

Oxygenases/deoxygenases containing the molybdopterin cofactor, Fig. 19.

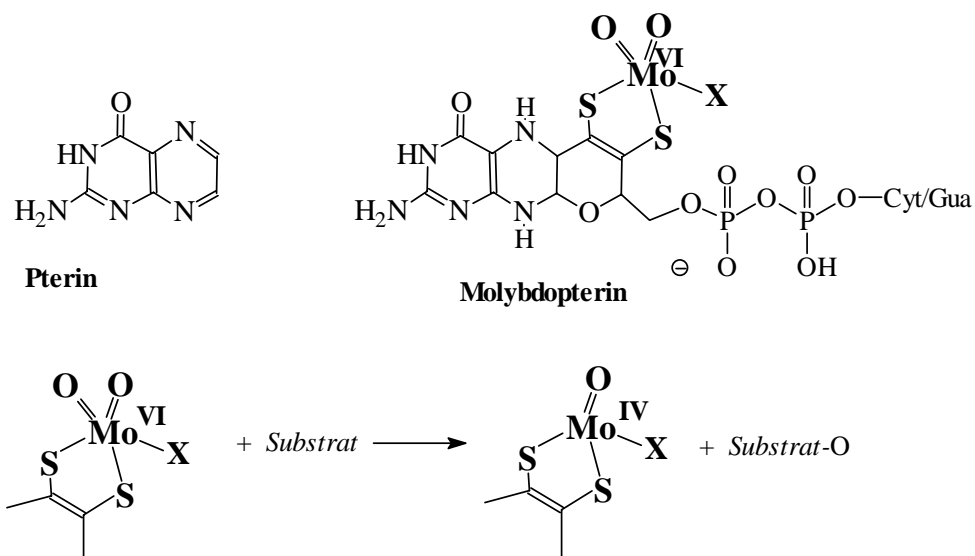
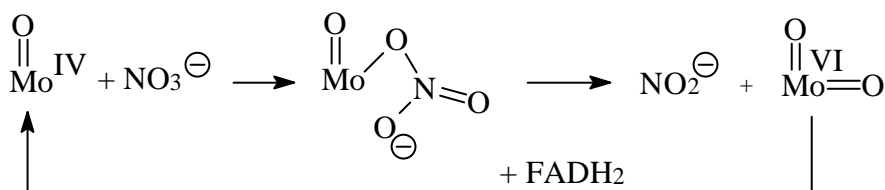
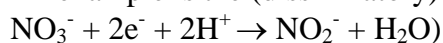


Figure 19. Oxidised form of the molybdopterin cofactor (upper right) of the sulphite reductase family. X usually is Cys⁻. Molybdopterin transfers oxido groups to a substrate (shown) or off a substrate.

An example is the (dissimilatory) **nitrate reductase**:



Vanadate-dependent haloperoxidases from marine algae catalyse the oxidation of halide (Hal⁻) to a Hal⁺ species such as hypohalous acid. Oxidant is hydrogen peroxide. The Hal⁺ species halogenates organic substrates non-enzymatically. For the mechanism, see Fig. 20.

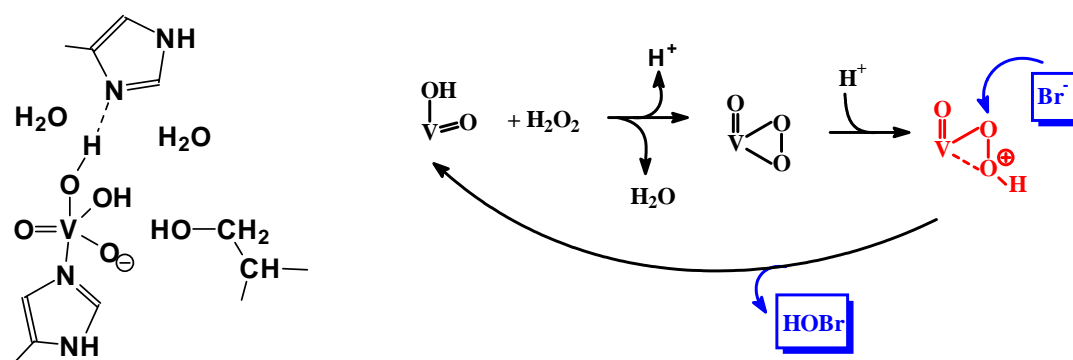
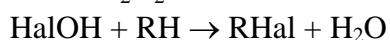
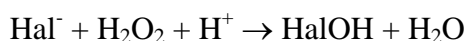


Figure 20. Active centre of vanadate-dependent haloperoxidase (left), and mechanism of the enzymatic formation of hypobromous acid from bromide (right).

Cu,Zn-Superoxidedismutase contains a catalytically active type-I copper centre and a structural zinc centre, linked by bridging His⁻; Fig. 21. The enzyme catalyses the disproportionation (dismutation) of superoxide to hydrogenperoxide and oxygen.

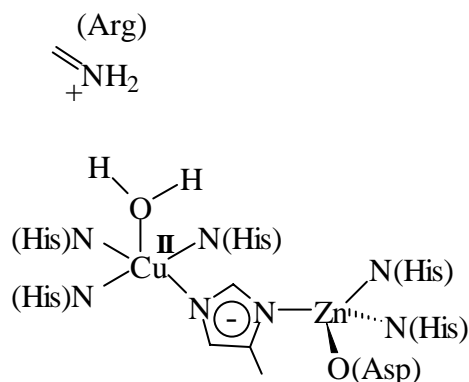
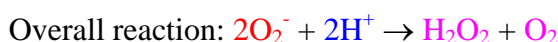


Figure 21. Active centre of Cu,Zn-superoxidedismutase



Reaction sequence:

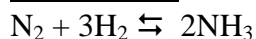
- (1) $\text{Cu}^{\text{II}}(\text{H}_2\text{O}) + \text{O}_2^- \rightarrow \text{Cu}^{\text{II}}(\text{O}_2^-) + \text{H}_2\text{O}$ (exchange of water for hyperoxide)
- (2) $\text{Cu}^{\text{II}}(\text{O}_2^-) + \text{O}_2^- \rightarrow \text{Cu}^{\text{I}}(\text{O}_2^-) + \text{O}_2$ (oxidation of external O_2^- to O_2)
- (3) $\text{Cu}^{\text{I}}(\text{O}_2^-) + \text{H}^+ \rightarrow \text{Cu}^{\text{II}}(\text{HO}_2^-)$ (internal reduction of O_2^- to peroxide at the Cu centre)
- (4) $\text{Cu}^{\text{II}}(\text{HO}_2^-) + \text{H}^+ + \text{H}_2\text{O} \rightarrow \text{Cu}^{\text{II}}(\text{H}_2\text{O}) + \text{H}_2\text{O}_2$ (separation of hydrogenperoxide)

7. Nitrogen fixation

Nitrogen fixation is the biogenic and non-biogenic transformation of elemental N_2 into nitrogen compounds, affording to overcome the bonding energy between the two trebly bonded nitrogen atoms (949 kJ/mol). The biogenic fixation, carried out by free living nitrogen-fixing bacteria (*Azotobacter*) and cyanobacteria (“blue-green algae”, *Anabaena*), some archaea, and by symbiotic bacteria associated with leguminous plants (*Rhizobium*), leads to ammonium ions. Biogenic fixation accounts for about 60% of the overall nitrogen supply. Non-biogenic non-anthropogenic fixation, which can occur by electric discharge (lightning) in the troposphere, and by cosmic radiation in the stratosphere [$\text{N}_2 \rightarrow 2\text{N}$; $\text{N} + \text{O}_2 \rightarrow \text{NO} + \text{O} \rightarrow \text{NO}_x$], accounts for 10%. For the overall conversions cf. Fig. 22. The remaining 30% of worldwide N_2 fixation go back to the Haber-Bosch process and combustion of fossil fuels (natural gas, coal, crude oil) and products produced from crude oil (petrol, gasoline, diesel).

Comparison between the Haber-Bosch process and the biogenic N_2 fixation:

Haber-Bosch



Temperature: 500 °C

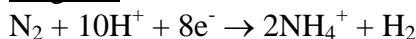
Pressure: 200-450 bar

Catalyst: Fe (+ Al_2O_3 + K_2O + ...)

Yield: 17%

Annual production ca. 10^8 t

biogenic



(energy driven by: $16\text{ATP} + 16\text{H}_2\text{O} \rightarrow 16\text{ADP} + 16\text{P}_i$)

Temperature: ca. 20 °C

Pressure: 1 bar

Catalyst: Nitrogenase (Fe/Mo- or Fe/V-S cluster)

Yield: 75%

Annual production ca. 10^8 t

Nitrogen cycle

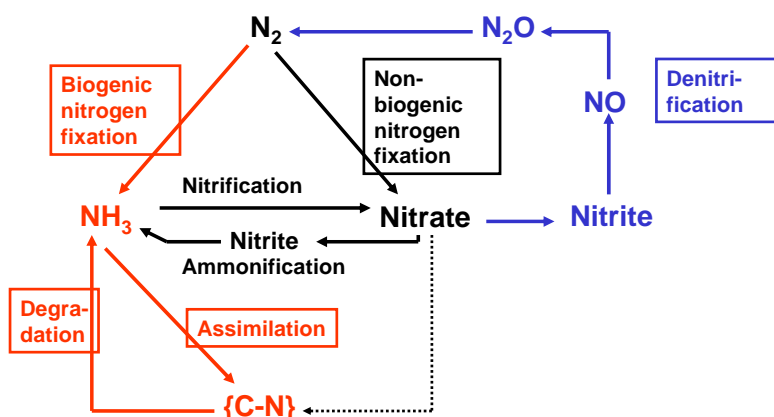


Figure 22: The nitrogen cycle. Processes involving the $-III$ oxidation state of nitrogen are in red. For nitrite and nitrate reductases see the previous chapter

In Fig. 23, the organisation of the metal centres of nitrogenase is depicted. The electrons necessary for the reduction of dinitrogen are delivered by an iron protein containing a cubane-like $[4Fe,4S]$ ferredoxin. Primary e^- acceptor is the P cluster of the $FeMoCo$, the iron-molybdenum-cofactor. Two ATP (activated by Mg^{2+}) are hydrolysed per electron transferred. The $FeMoCo$ contains two P and two M clusters, arranged in such a way that the complete cofactor attains C_2 symmetry. The P cluster is a double cubane containing the Fe_8S_7 core. The reduction equivalents are then further transported to the M cluster, a Fe_7MoS_9 core, which is responsible for the final activation and reduction of N_2 . The cage formed by the metal centres of the M cluster contains electron density which can be interpreted in terms of a nitrogen atom. The M cluster is connected to the protein matrix by just one Cys and a His, the latter coordinated to Mo. The coordination environment of Mo is supplemented by the vicinal hydroxide and carboxylate of homocitrate. In which way activation and reduction of N_2 takes place is unknown. In the case of insufficient molybdenum supply, or at low temperatures, a vanadium-nitrogenase is activated (which is more efficient at lower temperatures than the Mo version). An iron-only nitrogenases is also known.

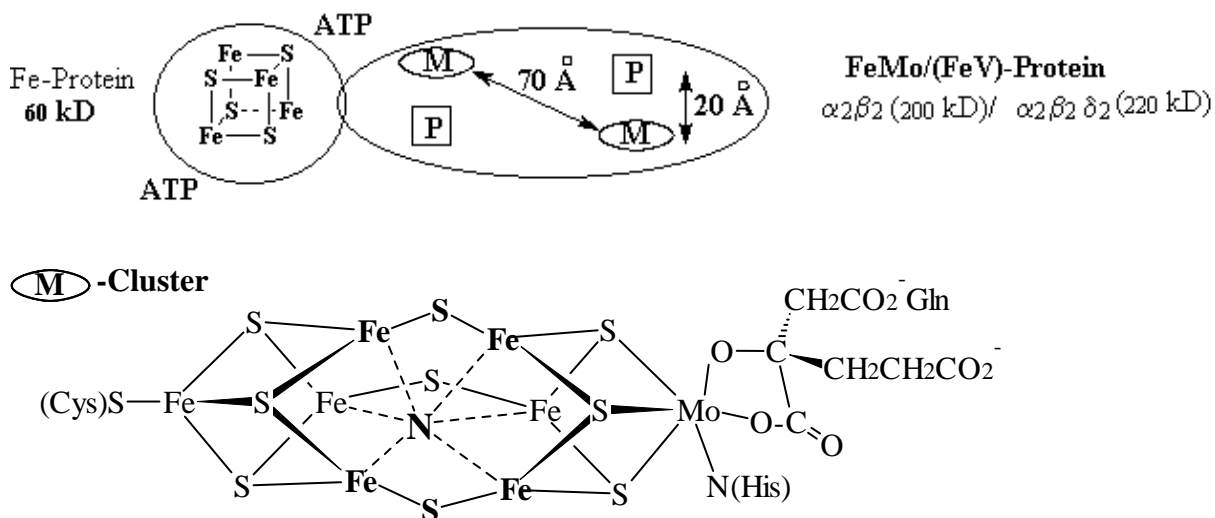


Figure 23. Organisation of nitrogenase (top), and the structure of the M cluster (bottom).

Tutorial: Nitrogen

Abundance: Atmosphere (in the form of N_2 , 78.1 Vol-%; $4 \cdot 10^{15}$ t); hydrosphere (N_2 dissolved in water, 10^{12} t); in minerals (saltpetre $NaNO_3$) and rocks ($2 \cdot 10^{17}$ t); in organic form in the biomass of soil-bound microorganisms ($3 \cdot 10^{11}$ t), plants and animals (10^{10} t).

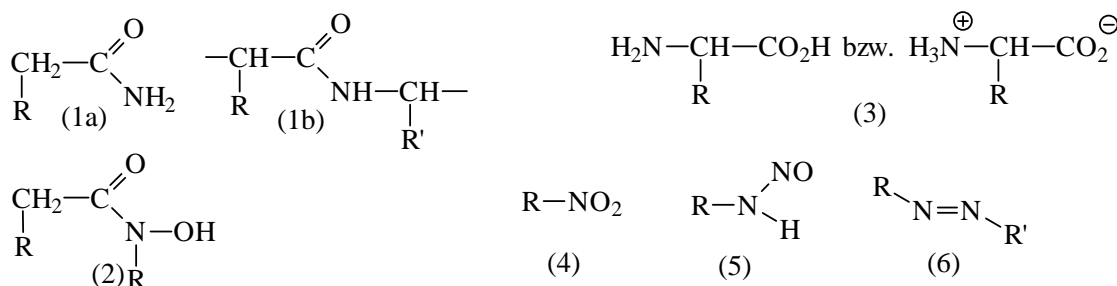
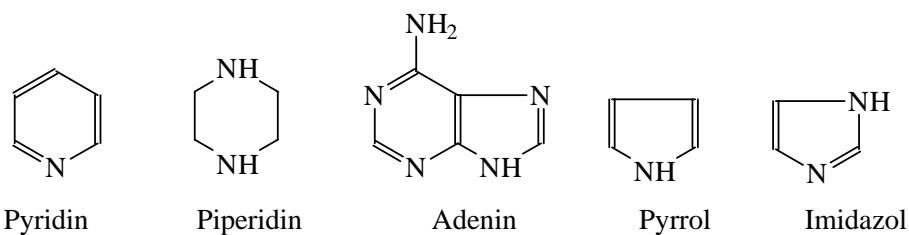
The bond in dinitrogen is a triple bond; the bond energy amounts to 949 kJ/mol, i.e. N_2 is particularly inert.

Hydrogen compounds: NH_3 (ammonia; synthesis from H_2 and N_2 according to the Haber-Bosch process) and ammonium ions (NH_4^+), N_2H_4 (hydrazine), HN_3 and salts derived thereof (azides, e.g. NaN_3 , commonly used as fungicide and bactericide in bio-assays). Nitrides, e.g. Na_3N , formally derive from ammonia. Ammonia is an efficient complexing agent, e.g. for Ag^+ : $AgCl$ dissolved in aqueous NH_3 forms soluble $[Ag(NH_3)_2]^+$, which gradually converts to silver nitride Ag_3N (highly explosive). The ammonium ion is a Brønsted acid; aqueous solutions of ammonium salts consequently are acidic.

Oxygen compounds: N_2O (dinitrogen monoxide, “laughing gas”), NO (nitrogen monoxide; synthesis by combustion of ammonia according to the Ostwald process), NO_2 (nitrogen dioxide, in equilibrium with N_2O_4 . NO_2 reacts with water to form nitrous acid HNO_2 + nitric acid HNO_3), N_2O_5 (dinitrogen pentoxide). Salts derived from HNO_3 are termed nitrates, those derived from HNO_2 nitrites.

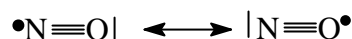
Use: Fertilisers (ammonium compounds, nitrates), explosives (nitrate; gun powder is a mixture of saltpetre, charcoal and flower of sulphur). HNO_3 is used for nitrosylations in organic synthesis.

Organic nitrogen compounds: Amines (NH_2R , R = phenyl: aniline; NHR_2 ; NR_3), heterocyclic nitrogen compounds (for a selection see below), amides (1a) and peptide (1b), hydroxamic acids (2), amino acids (3), nitro compounds (4), nitrosamines (5), diazo compounds (6).

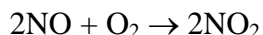
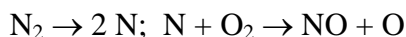


Other nitrogen compounds: Cyanide CN^- , cyanate NCO^- and thiocyanate NCS^- (can be formed in metabolic processes and coordinate to transition metal ions). Cyanide in particular is toxic, but may also occur as ligand in enzymes (iron-only hydrogenase). Amides of carbonic acid: carbamate, e.g. $NH_4^+(CO_2NH_2)^-$ = sal volatile, and urea $O=C(NH_2)_2$.

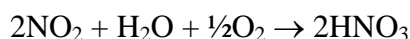
8. Nitrogenmonoxide



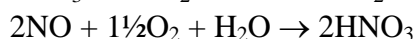
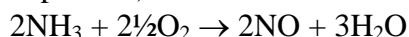
NO forms in the troposphere by electric discharges, and under stratospheric conditions under the influence of cosmic rays and high-energy UV. Further oxidation to NO₂ readily occurs:



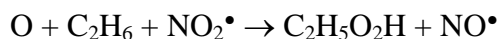
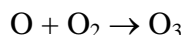
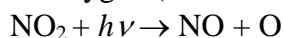
With additional oxygen and moisture, nitric acid is formed, one of the constituents of "acid rain":



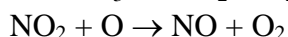
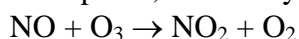
Industrially, NO is obtained by passing a mixture of ammonia and oxygen through a platinum net (contact time 10⁻³ s, temperature 1000 °C), which is further processed to form nitric acid (Ostwald process):



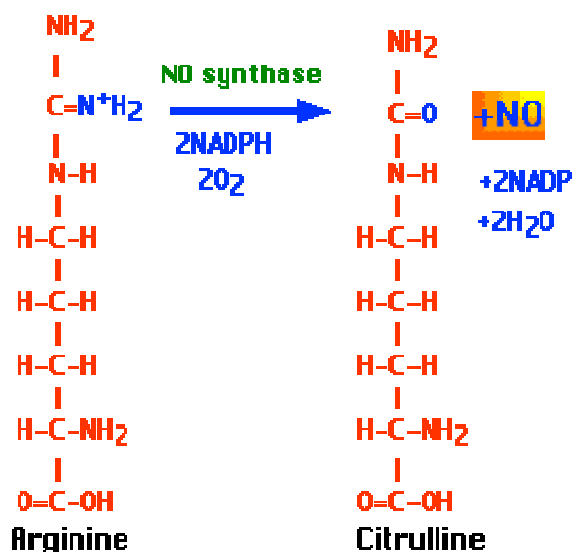
A large amount of HNO₃ goes into the production of ammonium nitrate for artificial fertiliser. NO is also contained in the exhaust gases of automobiles (along with water and CO₂ as the main components, and fuel constituents), as well as in industrial and domestic exhaust, and rapidly is oxidised to NO₂. Under the influence of UV, i.e. on sunny days, NO₂ is split into NO and oxygen atoms, which oxidise alkanes to alkylhydroperoxides, and form ozone with molecular oxygen ("summer smog"):



In the stratosphere, NO catalyses ozone depletion (see also the tutorial 'oxygen' on p. 11):



In organisms, NO is an important multi-functional messenger and neurotransmitter, targeting, inter alia, metal centres in haeme-type proteins, and cyclic guanosine-monophosphate (cGMPase). Biosynthesis of NO is achieved by oxidation of one of the NH₂ groups of arginine, catalysed by a NO-synthase (NOS), thereby converting Arg via hydroxyarginine to citrulline; see the illustration to the right. Three functionally different NOS are known: (1) nNOS, in the neurons, initiates signal transduction and thus takes part in mnemonic functions; (2) iNOS, in macrophages, induces the liberation of NO in case of infections and



thus participates – as a killer agent for infectious germs – in the functioning of the immune system; (3) eNOS, in the endothelial tissue cells, where it controls the tonicity of the vascular muscles and thus the blood pressure. The NO-induced relaxation of the vascular muscles is also the basis for the medication of hypertension and angina pectoris with compounds which set free NO under physiological conditions, such as amylnitrite, (C₅H₁₁NO₂); nitroglycerin (glyceroltrinitrate CH₂(ONO₂)-CH(ONO₂)-CH₂(ONO₂)) and nitroprussid sodium (disodium pentacyanido-nitrosylferrate Na₂[Fe(NO)(CN)₅]).

NO is also used by the glow worm (lightning bug, firefly) to switch on its glow organs. This luminescence can be traced back to the oxidation, by O₂, of luciferyl-AMP (AMP = adenosine-monophosphate) via peroxoluciferin to oxiluciferin; cf. Fig. 24. To start this oxygen consuming formation of peroxoluciferin, the glow worm triggers NO synthesis. The NO is used to block mitochondrial cytochromes (by coordination of NO to Fe) and thus the consumption of oxygen in respiration. The O₂ thus becomes available for triggering luminescence. Other organisms capable of bioluminescence also employ this mechanism. An example is *Nocticula scintillans*, a dinoflagellate responsible for marine phosphorescence.

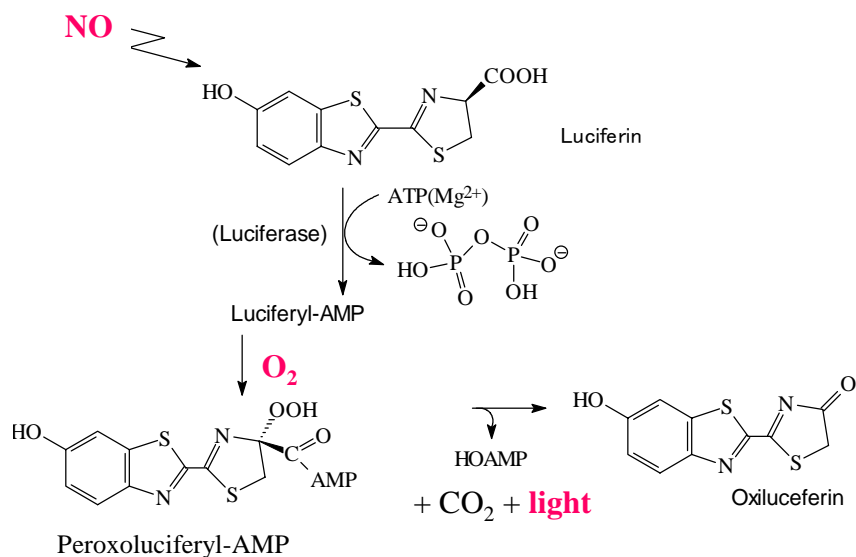
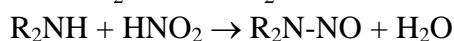


Figure 24. NO induced luminescence in the glow worm.

Larger amounts of NO are toxic, because NO binds to the iron and copper centres of enzymes depending on these metals. Haemoglobin binds NO ten-thousand fold more effective than O₂. On coordination, NO is reduced to NO⁻, which is isoelectronic with O₂ and binds, as O₂, in the bent end-on mode. Additional NO toxicity arises from the fact that NO nitrosylates amines, via the intermediate formation of nitrous acid, to form carcinogenic nitrosamines:



(Nitrous acid is also formed, under physiological conditions, by reduction of nitrate, present in e.g. leafy vegetables.)

9. The biochemistry of zinc

2.5 g of zinc per 70 kg body weight makes Zn the second-to-most abundant transition metal of biological importance. Contrasting iron, copper, manganese and molybdenum, zinc is not redox active (valence electron configuration d¹⁰). In zinc proteins, Zn²⁺ takes over either a

catalytic or a structural function; see the classification below. The daily requirement for zinc is 3-25 mg, a demand which, since zinc is ubiquitous, is commonly satisfied by our nutrients. Diseases due to zinc deficiency encompass disturbance of growth, arthritis-related health problems, break-down of the immune system and loss of taste. They are usually a consequence of impaired zinc absorption rather than of undersupply. In the blood stream, zinc is transported by albumin and transferrin. Zinc storage is achieved by thioneins (vide infra).

Interlude: Inorganic chemistry of zinc

Important minerals: ZnS (zinc blende, wurtzite, sphalerite), ZnCO₃ (zinc spar, calamine). Earth's outer sphere (17 km crust + hydro- + atmosphere) contains 0.007 % (by weight) of zinc.

Metallic zinc is obtained by firing of ZnS (\rightarrow ZnO + SO₂) followed by reduction of ZnO with coal, or by electrolytic reduction of aqueous zinc sulphate. Applications include alloys (such as brass, a Cu-Zn alloy), galvanisation (of iron), torch batteries (Lechlanché element).

The redox potential is $E^0 = -0.763$ V, i.e. Zn is oxidised by H⁺. In air, Zn is, however, stable due to passivation [formation of compact layers consisting of ZnO, Zn(OH)₂ and Zn(OH)(HCO₃)]. In aqueous media, Zn²⁺ exists in the form of the Brønstedt acid [Zn(H₂O)₆]²⁺; Zn²⁺ itself is a Lewis acid (and this is determinant for its enzymatic actions). Zinc hydroxide is amphoteric: Zn(OH)₂ + 2H⁺ \rightarrow Zn²⁺ + 2H₂O; Zn(OH)₂ + 2OH⁻ \rightarrow [Zn(OH)₄]²⁻. With halogenated alkanes RX, zinc forms reagents of composition RZnX, which transform to ZnX₂ and ZnR₂ on heating. RZnX and ZnR₂ are alkylating agents.

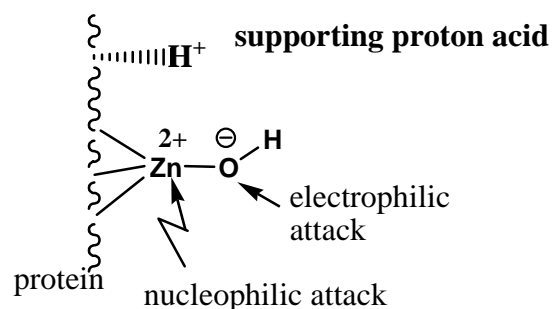
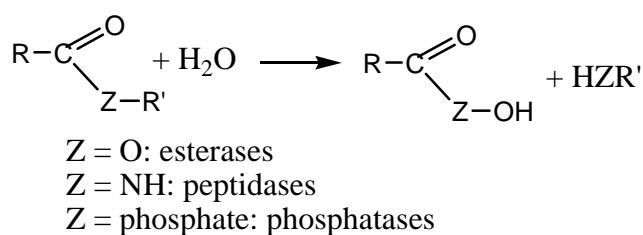
Zn²⁺ forms complexes mainly of coordination numbers 4 ([Zn(SR)₄]²⁻, tetrahedral; [Zn(Gly)₂], square planar), 5 ([Zn(acac)₂H₂O] [acac = acetylacetonate(1-), square-pyramidal] and 6 (octahedral).

Ointments containing zinc (in the form of ZnO, Zn(OH)₂, zinclactate) have already been employed in the Middle Age and are still employed today in wound healing. The essentiality of zinc for life had been discovered in 1869 by Raulin (inhibition of the growth of the mould *Aspergillus niger* caused by undersupply of zinc). In 1940, the first zinc-dependent enzyme, carboanhydrase, was isolated by Keilin and Mann, followed by the discovery, in 1954, of the second enzyme, pancreatic bovine carboxypeptidase-A. In 1985, the role of zinc in genetic transcription ("zinc fingers") became established, and in 1995, the Zn-based *Ada* repair protein (demethylation of DNA) was characterised. A role of Zn²⁺ in synaptic transmission was found in 2006.

Classification of zinc proteins according to function:

1. Enzymatic:

- Ligases and synthases (C-C-bond formation, e.g. aldolases)
- Hydrolases: Here, Zn²⁺ is coordinated by three amino acid side-chains of the protein matrix (His, Cys and/or Asp) plus water (inactive, resting form) or a hydroxy group (active form); see the scheme below. Examples: carboxypeptidase-A (an exopeptidase acting at the C-terminus of the peptide), thermolysin (a thermophilic exopeptidase acting at the N-terminus).



- Others: Carboanhydrase ($\text{CO}_2 + \text{OH}^- \rightleftharpoons \text{HCO}_3^-$; ch. 3 and Fig. 7)
Alcoholdehydrogenase ($\text{RCH}_2\text{OH} \rightarrow \text{RCHO} + 2\text{H}^+ + 2\text{e}^-$; see below)

Structural: Stabilisation of the tertiary structure of protein domains in enzymes (e.g. Cu,Zn superoxide dismutase, alcoholdehydrogenase, cytochrome-c oxidase). Here, Zn^{2+} is tetrahedrally coordinated by four amino acid residues.

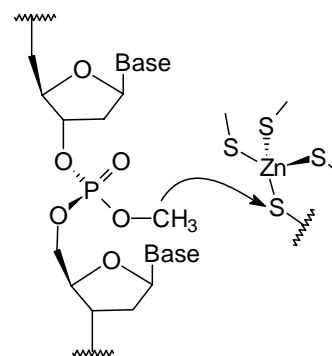
Stabilisation of quaternary structures, e.g. of the hexameric storage form of the peptide hormone insulin, where the monomers are linked by $[\text{Zn}(\text{His})_3(\text{H}_2\text{O})_3]^{2+}$.

Zinc finger are constituents of numerous genetic transcription factors. Coordination mode typically is $[\text{Zn}(\text{Cys})_2(\text{His})_2]$.

Ada repair protein: Repairs (by de-methylation) methylated phosphate linkers in DNA by transfer of the methyl group to cysteinate coordinated to Zn^{2+} .

Thioneins: Storage of Zn^{2+} /storage of cysteine; detoxification of Cd^{2+} and other thiophilic metal ions; mode of coordination: $[\text{Zn}(\text{Cys})_4]^{2-}$.

Synaptic transmission: Zinc ions modulate the synaptic activity of brain cells that use glycine as a neurotransmitter.

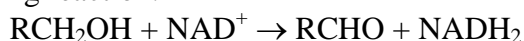


Examples for enzymes

(for carbonic anhydrase, see ch. 3)

Carboxypeptidase A (from bovine pancreas), is an exo-peptidase of molecular weight 36.4 kDa, which breaks down peptides from their C-terminus. The substrate (the peptide) is activated by coordination of the carbonyl oxygen of the peptide backbone to the Lewis-acid Zn^{2+} centre. For the several steps of the enzymatic reaction see Fig. 25.

Alcoholdehydrogenase is a homodimer of molecular weight 80 kDa, which catalyses the following reaction:



The cofactor NAD is bound by salt interaction and hydrogen bridges close to the active centre. Each subunit contains a catalytically active Zn^{2+} (Cys, H_2O , two His) and a structural Zn^{2+} centre (four Cys). The mechanism of alcohol dehydrogenation is sketched in Fig. 26: The alcohol is bound to and thus activated by Zn^{2+} , and deprotonated by the neighbouring OH^- . The alkoxido ligand thus generated then transfers a hydride to the cofactor NAD^+ , and the carbocation gets stabilised by formation of the aldehyde.

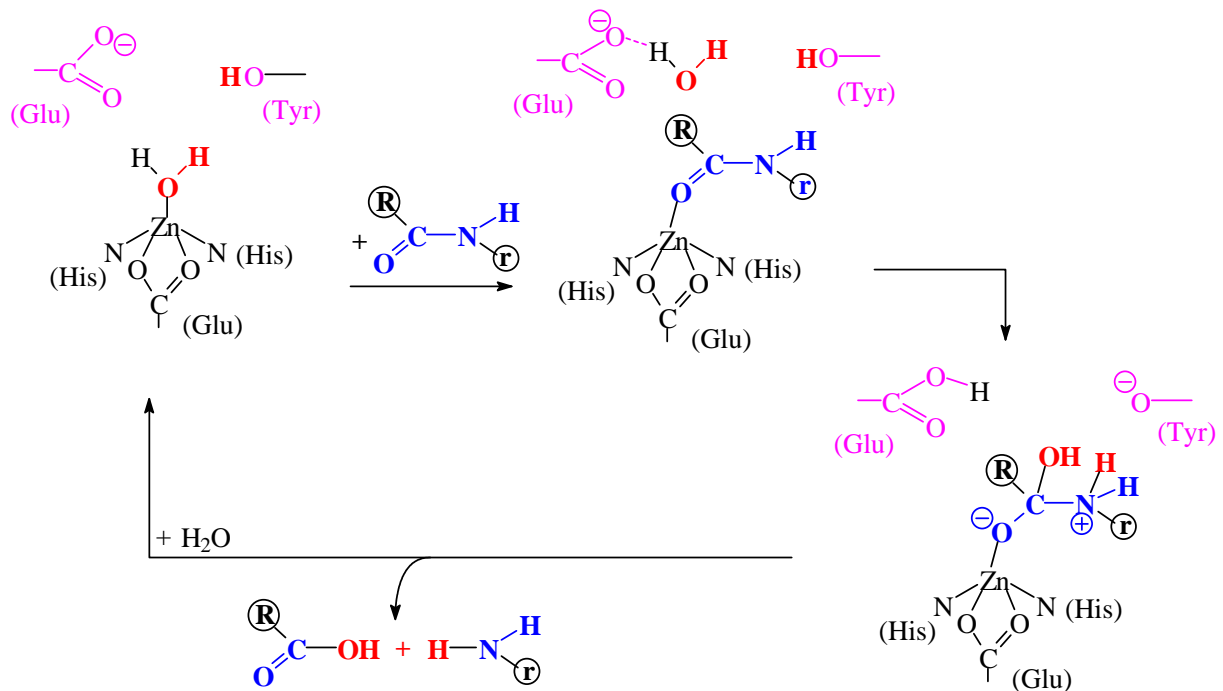


Figure 25. Reaction course of the **peptide hydrolysis** by carboxypeptidase. $\text{r} = -\text{CH}(\text{R}')-\text{CO}_2\text{H}$; R = residual protein.

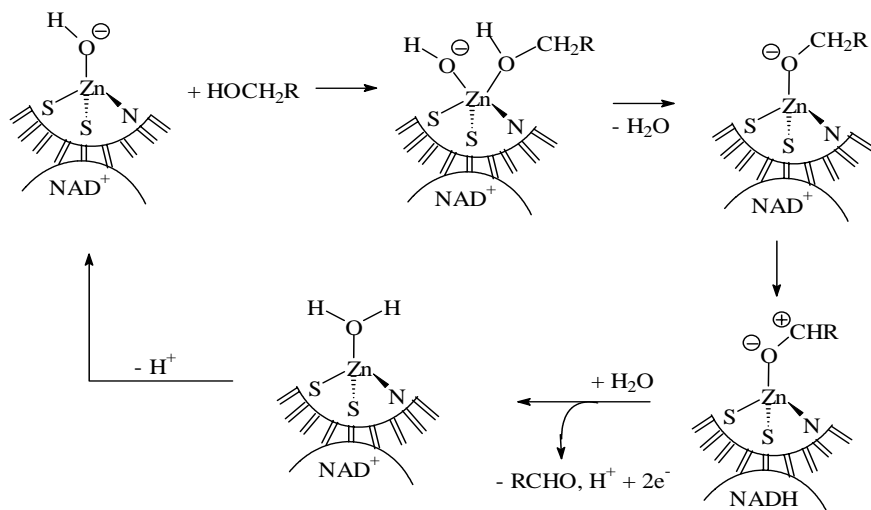
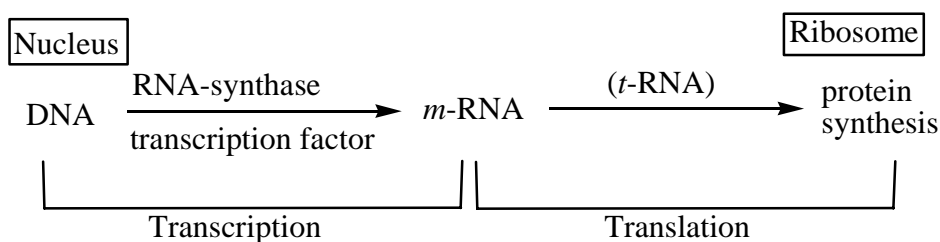
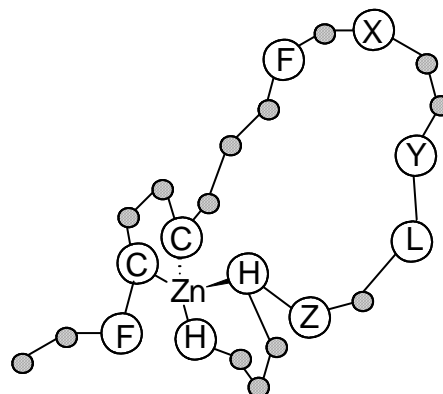


Figure 26. Reaction course of the enzymatic oxidation of primary alcohols by alcohol dehydrogenase.

Zinc and the genetic transcription (Zinc fingers)



The first step of the transcription of the genetic information stored in the DNA into a protein structure occurs in such a way that a complementary messenger-RNA (*mRNA*) is synthesised at a specific DNA segment (carrying this information). An RNA-synthase is needed, and a “pilot“ (the transcription factor) to “direct“ the RNA-synthase to the DNA section in question. This transcription takes place in the nucleus of the cell, while the locus for the protein synthesis, the so-called translation, is the ribosome. In many cases, the transcription factors are Zn^{2+} based, containing the zinc ion tetrahedrally coordinated to typically 2 Cys and 2 His. Zn^{2+} structurally stabilises a loop containing specific amino acid moieties necessary for recognising the DNA site, and thus binding to the respective large groove, where the information for the synthesis of the specific protein is contained. An example is shown to the right: C = Cys(1-), H = His, F = Phe, Y = Tyr, L = Leu, Z = Glx (Glu, Glu(1-) or Gln). Zinc fingers usually contain more than just one domain, which work in tandem for nucleic acid recognition; each domain interacts with three base pairs of DNA.



Thioneines

These are small proteins (ca. 6000 Da; 61 amino acids) with a high percentage of cysteine (ca. 1/3) and serine, and no aromatic amino acids. Thioneines can accommodate up to seven Zn^{2+} or other metal ions and probably serve as zinc and/or cysteines storage proteins. They are further used in the (transient) detoxification of heavy metal ions such as Cd^{2+} and Hg^{2+} Fig. 27 shows the two domains of a common thioneine.

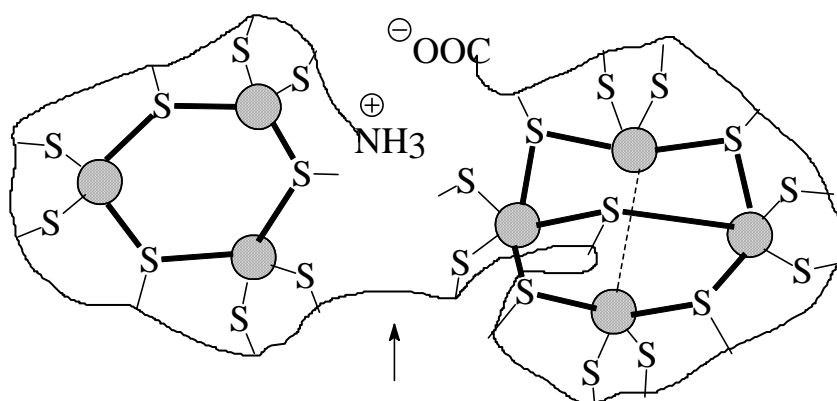


Figure 27. Structure of a $(M^{2+})_7$ thioneines. The metal centres are represented by hatched circles. The solid line represents the protein back-bone, the arrow the point where the protein can be enzymatically split into its two domains. One of the domains contains a cyclic M_3S_3 cluster in the chair conformation, the other domain an adamantane-like M_4S_5 cluster..

10. Cadmium and mercury

The two heavier homologues of zinc, cadmium and mercury, are toxic. The toxicity of Cd^{2+} can be traced back, in part, to its higher thiophilicity, allowing Cd^{2+} to replace zinc in its enzymes. Since Cd^{2+} has a larger ionic radius than Zn^{2+} (see Table), it is sufficiently less Lewis-acid, i.e. a replacement of Zn^{2+} for Cd^{2+} commonly results in a deactivation of the enzyme. An exception is the marine diatom *Thalassiosira weissflogii*, which contains Cd^{2+}

Interlude: Cadmium

Cadmium is contained in small amounts in zinc ores (zinc blende, e.g., contains 0.1-0.5% Cd). Small amounts are also present in shales, in black coal and phosphate minerals such as phosphorite. Since phosphate minerals are used in fertilisers, Cd becomes introduced into farmlands and thus into farming products and nutrients. Cd is used in cadmium-based alloys, as a protective for iron against rusting, in CdS-based yellow to orange pigments, cadmium soaps, and neutron absorbers in nuclear power stations

Coastal sea water can contain up to 30 µg/l Cd. Cd is enriched in sea weeds, sea shells, squids and, occasionally, fish, as well as in mushrooms and leafy vegetables. Other vegetables, wheat and grass accumulate Cd only when grown on contaminated farm land stemming from fertilisers and industrial emissions.

According to the WHO, 70 µg Cd per day are tolerable. About 35 µg are taken up daily via non-contaminated food. Smokers are subjected to an additional uptake of ca. 35 µg per day. Acute syndromes of poisoning can emerge with a singular uptake of 15 mg; about 500 mg are lethal. The threshold limit value (TLV) is 0.1 mg/m³, the biological tolerance value (BTV) 15 µg/l (urine) and 1.5 µg/l (blood). Cd is carcinogenic (A2), mutagenic (C2) and teratogenic.

Interlude: Mercury

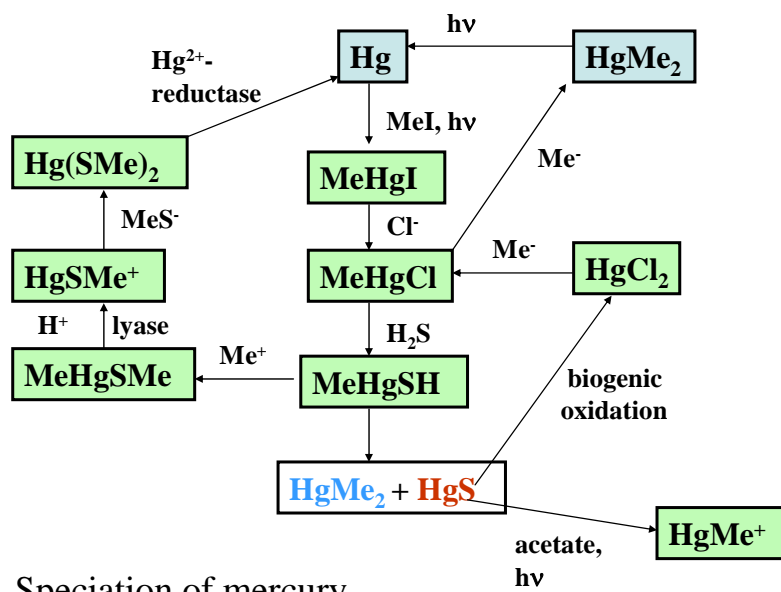
In nature, Hg occurs mainly in elemental form and in the form of cinnabar (HgS). Metallic mercury does not corrode in air. Earlier applications were fillings of thermometers, barometers and the like. Nowadays it is used in chloralkali electrolysis, dental fillings, and batteries. Coal fired power plants and volcanic exhalations are important sources for mercury pollution

The TLV is 0.1 (Hg and HgCl₂) and 0.01 (MeHgCl) mg/m³, respectively, the BTV 50 (blood) and 200 µg/l (urine) for inorganic Hg compounds and 100 µg/l for organic compounds. The LD₅₀ value (Rat, oral) amounts to 57 mg/kg (LD₅₀: lethal dose for 50% of the test animal).

Paracelsus employed mercury preparations against skin diseases, syphilis and inflammation. Hg preparations have also a long standing tradition as disinfectants (sublimat = HgCl₂; mercurchrome). Contrasting its lighter homologues, Hg also forms monovalent compounds. An example is calomel = Hg₂Cl₂.

Mercury and its compounds are liable to speciation in the atmosphere, aquasphere and siderosphere. The speciation is partly abiotic (chemical and photochemical speciation), partly biotic (such as the methylation of Hg). Some of the more important paths of mutual interconversion are depicted in Fig. 28. The methylation of mercury under physiological conditions is carried out by methylcobalamin, a close relative to vitamin B₁₂ (adenosylcobalamin).

Figure 28. (following page). Speciation of mercury. Anthropogenic sources are framed, microbial sources are in red. *Su* = substrate



11. The role of the alkaline metals and alkaline earth metals

Physiologically relevant are the alkaline metal ions Na^+ and K^+ , and the alkaline earth metal ions Mg^{2+} and Ca^{2+} . Li^+ is of therapeutic interest (treatment of mood disorders such as manic depression; treatment of hypertension). The transition metal ion Mn^{2+} exhibits some similarities with Ca^{2+} and Mg^{2+} .

Amounts present in man (in g per 70 kg body weight): Na 105, K 140, Mg 35, Ca 1050 g
Daily demand: Na 1.1-3.3, K 2.0-5.0, Mg 0.3-0.4, Ca 0.8-1.2 g.

With the exception of Mg^{2+} , there are striking differences in the intra- and extra-cellular concentrations of these cations. The following Table summarises the intra- and extracellular concentrations of the more important cations and anions. The Table also contains concentrations of these ions in sea-water, often considered “the cradle of life“. To maintain the “correct” concentration gradients between the cytosol and the extracellular space is of prime importance to ascertain the specific functions of these ions, such as controlling the osmotic pressure and cell membrane potentials, triggering signal transduction, and activating enzymes.

Table: Concentrations of selected ions (*mM*) in the **intracellular** and **extracellular** space (mean values for all human cell types, and data for erythrocytes and blood plasma are listed). For comparison, ion concentrations in **sea water** are also provided.

	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
Intracellular in erythrocytes	92	11	0.1	2.5	-	155	190	
Blood plasma	5	152	2.5	1.5	130	195	30	
Sea water	10	500	10	50	500	variabel	0.002	29
Intracellular mean value	155	10	0.001	15	8	10	65	0.5
Extracellular mean value	4	142	2.5	0.9	120	27	1	10

Overview of the functions (selection):

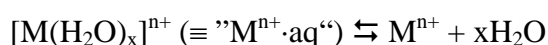
- Support (endo- and exo-skeletons, teeth): Ca (and Mg)
- Information transfer by migration along a concentration and/or electrochemical gradient: all ions
- Regulation of the osmotic pressure and of membrane potentials: Na, K
- Activation and regulation of enzymes Ca (and Mg, K)
- Signal transduction, e.g. in neurotransmission Ca, K
- Chlorophyll: Mg
- Phosphate und anaerobic energy metabolism; activation by phosphorylation: Mg
- Stabilisation of cell membranes by the formation of cross-links between membrane proteins and polysaccharides: Mg (and Ca)

For the physiological functions, the charge density (CD = ionic charge divided by the ionic radius) is of central importance:

	Li ⁺	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Mn ²⁺
r/Å*	0.76	1.02	1.38	0.72	1.00	0.83
LD	1.45	1.10	0.72	2.78	2.0	2.41

* for the coordination number 6

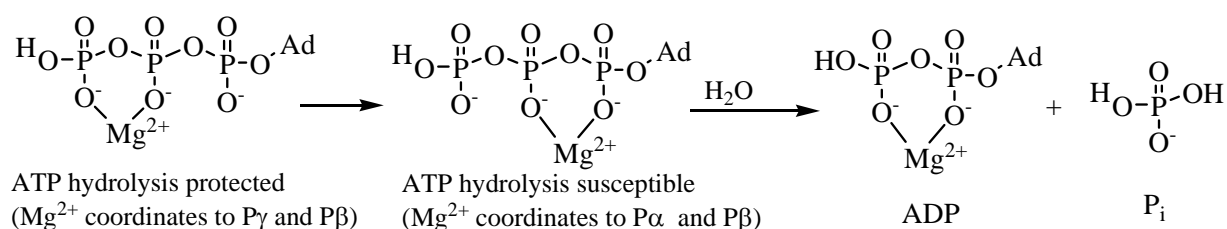
The larger the charge density, the higher is the ability of the ion to polarise a molecule. The charge density of Mg²⁺ is particularly high: contrasting Ca²⁺, but in accordance with transition metal ions, Mg²⁺ thus forms stable complexes also with N-functional ligands; see e.g. chlorophyll. Alkaline and alkaline earth metal ions are rather mobile; typically, their complexes are characterised by small stability constants. In aqueous media, and in the absence of other ligands, the ions are present in hydrated form. Unlike aqua complexes of transition metal ions, the number of water molecules in the hydration sphere is hardly defined, and the interactions are weak. The rate constants for the exchange of water in the hydration sphere and surrounding water, i.e. for the equilibrium



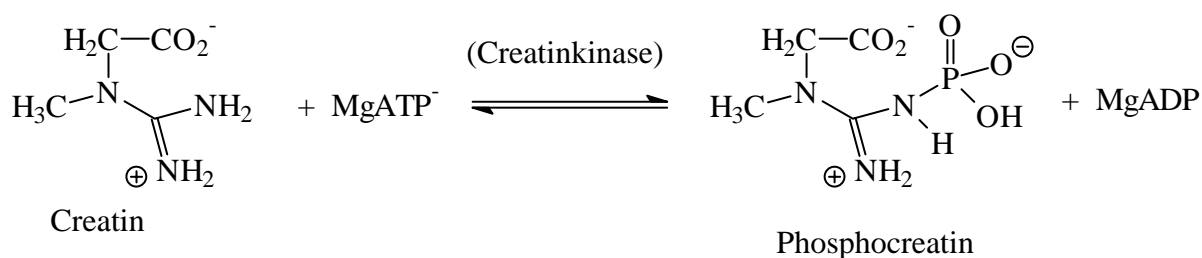
are in the order of magnitude of 10⁻¹⁰-10⁻⁷ s⁻¹ for Na⁺, K⁺ and Ca²⁺, and 10⁻⁷-10⁻⁵ s⁻¹ for Mg²⁺. Mg²⁺ complexes thus are not only thermodynamically but also kinetically more stable than those of the other ions.

Magnesium

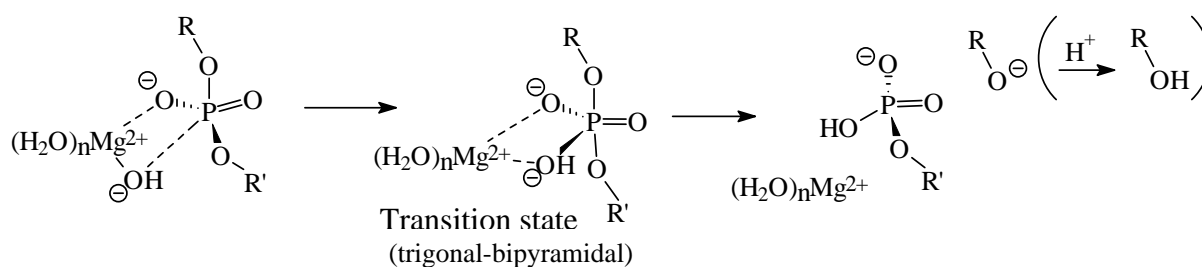
Magnesium takes over a crucial role in the phosphate (and hence the energy) metabolism in that it coordinates to diphosphate or phosphate + carboxylate, and thus activates molecules and triggers activation paths. Examples are kinases, ATPases, phosphatases, isomerases, enolases, proteinsynthases and –polymerases; see the following examples (ATP hydrolysis; creatinkinase):



The complex formation constant for the complex formed between Mg^{2+} and ATP ($\text{Mg}^{2+} + \text{ATP}^{3-} \rightleftharpoons [\text{MgATP}]^-$) is about 10^4 M^{-1} (the dissociation constant correspondingly 0.1 mM). The coordination sphere of Mg^{2+} is supplemented by water (and, depending on the pH, OH^-). The free enthalpy of reaction for the ATP hydrolysis amounts to $\Delta G \approx -35 \text{ kJ/mol}$. The phosphate group (P_i = inorganic phosphate) generated by ATP hydrolysis can also be transferred to suitable substrates. An example is the phosphorylation of sugars. In cells with a high turnover rate for ATP, phosphate can be transferred to creatine. Creatinephosphate on its part serves as a source for rapid regeneration of ATP. The daily turnover of ATP at rest corresponds to about half of the body weight.



Mg^{2+} also mediates the hydrolysis of phosphoester bonds by phosphatases by stabilising a trigonal-bipyramidal transition state for phosphorus:



Interlude: The Gibbs-Helmholtz Equation

This equation connects the reaction enthalpy (ΔH) with the free reaction enthalpy (ΔG , Gibbs free energy), the reaction entropy (ΔS) and the temperature T:

$$\Delta G = \Delta H - T\Delta S$$

According to this relation, part of the reaction enthalpy is converted to entropy changes in the reaction system. In the global system, the entropy always *increases* ($+\Delta S$); in a subsystem, the entropy can also *decrease* ($-\Delta S$). A reaction will only take place voluntarily in case of a negative ΔG .

Example: $\text{H}_2 + \frac{1}{2} \text{O}_2 \rightarrow \text{H}_2\text{O}$: $\Delta H = -286 \text{ kJ/mol}$, $T\Delta S = -49 \text{ kJ/mol}$ (at 298 K); $\Delta G = -239 \text{ kJ/mol}$

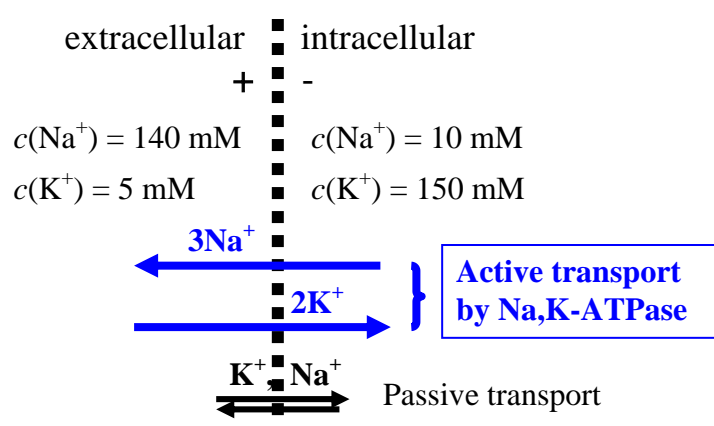
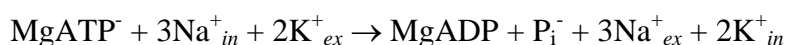
Even in case of a negative ΔG (*thermodynamically* allowed reaction), a reaction system may be metastable (i.e. the reaction does not take place) because it is *kinetically* hindered due to a high activation barrier (example: the formation of molecular oxygen from ozone and oxygen atoms in the stratosphere). Catalysts reduce this activation barrier.

Sodium and potassium

In order to adjust to the intra- and extra-cellular concentrations of Na^+ and K^+ it is essential that these ions can cross the membrane of the cells. Such a trans-membrane transport can be passive (via diffusion) or active by use of an ion pump. Since the lipophilic cell

membrane is usually impermeable for the hydrated, hydrophilic sodium and potassium ions, transport mediators, so-called ionophores can be employed. Alternatively, and more efficiently, the transport can occur along ion channels in the membrane.

The *active* transport for Na^+ and K^+ is achieved by a Na^+/K^+ -specific pump, an ATPase, abbreviated E (for enzyme) in the following discussion. The energy necessary for the transport $\text{Na}^+(\text{in}) \rightarrow \text{Na}^+(\text{ex}) // \text{K}^+(\text{ex}) \rightarrow \text{K}^+(\text{in})$ against a concentration gradient is provided by the hydrolysis of ATP. E consists of two glycoprotein subunits of molecular mass 131 and 62 kDa, the larger of which is the transport unit. In the course of the ion transport, coupled to phosphorylation/dephosphorylation of E , this unit switches between the conformations E_1 (Na^+ sensitive) und E_2 (K^+ sensitive). Per MgATP hydrolysed two K^+ are locked in and 3 Na^+ are locked out:



The charge imbalance thus produced is balanced, in part, by a $\text{Na}^+, \text{Ca}^{2+}$ -ATPase, and in part by passive transport. The various steps of the transport as catalysed by the Na, K -ATPase are assembled in Fig. 29; for the conformational changes of E see Fig. 30.

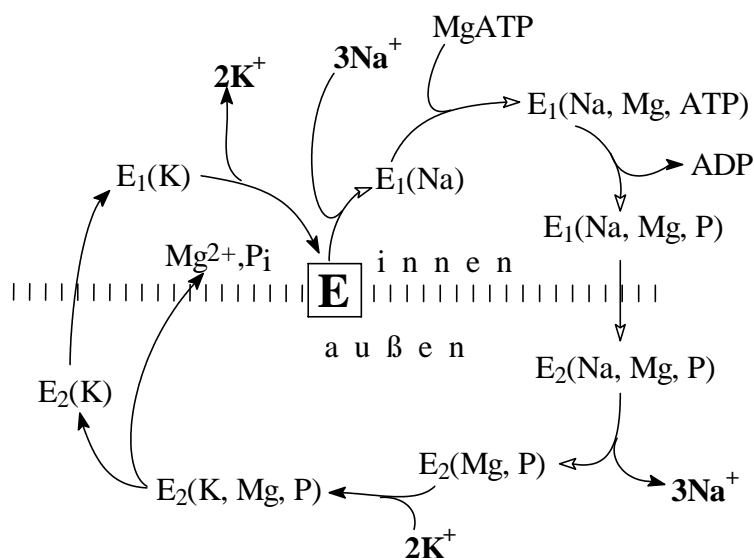


Figure 29. Function of the Na, K pump (Na, K -dependent ATPase), E .

Individual events (see Fig. 29):

- (1) Uptake of intracellular Na^+ , Mg^{2+} and ATP by E_1 ;
- (2) Phosphorylation of the enzyme (cf. Fig. 31);
- (3) Conformational change $E_1 \rightarrow E_2$ (cf. Fig. 30);

- (4) Extrusion of Na^+ into the extracellular space, and uptake of K^+ ;
- (5) Dephosphorylation of the enzyme, and release of phosphate and Mg^{2+} into the intracellular space;
- (6) Conformational rearrangement $E_2 \rightarrow E_1$;
- (7) Release of K^+ into the intracellular space;
- (8) Reactivation of the enzyme by uptake of Mg^{2+} : $E_1 + \text{Mg}^{2+} \rightarrow E_1(\text{Mg}^{2+})$.

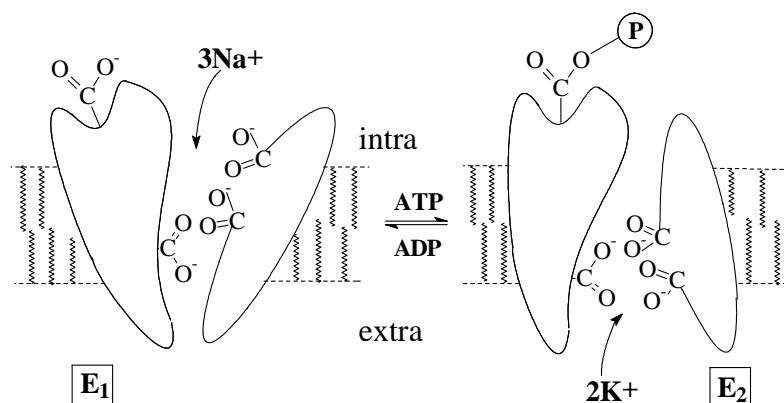


Figure 30. Transmembrane transport of Na^+ and K^+ by ATPase. E_1 and E_2 represent different conformers of the ATPase.

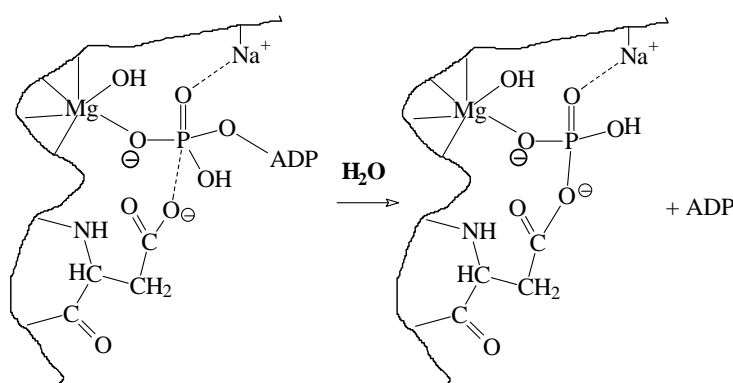


Figure 31. Phosphorylation of the ATPase. Phosphatases can be inhibited by the phosphate analogue vanadate (H_2PO_4^-), because vanadium freezes in the trigonal-bipyramidal transition state (shown on the left).

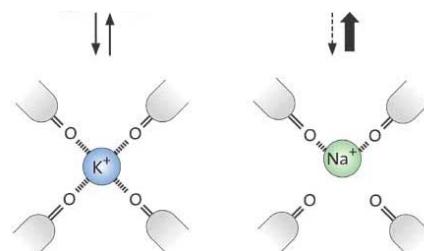
Ion channels and ionophores

Along with the ATP driven transport, alkaline (and alkaline earth) metal ions can surmount the membrane also by “passive“ transport along ion channels (hydrophilic transport) or with the help of transport vehicles, so-called ionophores (hydrophobic transport).

Ion channels are trans-membrane proteins, with their inner surface aligned with carboxylate (stemming from Glu and Asp) or/and carbonyl groups, allowing for the transport of ions, which are usually partly or completely deprived of their hydration shell while transported through weak coordinative interaction with the oxygen functionalities in the channel. The transport can also occur in the sense of a symport (concomitant transport of two ionic species) or antiport (counter transport; two ionic species being transported in opposite directions). The following types of ion channels are distinguished:

- Leak channels; only for K^+ : they are always open;
- Gated channels; these are channels with “gates“ (or locks) which are usually closed, but can be opened by a stimulus when required:
 - voltage gated: by a change in the electrochemical membrane potential;
 - ligand gated: by a chemical stimulus, e.g. neurotransmitters (acetylcholine, dopa, glutamate, NO) or toxins (nicotine), or Ca^{2+} ;
 - stretch gated: by a mechanical stimulus, e.g. a physical change in the membrane as a consequence of strain.

The selectivity of ion channels for K^+ and Na^+ is implemented by geometrical factors, as shown at the right hand side for a K^+ selective channel: The Na^+ cation is too small to fit properly into the opening provided by four oxygen functionalities of the channel.



Ionophores are macrocyclic compounds with mainly *O*-functional groups, which can form stable complexes *in vivo* specifically with Na^+ or K^+ . Models for ionophores are crown ethers, cryptands and calixarenes; Fig. 32. The selectivity for Na^+ and K^+ is determined by the size (diameter) of the space available within the functionalities. Examples for naturally occurring ionophores are displayed in Fig. 33: Nonactin and enniatine (both for K^+) and antamanide (for Na^+). Antamanide is a cyclic decapeptide of composition -Val-Pro-Pro-Ala-Phe-Phe-Pro-Pro-Phe-Phe-.

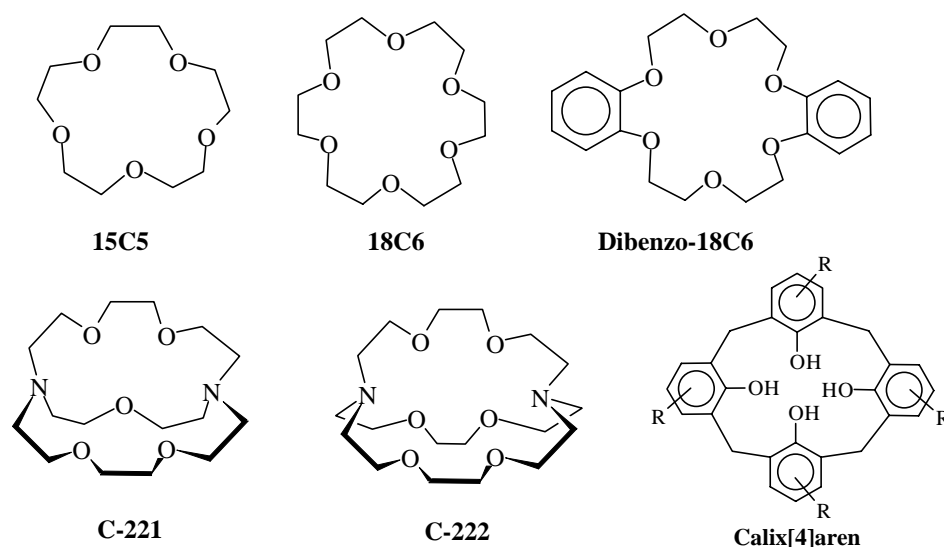


Figure 32. Ionophore models suited for the coordination of Na^+ and K^+ : Crown ethers (top row), cryptands (bottom left and centre), and a calixarene (bottom right). 18C6 = 18-crown-6 (18-membered ring, 6 O-functions); C221 = cryptand-221 (221 denotes the number of O-functions in the three bridges between the amine-nitrogens). The symbol [4] in the calixarenes indicated the number of phenolic units.

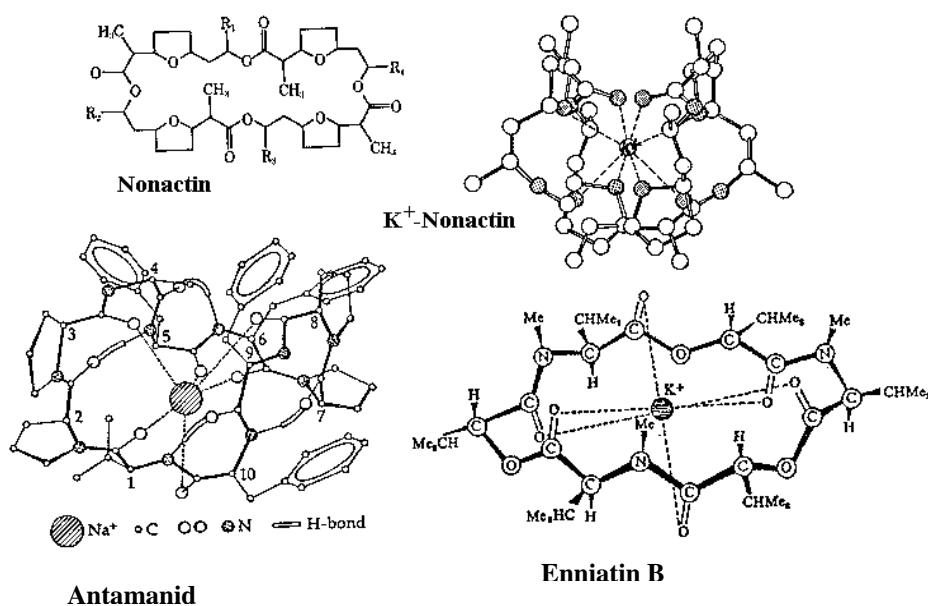
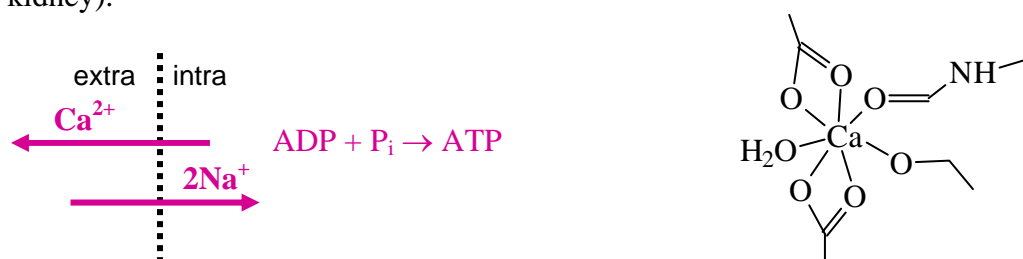


Figure 33. Ionophores for K^+ (nononactin and enniatine B) and Na^+ (antamanide).

Calcium

Sparingly soluble calcium compounds (such as carbonates and phosphates) can act as bearings by being incorporated into exo- and endo-skeletons. Examples are bone (calcium phosphate: hydroxyapatite) and sea shells (calcium carbonate: aragonite, calcite). The bones of the vertebrates are composite materials, containing about 50% collagen (a fibrous protein) and 50% hydroxyapatite $Ca_5(PO_4)_3(OH)_x F_{1-x}$ ($x \leq 0.01$). A person of average weight (70 kg) contains ca. 1.1 kg calcium, mainly as a constituent of the bone tissue. Only about 10 g are not confined to bony materials. These 10 g are used for a variety of functions in the organism, including the regulation of cell function, muscle contraction, blood clotting, and enzyme regulation, the latter with the help of specific Ca^{2+} binding proteins (calmodulins; see below).

On a general basis, Ca^{2+} acts as a second messenger by activating, regulating and reinforcing signals. Additionally, Ca^{2+} can take over the role of a co-factor in hydrolases (e.g. in nucleases responsible for the hydrolysis of the phosphodiester bonds), and exert structure function in proteins (e.g. in thermolysin and in proteinase-K). In this respect, it resembles Zn^{2+} . As a rule, only very low cytosolic calcium concentrations are necessary (about 0.1 to 1 μM). The extracellular Ca^{2+} concentrations are about 1 mM, and they can go up to 5 mM in special cellular compartments (SR; see below). The exchange between the extra- and intracellular space is achieved by Ca-ATPases (formation of ATP in case of a transport *with* the concentration gradient; see for a Na,Ca-ATPase below left). Malfunctions of the calcium metabolism can result in the deposition of sparingly soluble calcium compounds (oxalate, phosphate, steroids) in the blood vessels (where they cause calcification and cardiovascular diseases) and secretion organs (where these deposits are responsible for stones in gall, bladder, kidney).



Contrasting Mg^{2+} , which prefers octahedral coordination, Ca^{2+} has a tendency to form complexes of coordination number 7 or 8. Preferential ligands are H_2O , carboxylate (Asp, Glu), the carbonyl groups of the peptide bonds, and alcoholate (Ser). An example is parvalbumin (see the picture above, right), a Ca^{2+} protein in the smooth muscles, which takes part in muscle relaxation.

Ca^{2+} also plays an essential role in muscle contraction. Muscle cells contain protein filaments (so-called myofibrils), which are embedded within the sarcoplasmic reticulum (SR). The SR contains vesicles (*ves*) which store Ca^{2+} at concentrations of 1-5 mM. Ca^{2+} storage is provided by calsequestrin, an acidic protein of 50 kDa molecular weight, which can bind up to 50 Ca^{2+} to Asp and Glu. Muscle contraction is achieved by release of Ca^{2+} into the cytoplasm (*cyt*) of the SR via the SR membrane, a process where there is again an ATPase (*E*) involved, which switches between two conformations E_1 and E_2 :

- Transport out of the vesicles into the cytoplasm (following the concentration gradient), coupled with the synthesis of ATP, triggers the contraction of the muscle fibrils:
 $2Ca^{2+}(ves) + E_2\text{-phosphate} + ADP \rightarrow 2Ca^{2+}(cyt) + E_1 + ATP$
- Return transport of the Ca^{2+} ions from the cytoplasm into the vesicles results in muscle relaxation and consumption of ATP:
 $2Ca^{2+}(cyt) + E_1 + ATP \rightarrow 2Ca^{2+}(ves) + E_2\text{-phosphate} + ADP$

The activation of Ca^{2+} -dependent enzymes is initiated by proteins of the calmodulin family. Calmodulin = calcium modulating protein. These are small proteins of 17 kDa molecular weight, which can bind four Ca^{2+} ions. Ca^{2+} binding leads to a conformational change (Fig. 34 top), allowing for coupling of Ca^{2+} -calmodulin to the enzyme (grey in Fig. 34), the substrate of which (blue) becomes activated (red). Examples are Ca-ATPases, NO-synthases [see. ch. 8], NAD-kinases, adenylate-cyclase.

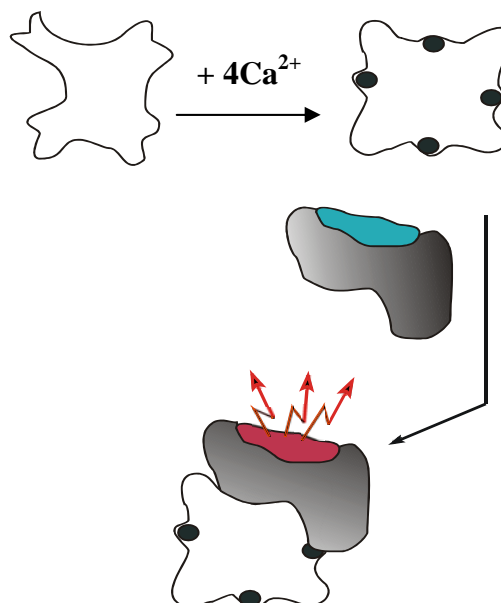
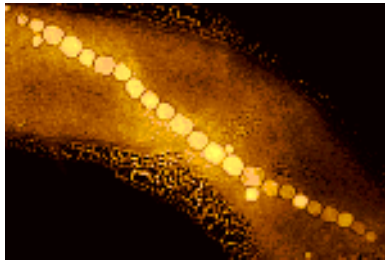


Figure 34. Model for the activation of enzymes (grey; e.g. NO-synthase) by Ca^{2+} -calmodulin. Blue: substrate (e.g. arginine); red: activated substrate.

Exkurs: Biomineralisation

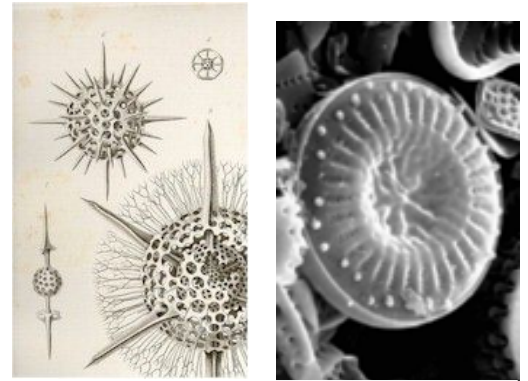
Hierunter versteht man die Generierung anorganischer („mineralischer“) Materialien oder anorganisch-organischer Kompositmaterialien durch biologische Aktivität. Beispiele sind Magnetit (Fe_3O_4) und Greigit (Fe_3S_4) in den Magnetosomen magnetostatischer Bakterien, Calciumcarbonate (CaCO_3 : Calcit, Aragonit) als Exoskelette von Muscheln, Schnecken, Seeigeln und Korallen sowie in den Zähnen der Raspelzungen von Schnecken, Gips ($\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$) als Schwerkraftsensoren in Tiefseequallen (*Periphylla*) und in Algen der Gattung *Closterium*, und Siliziumdioxid-Hydrate in den Exoskeletten von Radiolarien und Diatomeen (Kieselalgen). In allen Fällen werden die mineralischen Stoffe an einer Proteinmatrix aufgebaut (was zu den oft filigranartigen Strukturen führt), die sich – im 0.1%-Bereich – in solchen Materialien auch wiederfinden.



Magnetit und Greigit in einem magnetostatischem Bakterium



Calcit der Seeigelschale



SiO_2 -Skelette von Radiolarien (links) und Diatomeen