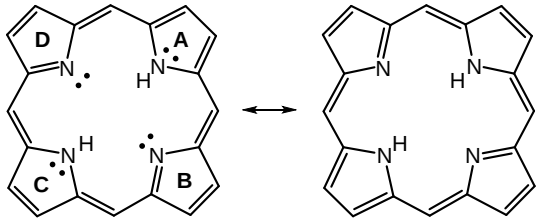
№ 35. NATURAL PYRROLE PIGMENTS. MYOGLOBIN, HEMOGLOBIN, BILIRUBIN.

(Refresh also knowledge on "Coordination compounds" and "Aromaticity of heterocyclic compounds")

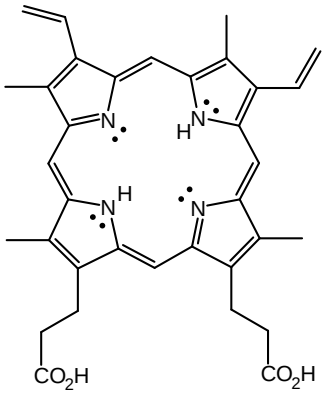
Iron is abundant in biology and plays a central role in almost all living cells. Iron is a necessary trace element found in nearly all living organisms. Iron-proteins are found in all living organisms, ranging from the evolutionarily primitive organisms to humans. Iron-containing enzymes and proteins, often containing **heme** prosthetic groups, participate in many biological oxidations and in transport. Examples of proteins found in higher organisms include hemoglobin, cytochrome, and catalase.

The color of blood is due to the hemoglobin, an iron-containing protein. As illustrated by hemoglobin, iron often is bound to cofactors, e.g. in hemes. The heme structure relates to a fundamental structure named **porphin** (porphine). Porphin is the parent for several types of biochemically significant compounds called



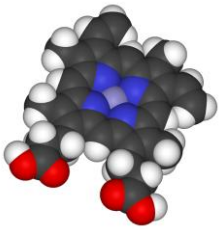
porphin

porphyrins. Porphyrins have all β-pyrrolic positions substituted with various groups (alkyls, vinyl, residues from propionic acid). The porphin molecule is a macrocycle having four pyrrole units connected with -CH= bridges. The entire porphin molecule is cyclic, planar, containing 11 C=C double bonds and 2 lone pairs. Rings **A** and **C** are of pyrrole type whereas **B** and **D** are of isopyrole type. The latter have pyridine-like nitrogens but equivalency of all 4 N has been determined experimentally. The two shown above structures are equivalent, therefore tautomerism is in place. The π electronic system of porphyn consists of 26 π electrons. It is an aromatic system according to the Hückel's rule for n=6 (4.6+2). The delocalization energy is very high. Therefore the system is very stable. The red color of porphin arises from relatively small energy of an absorbed quant of light in the visible range because of small HOMO-LUMO gap.

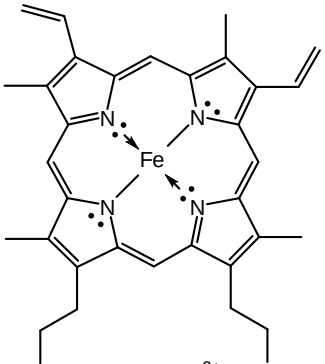


protoporphyrin IX

Protoporphyrin is the biochemical precursor for heme. Protoporphyrin in humans is biosynthesized after linear attachment of four pyrrole rings via saturated $–CH_2–$ bridges. Later in a macrocyclization reaction the first pyrrole ring is connected with the fourth. Last, aromatization is achieved through oxidation (dehydrogenation) and the iron atom is inserted. Protoporphyrin IX is a biochemically widely used carrier molecule for divalent metal cations. In such complexes protoporphyrin is a tetradentate ligand. Together with iron (**Fe^{2+}**) the **heme**-group of hemoglobin, myoglobin and many other heme-containing enzymes like cytochrome *c* and catalase is formed. Complexed with magnesium ions (Mg^{2+}) the main part of the chlorophylls is formed. Complexed with zinc ions (Zn^{2+}) it forms zinc protoporphyrin. When the iron in heme is oxidized to Fe^{3+} hemin is obtained. The structure of hemin from blood was determined using classical methods that led to Nobel prize in 1930 awarded to Hans Fischer.



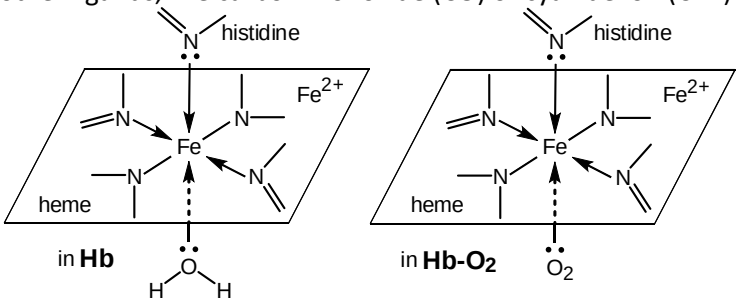
A **heme** or haem (British English) is a prosthetic group that consists of an iron atom contained in the center of a porphyrin. Upon coordination to a metal ion, the two hydrogen atoms in protoporphyrin are displaced. The metal complexes are generally called metalloporphyrins. There are several kinds of hemes that differ by the peripheral groups. Hemes are most commonly recognized in their presence as components of hemoglobin but they are also components of a number of other hemoproteins. The organs mainly involved in heme synthesis are the liver and the bone marrow, although every cell requires heme to function properly.



The enzymes involved in heme biosynthesis work in fast succession, without any accumulation of intermediates – like an assembly line. The process is highly conserved across biology. The rate-limiting enzyme responsible for this reaction, *ALA synthase*, is strictly regulated by intracellular iron levels and heme concentration. Therefore, if an enzyme on the pathway is deficient, overproduction of the intermediate up to the point of deficiency occurs. The principal problem in these deficiencies is the accumulation of porphyrins, the heme precursors, which are toxic to tissue in high concentrations. The accumulation of such precursors leads

to a group of diseases, known as porphyrias. Some of them are cruelly referred to as a "Vampire's" disease and are associated with many vampire legends, such as extreme sensitivity to sunlight (going out only during nighttime), pale faces (from anemia), drinking blood (it might be on the theeth because of the disease).

The iron in the heme group is in +2 oxidation state. Iron forms two ionic and two coordinate bonds to the porphyrin nitrogens. Fifth coordination place is occupied by a histidine nitrogen from the protein chain. Since the coordination number of Fe^{2+} normally is six, it has one more coordination position open for reversible attachment of water molecule or oxygen (in normal metabolism). This site can be occupied also by other ligands, like carbon monoxide (CO) or cyanide ion (CN^-) which may lead to organism's death.

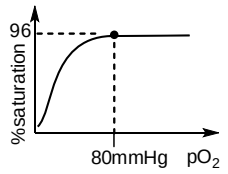


Hemoglobin (abbreviated **Hb** or Hgb) is the iron-containing oxygen-transport metalloprotein in the red blood cells of vertebrates. Hemoglobin consists of four globular protein subunits and four heme groups, therefore it can bind and transport 4 O_2 molecules at once. Each human red blood cell contains approximately 270 million of these hemoglobin biomolecules.

The ability of hemoglobin to deliver O_2 to tissues is due to spatial proximity of a histidine residue from the protein. In the absence of oxygen (low partial pressure in the tissues), the sixth ligand is a water molecule that binds to Fe^{2+} on the opposite side of the histidine residue to complete the octahedral complex. This paramagnetic complex is called **deoxyhemoglobin**. It has deeper red-purple color characteristic of venous blood. The water ligand can be replaced readily by oxygen molecule (at higher partial oxygen pressure in the lungs) to form bright red **oxyhemoglobin** that is present in arterial, oxygenated blood. These two binding modes of hemoglobin result in change of the spacial arrangement of the protein subunits – a conformational change. The binding process is reversible. The O_2 binding in hemoglobin occurs in the lungs where the pO_2 is high and pCO_2 is low.

$$Hb + O_2 \rightleftharpoons Hb\text{-}O_2 + H_2O$$

In this exchange process the back transported CO_2 is taken away from Hb. The O_2 release in the tissues is due to lower pO_2 but also lower pH which causes protonation of the histidine N (of pyridine type). When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron. With water bound, the iron atom is out of the ideal plane of porphyrin. When oxygen binds to the iron complex, it causes the iron atom to move back toward the center of the porphyrin ring into the plane. At the same time, the imidazole side-chain of the histidine residue moves nearer to the iron atom. This strain is transmitted to the remaining three monomers in the tetramer, where it induces a similar conformational change in the other heme sites such that binding of oxygen to these sites becomes easier. Such effect is called a cooperative process. The binding affinity of hemoglobin for oxygen is increased by the oxygen saturation of the molecule, with the first oxygen bound influencing the shape of the binding sites for the next oxygens, in a way favorable for binding. Evidence for this cooperative effect is the oxygen binding curve of hemoglobin which is sigmoidal, or *S*-shaped.



If hemoglobin has Fe^{3+} it is **methemoglobin**. It cannot bind oxygen. In binding, oxygen temporarily oxidizes Fe^{2+} to Fe^{3+}, so iron must exist in the +2 oxidation state to bind oxygen. Modern view assumes that several forms Hb contribute and iron is in +3 oxidation state but binding superoxide anion.

Carbon monoxide is a very good ligand for iron When present, CO forms stronger complex with hemoglobin than oxygen. The resulting stable complex, called carboxyhemoglobin, prevents the normal uptake of O_2, thus depriving the body of needed oxygen.

$$Hb\text{-}O_2 + CO \rightleftharpoons Hb\text{-}CO + O_2$$

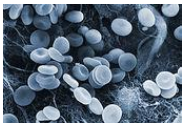
Carbon monoxide is an insidious poison because the gas is colorless, odorless, and tasteless but even in the presence of enough oxygen prevents its binding to hemoglobin. This poisonous action of CO is due to the above equilibrium lying to the right.

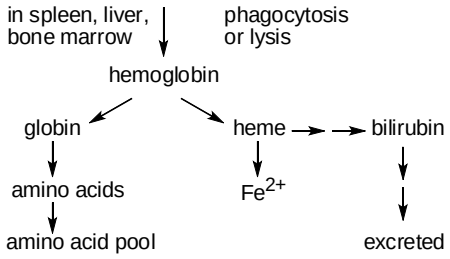
Myoglobin is a single-chain globular protein, containing a heme. Myoglobin is the primary oxygen-carrying metalloprotein of muscle tissues. Unlike the blood-borne hemoglobin, to which it is structurally related, this protein does not exhibit cooperative binding of oxygen, because it has only one chain and one heme units. Instead, the binding of oxygen by myoglobin is unaffected by the oxygen pressure in the surrounding tissue. Myoglobin is often cited as having an "instant binding tenacity" to oxygen. High

concentrations of myoglobin in muscle cells allow organisms to hold their breaths longer. Diving mammals such as whales and seals have muscles with particularly high myoglobin abundance. Myoglobin was the first protein to have its three-dimensional structure revealed with certainty. In 1958, John Kendrew and associates successfully determined the structure of myoglobin by high-resolution X-ray crystallography, then a modern metod which is widely used today for structural determinations. For this discovery, John Kendrew shared the 1962 Nobel Prize in chemistry with Max Perutz.

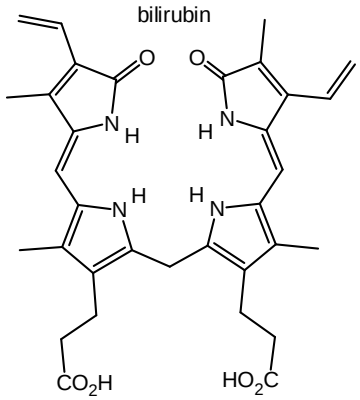
The porphyrin group is a very effective chelating agent, found also in cytochromes, catalase, and peroxidase.

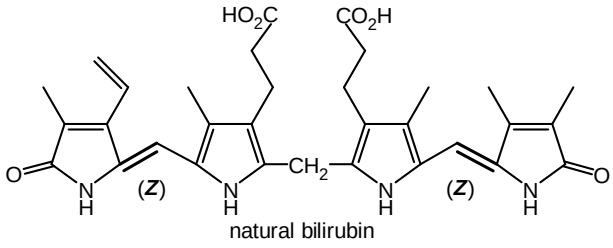
Bilirubin. Bilirubin is catabolitic product of heme degradation. The life span of red blood cells (erythrocytes) in blood stream is 100-120 days. When the red blood cells age or are damaged during their passage through narrow capillaries, their cell membrane changes. Such change is recognized by specialized cells in the spleen, bone marrow, and liver and senescent red blood cells are phagocytosed and/or lysed, thus removed from blood circulation. Most of the chemical materials in hemoglobin are reused. The most "expensive" for an organism is iron. Human beings use 20 mg of iron each day (very large amount that cannot be supplied with food) for the production of new red blood cells, much of which is recycled from old red blood cells. Most well-nourished people in industrialized countries have 3-4 grams of iron in their bodies. Of this, about 2.5 g is contained in the hemoglobin needed to carry oxygen through the blood. Another 400 mg is devoted to cellular proteins that use iron for important cellular processes like storing oxygen (myoglobin), or performing energy-producing redox reactions (cytochromes). The heme degradation begins with removal of the globin (protein) portion which later is reused as amino acids. The iron from the released prosthetic group – the heme, is removed, put onto transferrin molecules, and then exported as transferrin-iron complexes back out into the blood. Most of the iron used for blood cell production comes from this cycle of hemoglobin recycling.





The organic portion of heme is oxidized selectively at the α-CH= bridge giving an open chain, linear tetrapyrrole – biliverdin (blue compound), that is quickly reduced to **unconjugated bilirubin** (yellow-orange compound). The α-carbon from heme is removed as CO. This is one of the few natural sources of carbon monoxide production in the human body, and is responsible for the normal blood levels of carbon monoxide even in people breathing pure air. The unconjugated bilirubin is not soluble in water despite the presence of two COOH groups and numerous other polar groups. Due to the lack of solubility, bilirubin is tightly bound to serum albumin and transported to the liver, as unconjugated bilirubin. In the liver, bilirubin is made water soluble by esterification with glucuronic acid to form **conjugated bilirubin**. This process requires specific enzyme and produces bilirubin diglucuronide. This bilirubin derivative is secreted from the liver cells into the bile which enters the gall bladder and from there - the gastrointestinal tract. In the ileum and colon, bacteria convert bilirubin into more reduced tetrapyrroles. Later oxidation of them gives compounds that are excreted in the feces. A small amount of the partially saturated analogs is reabsorbed into the blood, chemically modified and excreted in the urine. Urine does not contain bilirubin. Collectively linear tetrapyrroles similar to bilirubin are called **bile pigments** that are usually yellow, orange, brown in color.

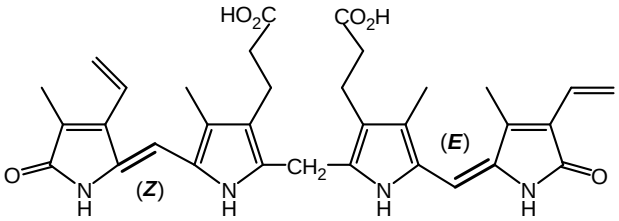




The bilirubin structure can be shown as resembling a porphyrin. When the structure is written in linear form, as on the left, care must be exercised to show the correct double bonds' configuration which in the natural bilirubin is (*Z, Z*).

Bilirubin shows its presence as yellow coloration in jaundice. Jaundice itself is not a disease, but rather a sign of one of many possible underlying pathological processes that occur at some point along the normal physiological pathway of the metabolism of bilirubin. Jaundice is the visible manifestation of increased levels of either unconjugated or conjugated bilirubin. Often

the white sclera of the eye becomes yellow because the patient has jaundice, or icterus. Of particular importance is the jaundice in newborn babies – so called neonatal jaundice. Some newborn babies have increased bilirubin levels as a result from overproduction of unconjugated bilirubin due to breakdown of fetal hemoglobin as it is replaced with adult hemoglobin and the relatively immature hepatic enzymes which are unable to conjugate and to excrete bilirubin as quickly as an adult. In severe cases the accumulation of unconjugated bilirubin can cause brain damage. Therefore removal of bilirubin is necessary. One common way now to do this removal is phototherapy. Like other pigments, some of the double-bonds in bilirubin isomerize when exposed to light. This geometrical isomerization is used in the phototherapy of jaundiced newborns. The (*Z, E*) isomer of bilirubin formed upon light exposure is much more soluble than the unilluminated (*Z, Z*) isomer. Therefore, the (*Z, E*) bilirubin isomer can be excreted by the babies without the need for glucuronidation. The (*Z, E*) isomer of bilirubin is thermodynamically unstable and reverts back to the natural pigment.



photoisomer (geometrical) of bilirubin

Bilirubin is responsible also for the yellow color of bruises.