# Questions & Answers on Bioinorganic Chemistry (D Ray)

**12. Explain the iron binding by transferrin.**

Ans. Iron, when taken up with the food and processed in the mouth is mostly present in Fe^{3+} form and thus gets into the gastrointestinal tract as Fe^{3+}. In case of an intact milieu in the small intestines, ferric iron is reduced to its ferrous form (Fe^{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 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). Transferrin is a glycoproteid of molecular weight 80 kDa (containing ca. 6% carbohydrate), having available two almost equivalent binding sites for iron(III), in the C- and N-teminal 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.
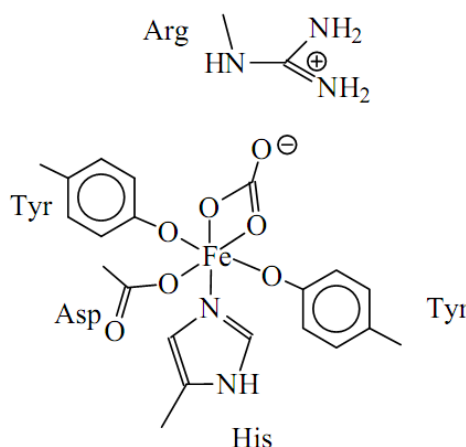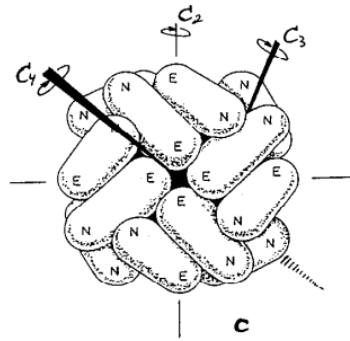

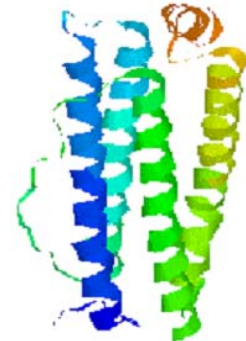**13. Show and explain the iron storage protein ferritin.**

Ans. 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 Fe3+. Up to 4500 Fe3+ 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. The overall composition of the iron nucleus is 8FeO(OH)·FeO(H2PO4). Channels of threefold symmetry and a width of 10 Å allow for an exchange of Fe3+ 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.
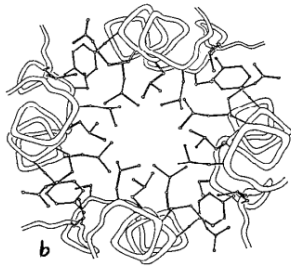
| Apoferritin (the inside of the hollow sphere is lined with carboxylates) | subunit structure and channels of C_2, C_3 (for iron exchange with the surroundings) and C_4 symmetry | one of the subunits |

14. Explain with a schematic view the tetrameric structure of the hemoglobin.

Ans. Tetrameric adult human hemoglobin is a heterotetramer. Hb in the human blood carries O_2 from the respiratory machines to the remaining part of the body where it releases it to burn nutrients to provide energy to power the functions of the organism, and collects the formed CO_2 to bring it back to the respiratory organs to be released from the organism.
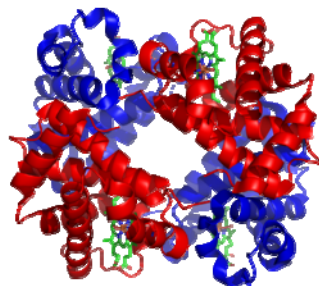

When O_2 binds to the iron complex, it causes the Fe atom to fit nicely move to the plane of the porphyrin ring. The imidazole side-chain of the histidine residue interacting at the other pole of the iron is pulled toward the porphyrin ring which forces the plane of the ring sideways toward the outside of the tetramer, and also induces a strain in the protein helix containing the histidine as it moves nearer to the Fe 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 O_2 to these sites becomes easier. This is known as the cooperative binding of O_2. The binding affinity of Hb for O_2 is increased by the O_2 saturation of the molecule. The positive cooperative binding is achieved through conformational changes of the Hb. Thus the oxygen

binding curve of hemoglobin is sigmoidal as opposed to the hyperbolic one for non-cooperative binding.

15. Graphically compare the O_2 affinity of hemoglobin and myoglobin.

Ans. Affinity of Hb and Mb to O_2 is shown below. The O_2 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 O_2.
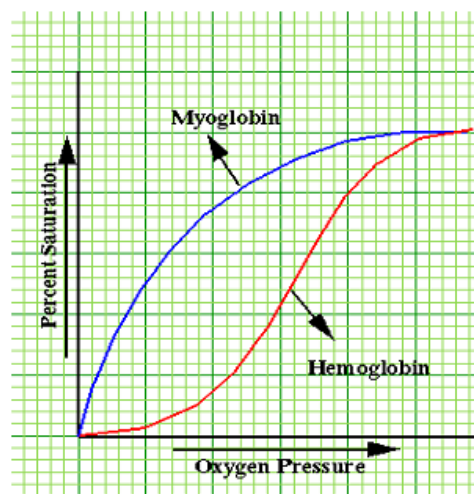

16. List the common forms of O2 and oxygen bearing species in relation to ROS.

Ans. One commonly distinguishes three oxygen modifications: **singlet**-O_2 ($^1O_2$; high energy content, unstable, diamagnetic), **triplet**-O_2 ($^3O_2$, stable, biradical and hence paramagnetic), and ozone (O_3; toxic; very reactive and strong oxidant).
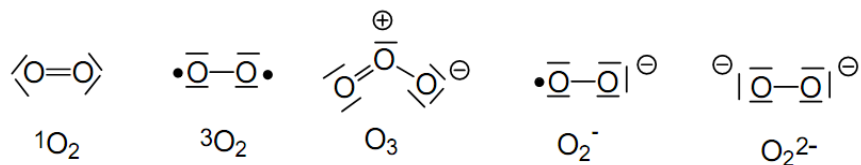
Reduction of O_2 produces **superoxide** ($O_2^{\bullet-}$) or **peroxide** (O_2^{2-}), both of which are strong oxidants and physiologically harmful (reactive oxygen species, ROS). To cope with these oxidants, the body holds ready catalases ($H_2O_2 \rightarrow H_2O + O_2$) and superoxide dismutases ($2O_2^- + 2H+ \rightarrow H_2O_2 + O_2$).

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

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + H_2O + HO^{\bullet}$$
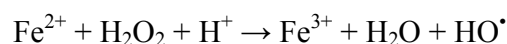
17. Show with a picturesque presentation the role of distal imidazole heterocycle for trapping of O2 by heoxy-Hb.

Ans. Deoxy and oxy forms of haemoglobin or myoglobin are shown below. The central ligand system, protoporphyrin IX, is shown here without the appropriate ring substituents.
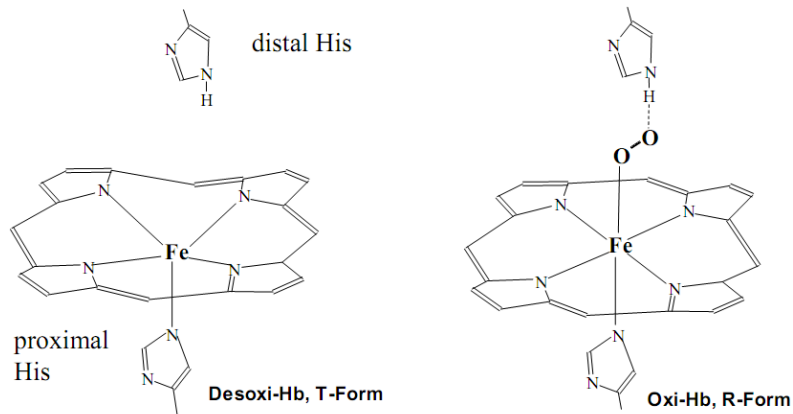
18. Explain transport, formation and degradation of hydrogencarbonate in our body.

Ans. Oxygen is ultimately reduced to water in the mitochondrial respiratory chain. 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 $CO_2 + OH^- \rightarrow HCO_3^-$, 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 Hb·H+, coupled with binding of O_2 to haemoglobin. Carbonic acid finally is enzymatically split into CO_2 und H_2O. The enzyme catalysing the formation and degradation of hydrogencarbonate/carbonic acid is called carbonic anhydrase (CA). CA has a molecular weight
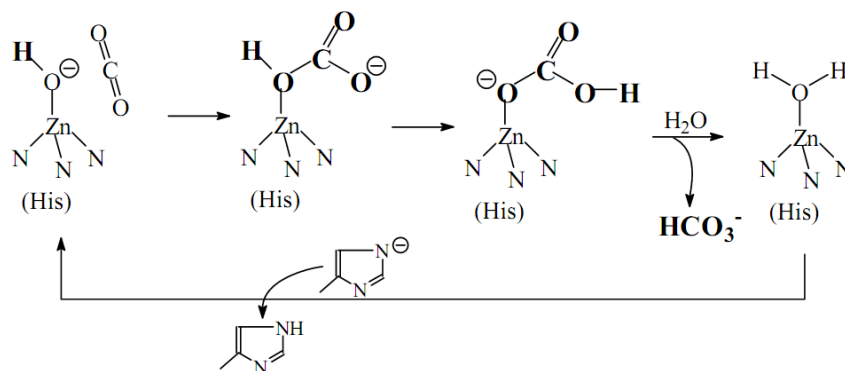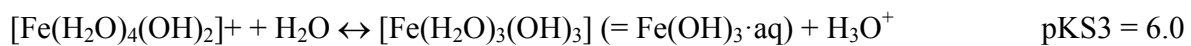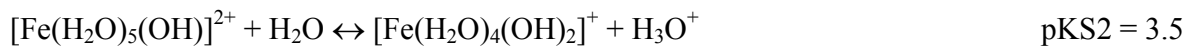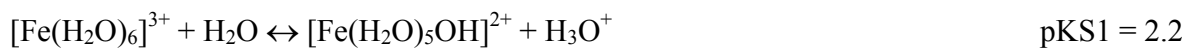

of 29.7 kDa and contains Zn^{2+} in its active centre. Zn^{2+} is coordinated to three histidine residues plus an aqua ligand (in its resting state) or a hydroxido ligand (in its active state). A histidine

close to the active centre participates in the proton shuttle. For the catalytically conducted mechanism see figure for the 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.

19. Briefly present the aqueous iron chemistry in relation to the mineralization.

Ans. The redox potential for the pair Fe^{2+}/Fe^{3+} at pH = 7 demonstrates that Fe^{II} is easily oxidized to Fe^{III} under aerobic conditions. Hexaaquairon(III) ions are cationic Brønstedt acids:

$[Fe(H_2O)_6]^{3+} + H_2O \leftrightarrow [Fe(H_2O)_5OH]^{2+} + H_3O^+$ $\qquad$ pKS1 = 2.2

$[Fe(H_2O)_5(OH)]^{2+} + H_2O \leftrightarrow [Fe(H_2O)_4(OH)_2]^+ + H_3O^+$ $\qquad$ pKS2 = 3.5

$[Fe(H_2O)_4(OH)_2]+ + H_2O \leftrightarrow [Fe(H_2O)_3(OH)_3] (= Fe(OH)_3 \cdot aq) + H_3O^+$ $\qquad$ pKS3 = 6.0

The formation of ferric hydroxide $Fe(OH)_3 \cdot aq$ 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 iron oxide hydrates correspond to that of the minerals FeO(OH) (goethite) and $5Fe_2O_3 \cdot 9H_2O$ (ferrihydrite). Goethite
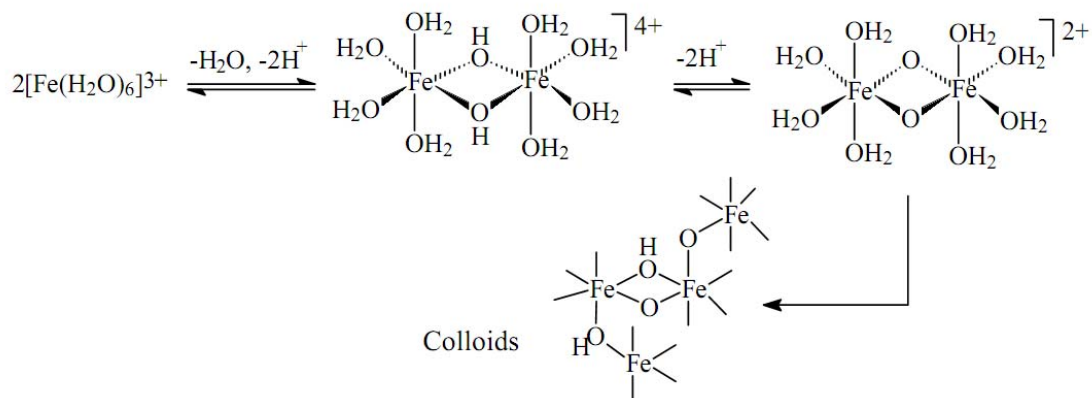
is an iron bearing oxide mineral found in soil and other low-temperature environments. Goethite has been well known since prehistoric times for its use as a pigment. Ferrihydrite is a widespread hydrous ferric oxo-hydroxido mineral at the Earth's surface, and a likely constituent in extraterrestrial materials. It forms in several types of environments, from freshwater to marine systems, aquifers to hydrothermal hot springs and scales, soils, and areas affected by mining.

20. How mobilization of Fe^{3+} is done by siderophores?

Ans. The extremely low solubility of Fe(OH)3 [solubility product L = 2·10-39, solubility (pH 7) l = 10-18 mol·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 Fe3+ (complex formation constants up to 1050 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 ferrichromes). 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

Hydroxamate

$$R-C{\overset{O}{\underset{\underset{R'}{N}}{\Big\backslash}}}-O^-$$

Ferrichrom
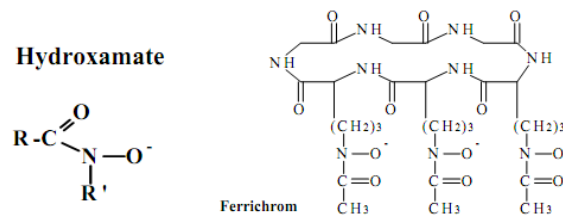
easy transport in the aquatic medium. Internalization of the Fe-loaded siderophore by the organism typically takes place by entocytosis. Endocytosis is an energy-using process by which cells absorb molecules (such as iron bearing siderophores) by engulfing them. It is used by all cells of the body because most substances important to them are large polar molecules that cannot pass through the hydrophobic plasma or cell membrane. The cytosolic remobilisation of the iron either by reduction of Fe3+ to Fe2+ and recycling of the siderophore, or by oxidative destruction of the siderophore is well established.

21. Explain the pathways for inactivation of hemoglobin.

Ans. Oxygen binding to haemoglobin can only occur if the Fe2+ 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 O2. Carbon monoxide exerts a comparable effect, which is responsible for the toxicity of CO. CO is bound 220 times more strongly to Fe2+ than O2: 0.25% of CO in air, i.e. the CO contents of inhaled cigarette smoke of

twenty 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 alanin) 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 O2 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:

$$Hb(Fe^{2+}) + H_2O \rightarrow MetHb(Fe^{3+}\text{-}OH) + e + H^+$$

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

22. Explain the spectroscopic characterization for the Mo centre in formate dehydrogenase.

Ans. Formate dehydrogenase, Fdh, from Escherichia coli contains a molybdopterin guanine dinucleotide cofactor and a selenocysteine residue in the polypeptide. An unusual Mo center of the active site is coordinated in the reduced Mo(IV) state in a square pyramidal geometry to the four equatorial dithiolene sulfur atoms from a pair of pterin cofactors and a Se atom of the selenocysteine-140 residue.
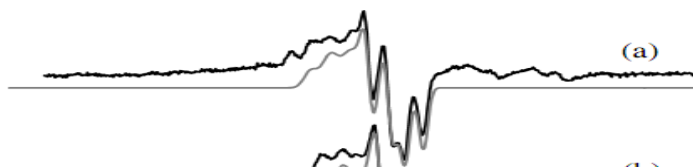
The Mo(V) EPR signals at 100 K of Fdh shown above is for as-prepared enzyme after 30 min reduction with HCOO$^-$. EPR data can also indicate the presence of Mo(V) pentacoordinated species formed in the presence of inhibitors. The Mo(V) species obtained in the presence of inhibitors (azide or cyanide) yields a nearly axial EPR signal showing only one detectable splitting given by nuclear species (I = ½) with typical and characteristic g-values as g_{max} = 2.012 (12), g_{mid} = 1.996 (8), g_{min} = 1.985 (8).

23. Explain O$_2$ binding in hemerythrin.

Ans. Hemerythrin is an oligomeric protein responsible for oxygen (O_2) transport in the marine invertebrate phyla of sipunculids, priapulids, brachiopods, and in a single annelid worm, magelona. Recently, hemerythrin was discovered in methanotrophic bacterium *Methylococcus capsulatus*. Myohemerythrin is a monomeric O_2-binding protein found in the muscles of marine invertebrates. Hemerythrin and myohemerythrin are essentially colorless when deoxygenated, but turn a violet-pink in the oxygenated state. The mechanism of dioxygen binding is unusual. Most O_2 carriers operate via formation of dioxygen complexes, but hemerythrin holds the O_2 as a hydroperoxide. The site that binds O_2 consists of a pair of iron centres. The iron atoms are bound to the protein through the carboxylate side chains of a glutamate and aspartates as well as through five histidine residues. Hemerythrin and myohemerythrin are often described according to oxidation and ligation states of the iron centre:

Fe^{2+}—OH—Fe^{2+}	deoxy(reduced)
Fe^{2+}—OH—Fe^{3+}	semi-met
Fe^{3+}—O—Fe^{3+}—OOH^-	oxi (oxidized)
Fe^{3+}—OH—Fe^{3+}—ligand	met (oxidized)

O_2 Binding to Hemerythrin

Deoxyhemerythrin

Fe_a^{II} (HS) is 5-coordinate,
Fe_b^{II} (HS) is 6-coordinate;
Ions are weakly AF-coupled (J ~10 cm^{-1})
via the bridging ligands;
d(Fe-Fe) 3.4-3.6Å; colorless

Fe$_a$ Fe$_b$

Oxyhemerythrin

both Fe^{III} are 6-coordinate, the Fe^{III} (HS) ions are stronger AF-coupled (J ~100 cm^{-1}) via the bridging ligands;
d(Fe-Fe) 3.2-3.3Å, v(^{16}O-^{16}O) 844 cm^{-1}
Purple red: 480 nm (ε ~4000 M^{-1} cm^{-1})

Fe$_a$ Fe$_b$

24. Why did the Fe(II) porphyrin bind O_2 when excess 1,2-dimethylimidazole was present but not at all when 1-methylimidazole was present?

Ans. The steric crowding of the bulky dimethyl-Im cause it to be farther below the porphyrin plane. This pulls the Fe down into the plane, making it unable to bind a second dimethyl-Im on top (it can, however, bind the smaller O_2). MeIm is not so sterically crowded and does not pull the Fe down far. Therefore the Fe can bind two MeIm's (one on top and the other on bottom), leaving no room for O_2.

25. Explain the different oxygen binding behavior of the Co(II) prophyrin.

Ans. Co(II) will only bind one axial imidazole, either 1,2-dimethylimidizole or 1-methylimidizole. Thus there is an open coordination site for O_2 in either case.

26. Indicate the oxidation state of the copper ions and of the O_2 ligand in oxyhemocyanin.

Ans. Two copper(II) ions and one peroxide ion (O_2^{2-}).

27. What single method: mass spec, UV-vis, NMR, microwave, or IR/Raman spectroscopy, would be best suited to verify the oxidation state of the O_2 ligand in oxyhemocyanin? Briefly explain your answer–for example, would oxygen isotopes such as $^{18}O_2$ be useful?

Ans. a) IR/Raman of O2 stretch would verify the oxidation staste of O_2 because more antibonding electrons have a lower stretching frequency.

b) With $^{18}O_2$ the frequency shift (isotope effect) can be used to confirm the assignment of the band.

28. Ferrochelatase is an enzyme which inserts iron into protoporphrrin IX; the iron complex is then taken up by hemoglobin. Some people are deficient in this enzyme and have some iron free protoporphyrin IX. Such individuals develop dark lesions on their skin when they are in the sun. Explain the underlying cause of this disease. Do you expect this disease to be congenital?

Ans. Porphyrins are excellent photosensitizers, exciting oxygen from triplet to singlet O2, using light energy (hv). Singlet oxygen is very reactive, and will oxidize funcionalities in the person's cells, etc, leading to the skin lesions.

Paramagnetic metal ions coordinated to the porphyrin rings quench the singlet oxygen. So, the lack of ferrochelatase, which inserts iron into protoporphyrin IX, is a problem because, without the iron, the singlet oxygen is not quenched.

This enzymatic defect is congenital. Enzymes are made by coding in the DNA, so this defect is inherited.

29. Would cytochrome-c be a useful oxygen carrier? Explain your answer.

Ans. No. The monohemeprotein, because it already has two axial ligands, has no vacant coordination sites to bind O_2.

30. What is the function of cytochrome-c?

Ans. It's an electron carrier.

31. Cobalt substituted cytochrome-c (Co substituted for Fe) is not known in nature; however, when a synthetic sample of cobalt substituted cytochrome-c was studied, it was found that the rate of electron transfer between the Co(II/III) states is much slower compared with the iron derivative. Explain the rate difference.

Ans. Redox changes in iron only involve changes in the nonbonding low spin t2g levels. In cobalt, an electron is removed from an eg antibonding orbitals, so there is a change in bond length. In general, changes in geometry slow down a reaction, so the cobalt reaction is much slower.

32. Compare the relative metal-oxygen (M-O) stretching frequencies (in the infrared or Raman spectra of oxygenated CoMb *vs* FeMb.

Ans. The Fe-O bond is shorter and stronger than the Co-O bond. Therefore, the Fe-O stretching frequency would be higher than the Co-O stretching frequency.

33. Explain why cyanide anion inhibits catalase activity for the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen but carbon monoxide does not.

Ans. Catalase goes between Fe(III) and the equivalent of Fe(V) (it's more like Fe(IV) with porphyrin being 1e⁻ oxidized as well) as H_2O_2 is oxidized and reduced by two 2e⁻ changes. Thus the thermodynamically allowed disproportionation is catalysed. The CN⁻ ion binds Fe(III) and inhibits binding of H_2O_2 and as a result stop the reaction. The CO molecule would bind Fe(II) but Fe(II) never occurs in the system resulting no binding.

34. Why does CO bind more tightly to iron(II) porphyrins? Explain in one or two sentences; comment on the vCO infrared frequency in this complex. Compare this frequency to that of free CO.

Ans. The Fe(II)-CO bond is strong because there are strong ¼-backbonding interactions which strengthen the Fe-C bond. This is much less important for Fe(III)-CO because the charge is higher on the metal. The IR frequency of free CO is higher than the IR frequency of CO in a porphyrin complex because backbonding added electron density to the p* antibonding orbital of the CO bond, decreasing its strength.

35. In what way would CO binding change the reduction potential of an iron(III) porphyrin?

Ans. Fe(III) does not bind CO, but Fe(II) binds CO strongly, creating a very stable complex (a complex more stable than Fe(II) by itself.) Since the Fe(II)-CO complex is more stable than Fe(II), the ΔG of forming the complex is lower (more negative) than the ΔG of forming Fe(II). Because $\Delta G = -nFE$, this also means that the reduction potential for Fe(III) and the complex is higher than the reduction potential for Fe(III/II).

36. In what way would cyanide alter the Fe(III)/Fe(II) reduction potential of an iron porphyrin complex?

Ans. Cyanide forms a stable complex with Fe(III) but not Fe(II). Thus, Fe(III) will not be reduced as easily in the presence of CN^-. The reduction potential would be lower.

37. What are oxidoreductases?

Ans. An oxidoreductase is an enzyme that catalyzes the transfer of electrons from one molecule, the reductant, also called the electron donor, to another the oxidant, also called the electron acceptor.

38. What are vanadium haloperoxidases?

Ans. Vanadium haloperoxidases catalyze the oxidation of halides (iodide, bromide, chloride) by hydrogen peroxide. Vanadium haloperoxidases are classified according to the most electronegative halogen oxidized. V-BrPO has been isolated and characterized from all the different classes of marine algae, including chlorophyta (green algae), phaeophyta (brown algae), and rhodophyta (red algae). The overall reaction that V-HPOs catalyse is

$$H_2O_2 + X^- + R\text{–}H + H^+ \rightarrow R\text{–}X + 2H_2O$$
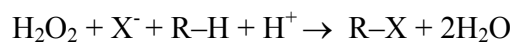
39. Show the vanadium chloroperoxidase-catalyzed oxidation of chloride by hydrogen peroxide.

Ans.
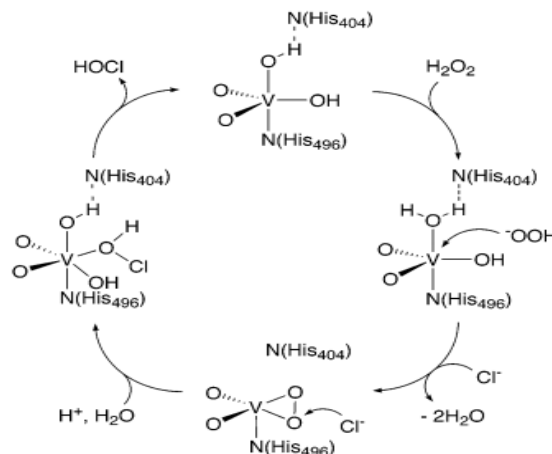
40. What are tungsten enzymes?

Ans. The biological importance of tungsten has been fully proved in recent times due to isolation of a number of tungsten-containing enzymes (W-enzymes) from hyperthermophilic archaea.

   True W-enzymes are

   1) formate dehydrogenase,

   2) aldehyde:ferredoxin-oxidoreductase (AOR from hyperthermophile),

3) formaldehyde:ferredoxin-oxidoreductase, where tungsten cannot be replaced by molybdenum.

W is able to replace Mo in Mo-enzymes, forming catalytically inactive or possesing very low activity analogs. Although a tungsten-containing xanthine dehydrogenase from bacteria has been found to contain tungsten-molydopterin and also non-protein bound selenium, a tungsten-selenium molybdopterin complex has not been definitively described. Tungsten-using enzymes typically reduce carboxylic acids to aldehydes. The tungsten oxidoreductases may also catalyse oxidations. The first tungsten-requiring enzyme to be discovered also requires selenium, and in this case the tungsten-selenium pair may function analogously to the molybdenum-sulfur pairing of some molybdenum cofactor-requiring enzymes. One of the enzymes in the oxidoreductase family which sometimes employ tungsten (bacterial formate dehydrogenase) is known to use a selenium-molybdenum version of molybdopterin.