International Graduate Program in Biological Inorganic Chemistry

Course: **Bioinorganic Chemistry**

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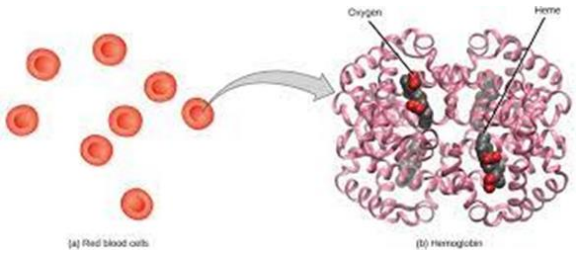
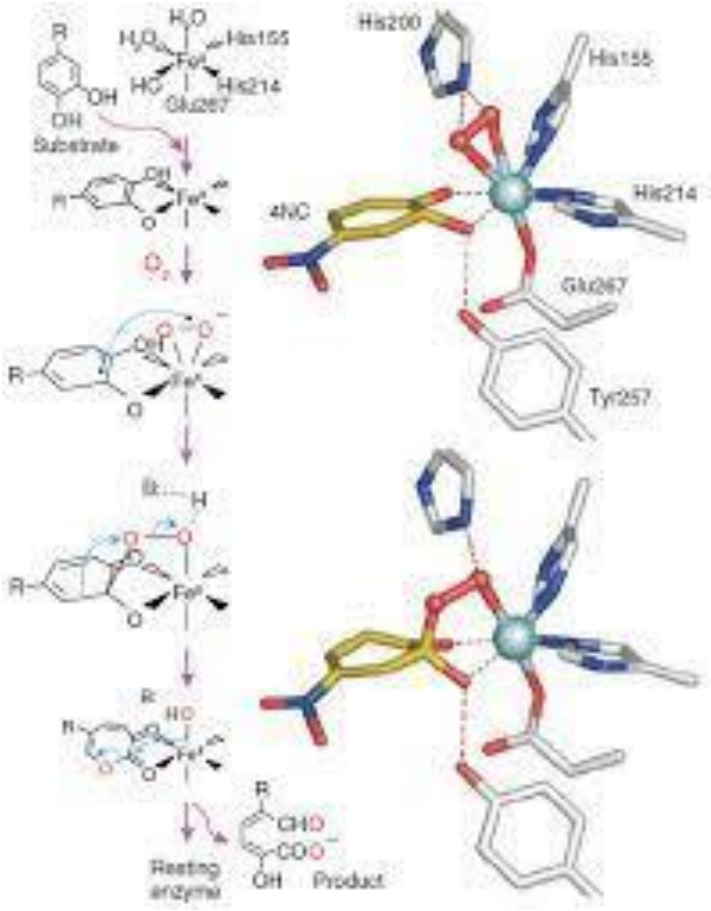
University of Patras, 2023

Outline

- Oxygen transporters
- Biological activation of O_2
- Biological nitrogen fixation





Jan Paulo T. Zaragoza and David P. Goldberg, CHAPTER 1:Dioxygen Binding and Activation Mediated by Transition Metal Porphyrinoid Complexes , in *Dioxygen-dependent Heme Enzymes*, 2018, pp. 1-36 DOI: 10.1039/9781788012911-00001
eISBN: 978-1-78801-291-1
From Book Series: Metallobiology

Biological Inorganic Chemistry: Structure and Reactivity 1st Edition
by Harry B. Gray (Editor), Edward I. Stiefel (Editor), Joan Selverstone Valentine (Editor), Ivano Bertini (Editor)

Oxygen (O_2) Transporters

(A) Properties of O_2

(i) O_2

- Dioxygen is obviously a very important molecule for biology:

(i) Energy: Respiration and the enzyme catalyzed reduction to H_2O.

(ii) Source of O atoms: Enzyme catalyzed biosynthetic reactions of organic substrates, referred to as O_2 activation.

- However, simple O_2 diffusion is not a sufficient means for complex multi-celled organisms to transport O_2.
- **Complex multi-celled organisms require soluble O_2 transporter proteins!**

Oxygen (O_2) Transporters

(A) Properties of O_2

(i) O_2

- **Problems with O_2 transport** in biological systems:

(1) Low solubility of O_2 in water (5.4 mL/L at 37ºC).

(2) O_2 is an oxidizing agent and therefore toxic. Combination of O_2 and highly reducing cellular environment is thermodynamically unstable.

(3) Reactive Oxygen Species (ROS): O_2^- (superoxide), H_2O_2, small amounts of these species can cause oxidative damage (antioxidants, catalase, peroxidase, SOD).

(8) Oxygen (O_2) Transporters

(A) Properties of O_2

(ii) Chemistry of O_2

(a) Thermodynamics

(1) Electron transfer reactions (products contain only O or H):

One electron reduction of O_2:

$O_2 + e^- \rightarrow O_2^-$ $\quad E_{red} = -0.33$ V

$O_2^- + e^- + 2H^+ \rightarrow HOOH$ $\quad E_{red} = +0.98$ V

$HOOH + e^- + H^+ \rightarrow H_2O + OH$ $\quad E_{red} = +0.38$ V

$OH + e^- + H^+ \rightarrow H_2O$ $\quad E_{red} = +2.31$ V

} One electron reduction of O_2 is not favorable.

Two electron reduction of O_2:

$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$ $\quad E_{red} = +0.281$ V

$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O$ $\quad E_{red} = +1.349$ V

$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ $\quad E_{red} = +0.815$ V

Oxygen (O_2) Transporters

(A) Properties of O_2

(ii) Chemistry of O_2

(a) Thermodynamics

(2) Atom transfer reaction

- Generate C-O bonds, very important biologically speaking:

$CH_4 + O_2 \rightarrow 2\ CH_3OH$ $\quad \Delta G = -30$ kcal/mol

$2\ C_6H_6 + O_2 \rightarrow 2C_6H_5OH$ $\quad \Delta G = -43$ kcal/mol

- These combustion reactions are very thermodynamically favorable, but they are slow! High E_a.

Oxygen (O_2) Transporters

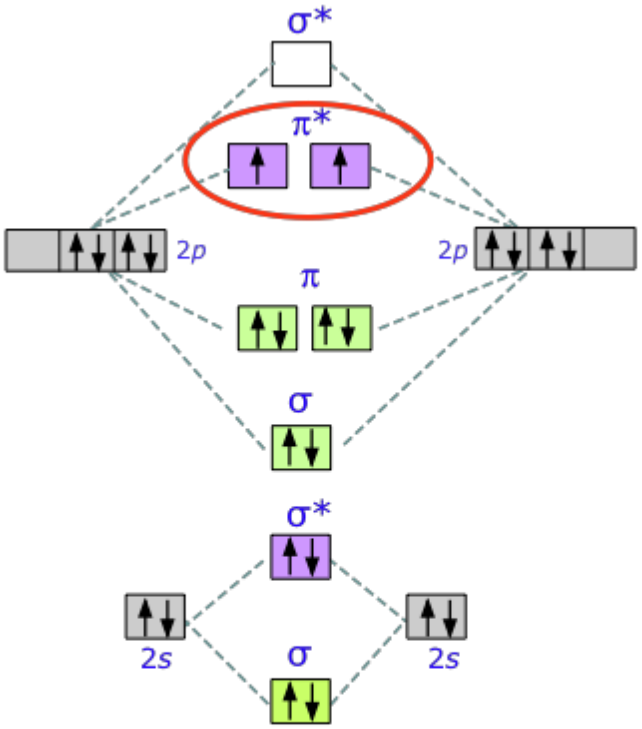
(A) Properties of O_2

(ii) Chemistry of O_2

(b) Kinetics

- O_2 has a triplet ground state (two unpaired electrons in $\pi^*_x\pi^*_y$ molecular orbitals, $S = 1$).
- Reactions between O_2 and most organic molecules (singlet ground states) are quantum mechanically forbidden (triplet to singlet conversions are forbidden).
- Results in high energy barrier to reaction.
- Reacts readily with radical species (Including transition metals with unpaired electrons).

Electronic structure of O_2:



dioxygen O_2

bond order 2
bond energy 498 kJ

	Bond Order	Internuclear Distance (pm)
O_2^+ (dioxygenyl)	2.5	111.6
O_2 (dioxygen)	2.0	120.8
O_2^- (superoxide)	1.5	135
O_2^{2-} (peroxide)	1.0	149

triplet singlet

Concerted: $^3O_2(\uparrow\uparrow) + {}^1X(\uparrow\downarrow) \not\rightarrow {}^1XO_2(\uparrow\downarrow)$

Allowed pathway 1:

$$^3O_2(\uparrow\uparrow) + {}^1X(\uparrow\downarrow) \rightarrow \boxed{{}^3XO_2(\uparrow\uparrow)}$$

$$^3XO_2(\uparrow\uparrow) \rightarrow {}^1XO_2(\uparrow\downarrow)$$

40-70 kcal > ground singlet state

Allowed pathway 2:

$$^3O_2(\uparrow\uparrow) \rightarrow \boxed{{}^1O_2(\uparrow\downarrow)}$$

$$^1O_2(\uparrow\downarrow) + {}^1X(\uparrow\downarrow) \rightarrow {}^1XO_2(\uparrow\downarrow)$$

22.5 kcal > ground triplet state

Allowed pathway 3:

$$^1X(\uparrow\downarrow) \rightarrow {}^3X(\uparrow\uparrow)$$

$$^3O_2(\uparrow\uparrow) + {}^3X(\downarrow\downarrow) \rightarrow {}^1XO_2(\uparrow\downarrow)$$

Reactions requiring spin flips all have high activation barriers, i.e., are slow.

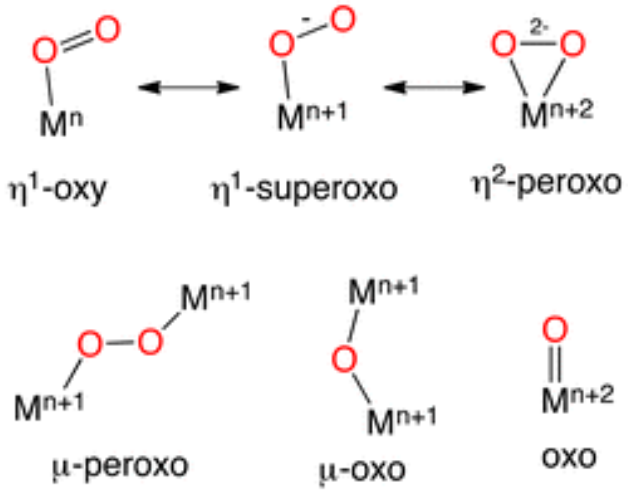
Oxygen (O_2) Transporters

(A) Properties of O_2

(ii) Chemistry of O_2

(c) Metal ion binding modes of O_2

- Mononuclear: Bent (σ, neutral, superoxo, or peroxo) and side on (π, dianion)
- Bridging dinuclear: Bent end on (μ-1,2-peroxo), side on planar (μ-η^2:η^2-peroxo or μ-dioxo), and side on bent.



Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(i) Introduction

- Special transport proteins have evolved to transport and store O_2 in organisms. Why?

(1) Low solubility of O_2 in water

(2) Supplying O_2 to buried tissue

- 3 types:

(1) Hemoglobin-myoglobin: Evolutionary diverse; heme

(2) Hemocyanin: Molluscs & anthropods; binuclear Cu

(3) Hemerythrin: Marine invertebrates; binuclear Fe

Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(ii) Hemoglobin-Myoglobin

(a) Myoglobin (Mb or deoxyMb)

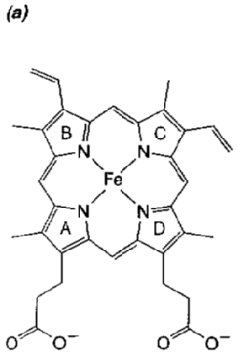
- Heme protein (17 kDa), coordinates O_2 reversibly and controls [O_2] in tissue.
- Contains one heme cofactor.
- Binds O_2 less tightly than hemoglobin at high O_2 pressure (non-cooperative binding).

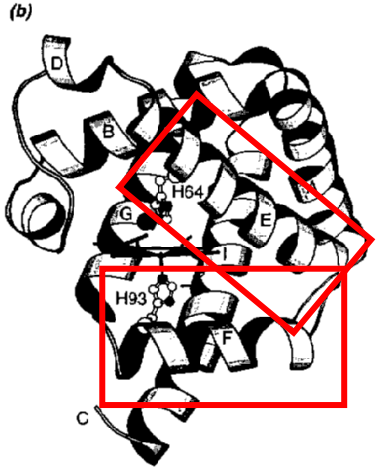
Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(ii) Hemoglobin-Myoglobin

(a) Myoglobin (Mb or deoxyMb)





Deoxy: Fe(II) and vacant 6th coordination site

Oxy: Fe(II) and O_2 binding in 6th coordination site

distal His (H64, or E7His)

DeoxyMb **OxyMb**

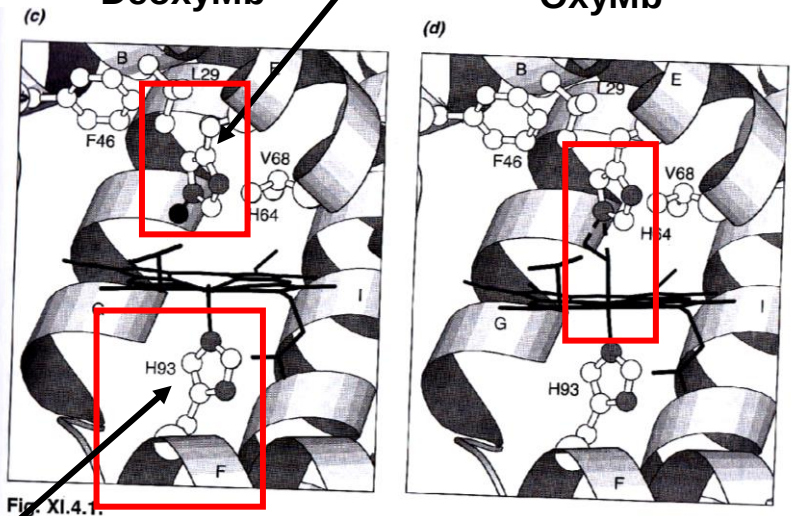


Fig. XI.4.1. The heme group in sperm whale myoglobin. (*a*) The heme *b* (iron protoporphyrin IX) group is the active site of myoglobin. (*b*) The heme *b* group sits in a cleft formed by helices E and F. The side chains of the proximal His (H93) and the distal His (H64) are shown. For deoxyMb a water molecule is hydrogen bonded to the distal His and sits inside the ligand-binding pocket. The view is directly into the ligand-binding pocket. (*c*) Closeup of the active site of deoxyMb (PDB code: 1A6N). In addition to the distal His, a Leu (L29), Val (V68), and a Phe (F46) line the ligand-binding pocket. (*d*) Closeup of the active site of oxyMb (PDB code: 1A6M). The coordinated O_2 hydrogen bonds to the distal His. Note how the Fe atom moves into the plane of the porphyrin on binding of O_2. The distal and proximal histidines are often designated by their position on the helix, a manner that is independent of the species of Mb or Hb: The distal His is the seventh residue along helix E and is denoted E7His; analogously, the proximal His is denoted F8His.

proximal His (H93, or F8His)

Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(ii) Hemoglobin-Myoglobin

(b) Hemoglobin (Hb or deoxyHb)

- Heme protein (68 kDa) found in red blood cells (erythrocytes).
- 1 L human blood = 150 g
- A $\alpha_2\beta_2$ tetramer contains four heme cofactors (structure is analogous to four Mb units with a central cavity).
- Binds O_2 more tightly (cooperative binding) than Mb at high O_2 pressure.

Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(ii) Hemoglobin-Myoglobin

(b) Hemoglobin (Hb or deoxyHb)

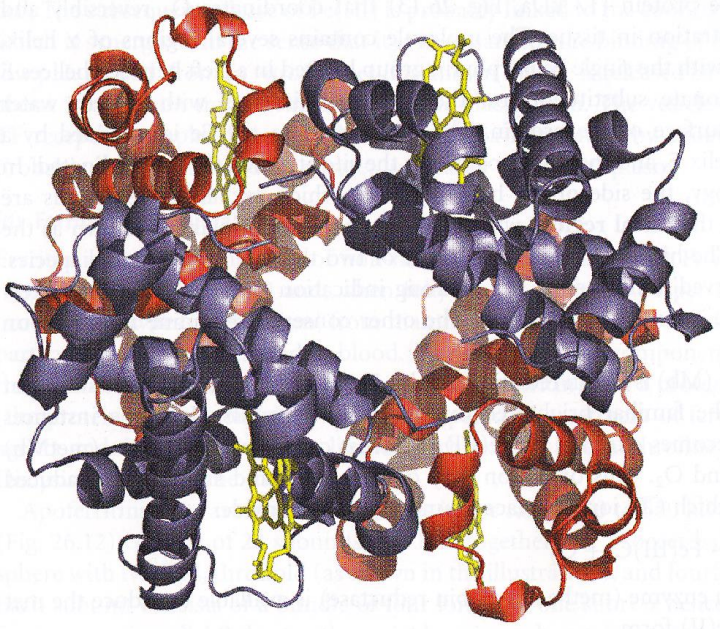


Fig. 26.16 Haemoglobin is an $\alpha_2\beta_2$ tetramer. Its α and β subunits are very similar to myoglobin. Haem groups are shown in yellow.

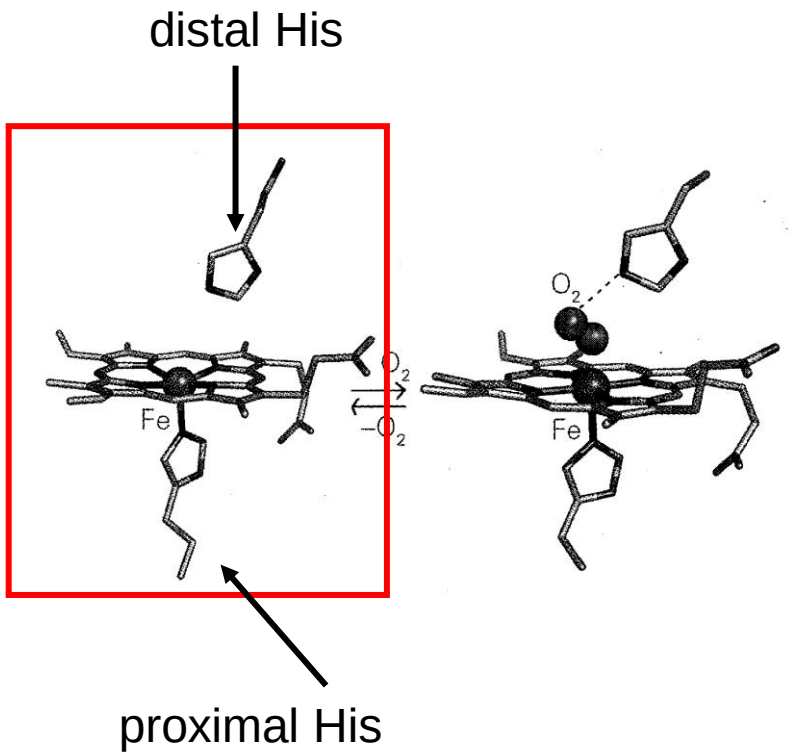
Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(ii) Hemoglobin-Myoglobin

(c) Structure of O_2 binding site:

- Similar for Hb & Mb



- Square pyramidal (5-coordinate)
- High-spin Fe(2+)
- Fe coordination is out-of-the-plane of the porphyrin.
- Red: π-π^* (Soret)

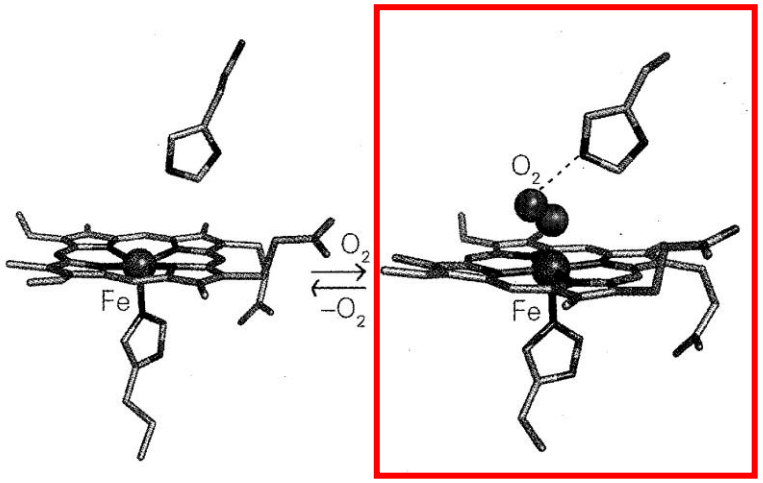
Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(ii) Hemoglobin-Myoglobin

(c) Structure of O_2 binding site:

- Similar for Hb & Mb



- 6-coordinate
- Fe oxidation/spin state?
- Fe shrinks and drops into-the-plane
- Red-purple: π-π^*
- EPR silent, 2 possibilities:

(1) LS Fe(2+)—O_2 (neutral)

(2) LS Fe(3+)—O_2^- (superoxide)

νO_2^- ~ 1145 cm^{-1}, found at 1103 cm^{-1} in oxyMb.

Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(ii) Hemoglobin-Myoglobin

(d) Cooperative binding of O_2 to Hb

- Cooperativity: Binding (or release) of one O_2 molecule to (from) Hb tetramer increases K_{eq} for binding (or release) of subsequent O_2 molecules.
- In mammals, the fourth O_2 molecule binds with 100-fold greater affinity than the first.
- Hemoglobin exists in two distinct conformations: T (*tensed*, deoxy) to R (*relaxed*, oxy) transition.

T to R transition is related to change in size of Fe ion upon O binding, movement down into-plane of porphyrin ring, and consequent tug on coordinated His residue.

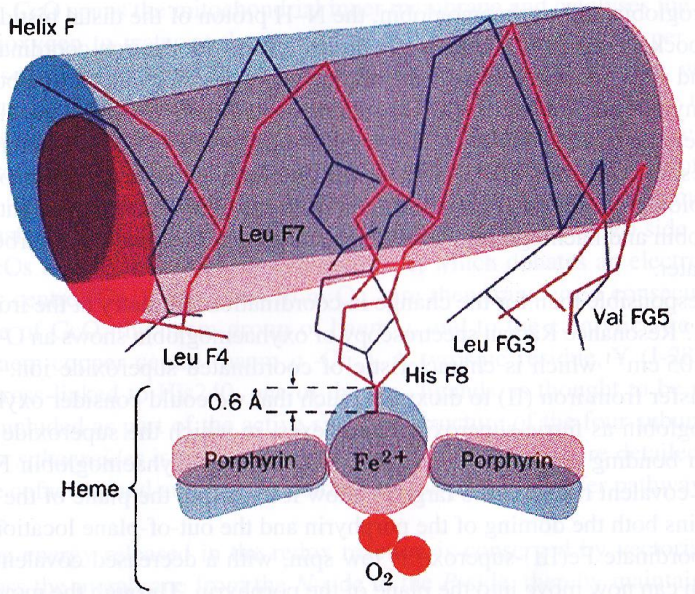


Figure 13.7 The triggering mechanism for the T to R transition in haemoglobin. (From Voet and Voet, 2004. Reproduced with permission from John Wiley & Sons., Inc.)

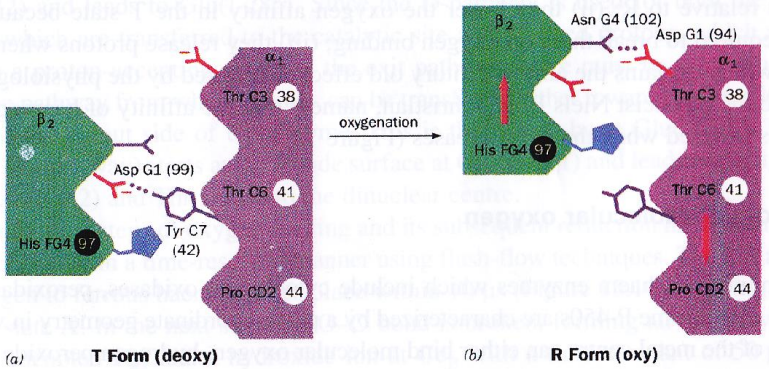


Figure 13.8 The α_1–β_2 interface in (a) human deoxyhaemoglobin and (b) oxyhaemoglobin. (From Voet and Voet, 2004. Reproduced with permission from John Wiley & Sons., Inc.)

Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(iii) Hemocyanin (Hc)

- Type 3 Cu protein.
- *Cyanos* is Greek for blue.
- Molluscs (octupus & snail).
- Anthropods (lobsters, scorpions, crabs, spiders, ….).
- Very complex, large protein structures; large assemblies of subunits.

"Blue blood"

Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(iii) Hemocyanin (Hc)

(a) Mollusc Hc

- 10-20 subunits, ~9000 kDa
- Each subunit contains 8 covalently linked domains
- Each domain has a mass of ~52 kDa and contains one bimetallic Cu center
- Hc proteins are cylindrical (SEM), 190-380 Å long and 350 Å in diameter.

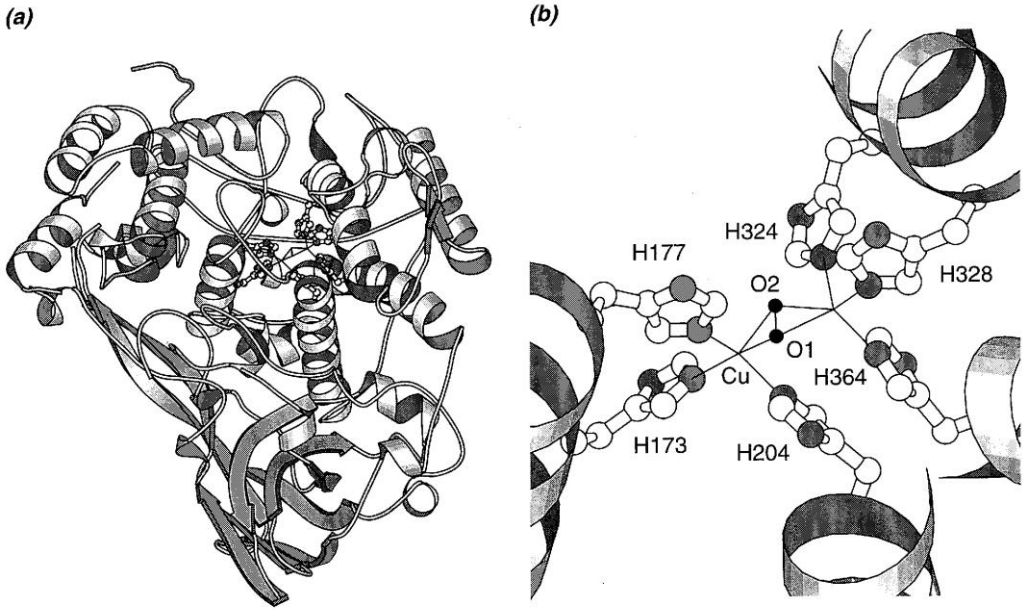
Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(iii) Hemocyanin (Hc)

(a) Mollusc Hc

Fig. XI.4.5.
Oxyhemocyanin of the arthropod horse crab, *L. polyphemus* [PDB code: 1NOL (oxy), 1LLA (deoxy)]. (*a*) One subunit with the side chains of the coordinated His residues shown. (*b*) Closeup of the active site showing the manner by which O_2 is bound by the pair of Cu atoms.



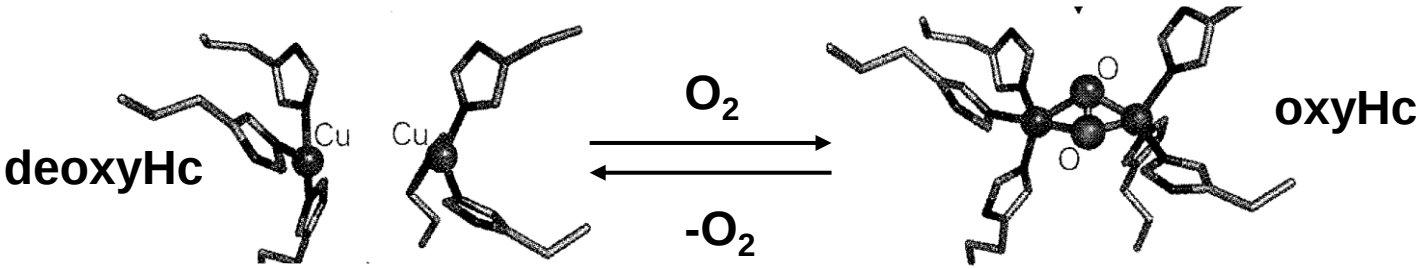
Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(iii) Hemocyanin (Hc)

(b) Structure of O_2 binding site in Hc:

- Established by XAS, EXAFS & X-ray diffraction experiments.
- Bimetallic Cu^+ with each Cu coordinated by 3 His residues:



- trigonal pyramidal
- Cu+, d^{10}, diamagnetic
- EPR silent & colorless

- 5-coordinate, μ-η^2:η^2-peroxo, Cu^{2+}, d^9
- Intense blue: LMCT (peroxo-to-Cu^{2+}), 580 nm
- EPR silent, strong AFM coupling
- νO_2 ~ 750 cm^{-1} (H_2O_2, 880 cm^{-1})

Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(iii) Hemocyanin (Hc)

(c) Cooperative O_2 binding

- In deoxyHc, Cu^+ centers are 4.6 Å apart; O_2 binding brings Cu^{2+} ions closer together (3.6 Å) in oxyHc.
- This movement displaces linked amino acids, which affects subunit interactions and leads to cooperativity in O_2 binding.
- Magnus, K. A. *Chem. Rev*. **1994**, *94*, 727-735.

Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(iv) Hemerythrin (Hr)

- Rare: Found in marine invertebrates, such as non-segmented worms, some segmented worms, shrimp and priapulid families.
- Monomeric (myoHr), trimeric, and octameric forms of Hr are known.
- All feature similar subunit of 13.5 kDa (8 subunits ~105 kDa)

Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(iv) Hemerythrin (Hr)

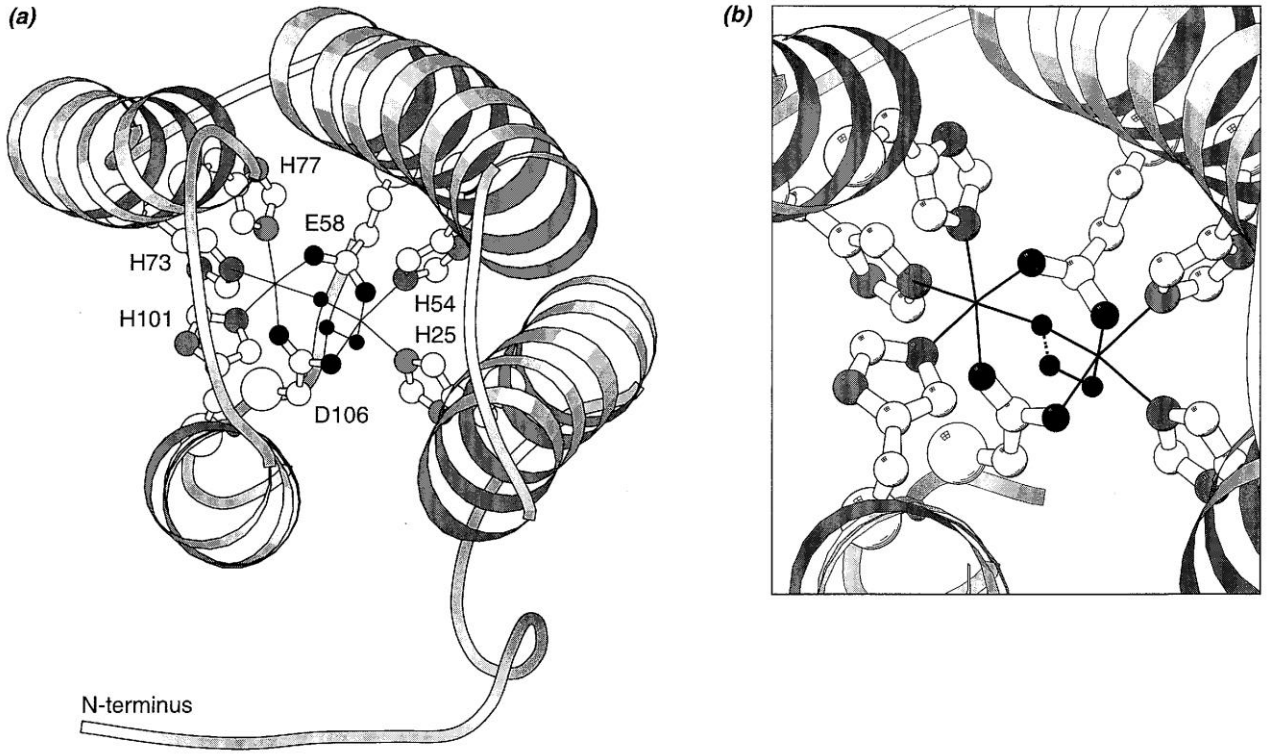


Fig. XI.4.6.
Oxyhemerythrin of the sipunculid worm, *Themiste dyscrita* [PDB code: 1HMO (oxy), 1HMD (deoxy)]. (*a*) One subunit of the octameric protein. (*b*) Closeup of the active site showing the manner by which O_2 is bound to a single Fe atom and is hydrogen bonded to the bridging oxo moiety.

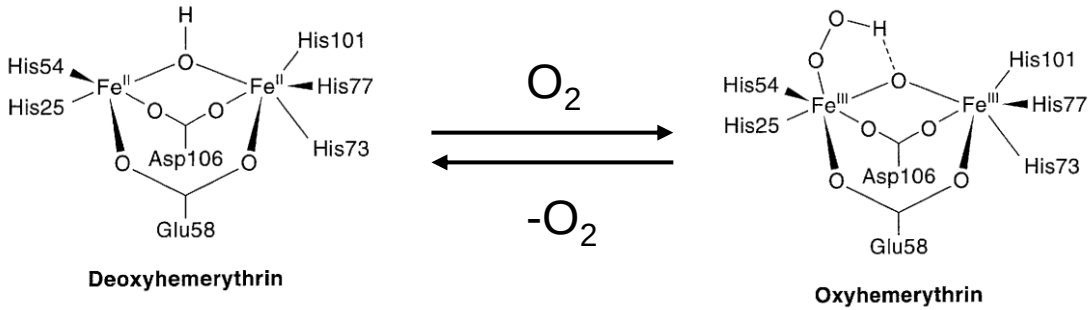
Oxygen (O_2) Transporters

(B) Biological O_2 transport systems

(iv) Hemerythrin (Hr)

(a) Structure of O_2 binding site:

- Bimetallic Fe^{2+} centers are triply bridged by OH^- (deoxyHr) or O^{2-} (oxyHr) and two μ-1,3-carboxylate bridges from asp and glu residues; 5 of 6 remaining coordination sites occupied by His.



Deoxyhemerythrin

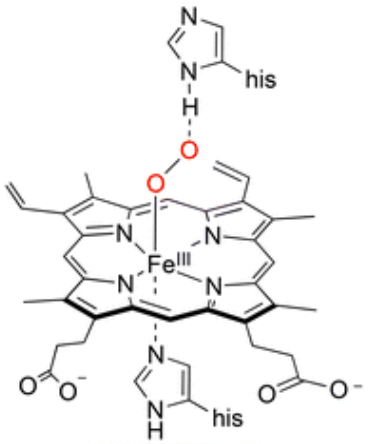
- Two high-spin Fe^{2+} ions
- Weak AFM coupling; J ~ -10 cm^{-1}
- Colorless

Oxyhemerythrin

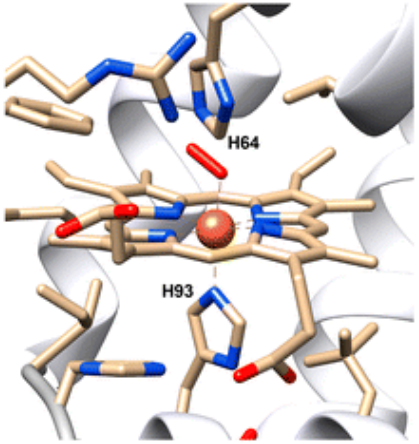
- Two high-spin Fe^{3+} ions
- Strong AFM coupling; J ~ -100 cm^{-1}
- Two electrons are transferred from Fe^{2+}, generating OOH (peroxide)
- Intensely purple (peroxo-to-Fe^{3+} LMCT)

Oxygen (O_2) Transporters

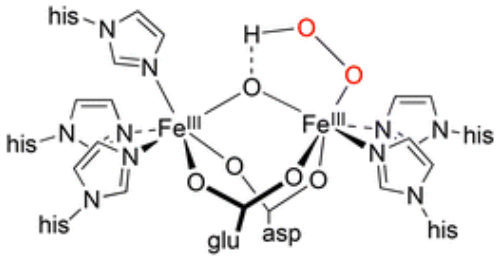
All together…



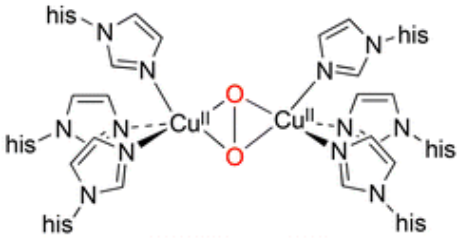
Hemoglobin



Myoglobin



Hemerythrin



Hemocyanin

Biological activation of O_2

Biological activation of O_2

(A) Introduction

O_2 activation: Insertion of O atoms from molecular O_2 to "inert" organic substrates.

- An extremely important biological reaction.
- Examples of important O_2 activation processes:

(1) $CH_4 + O_2 \rightarrow CH_3OH$ by methanotrophic bacteria.

(2) Amino acid modification & peptide processing.

(3) Biosynthesis of neutransmitters, hormones, antibiotics.

(4) Detoxification of xenobiotics (substances foreign to a biological system), i.e. dioxins or polychlorinated biphenyls (PCBs).

Biological activation of O_2

(A) Introduction

- O_2 activation is carried out by a diverse array of enzymes (active-site structures are very similar to O_2 transporters). Two classes:

1. Monooxygenases: Insert one O in substrate and one O into H_2O.

$$\mathbf{RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O}$$

2. Dioxygenases: Insert both O atoms into substrate.

$$\mathbf{H\text{-}R\text{-}R'\text{-}H + O_2 \rightarrow HO\text{-}R\text{-}R'\text{-}OH}$$

Biological activation of O_2

(B) Monoxygenases

(i) Cytochrome P450

- Most studied and best understood O_2 activating enzyme.

- Found in liver tissue, and functions in mammalian metabolism: Biosynthesis of steroids (progesterone); detoxification & carcinogenesis; drug metabolism.

- Active site structure is similar to hemoglobin, exception of proximate ligand (cysteine).

- “P450” = In CO adduct, Soret band at 450 nm.

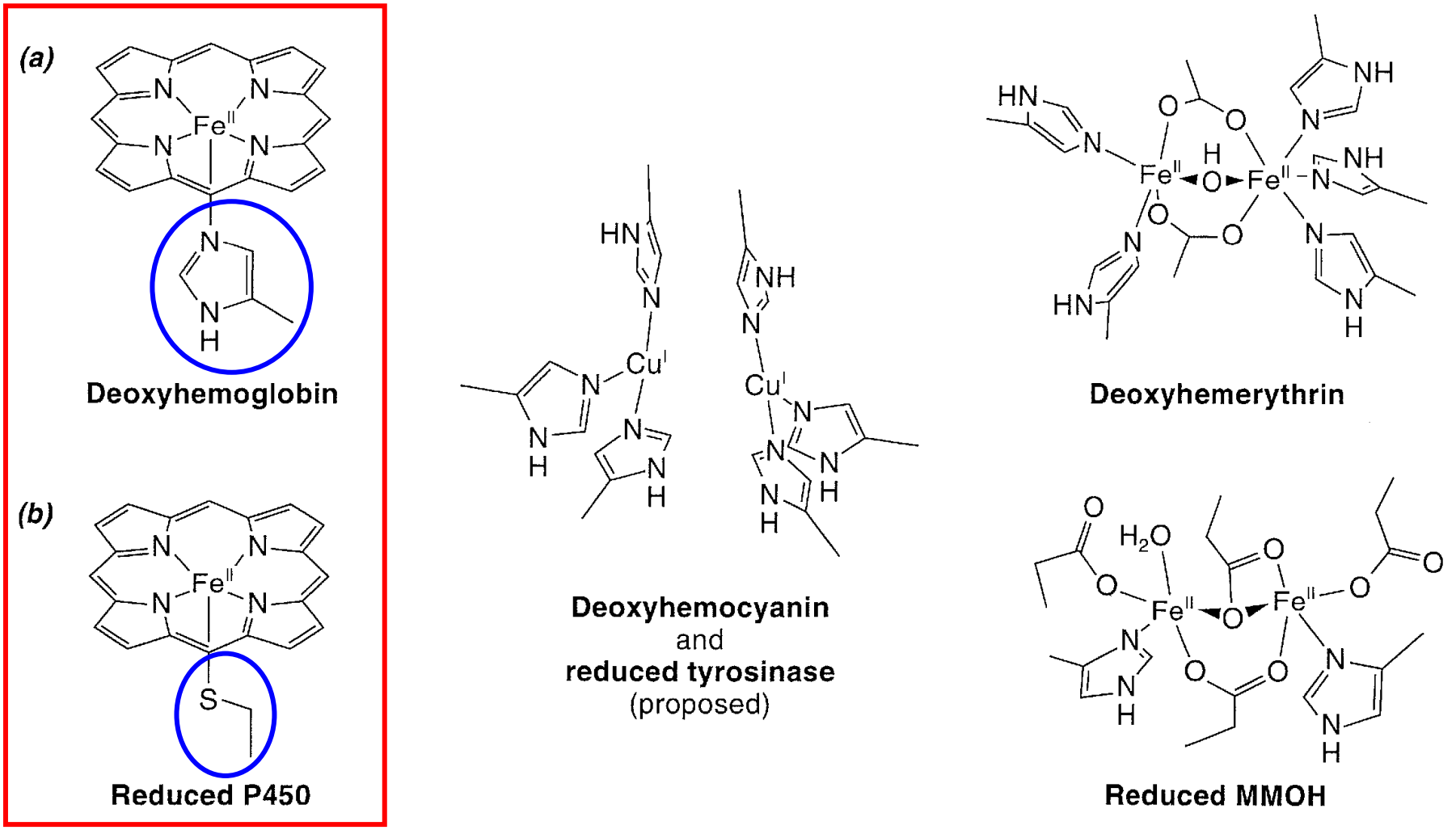


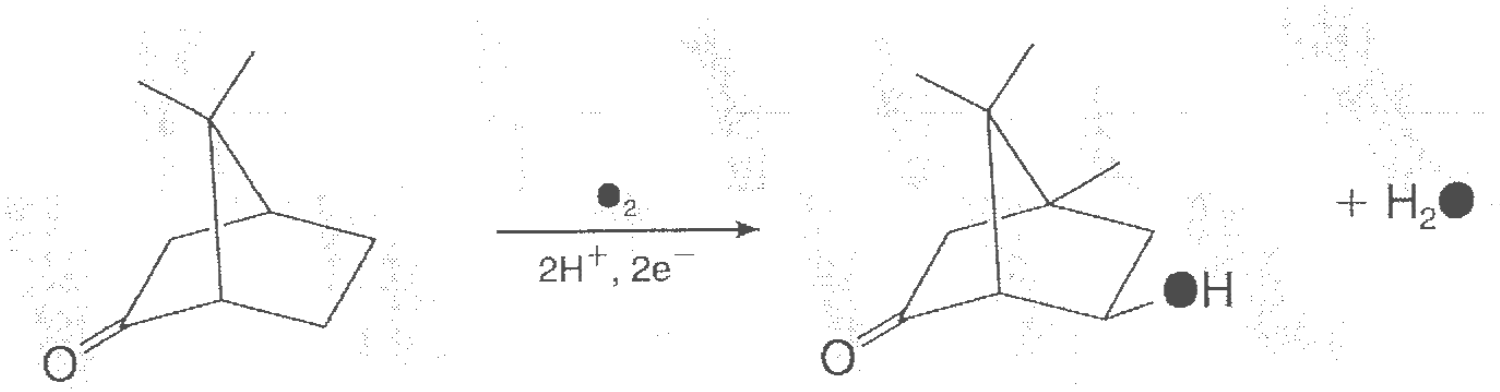
Fig. XI.5.1.
Active sites of the three known O_2 carriers (*a*) and their counterparts in oxygen activation (*b*). (MMOH = methane monooxygenase hydroxylase)

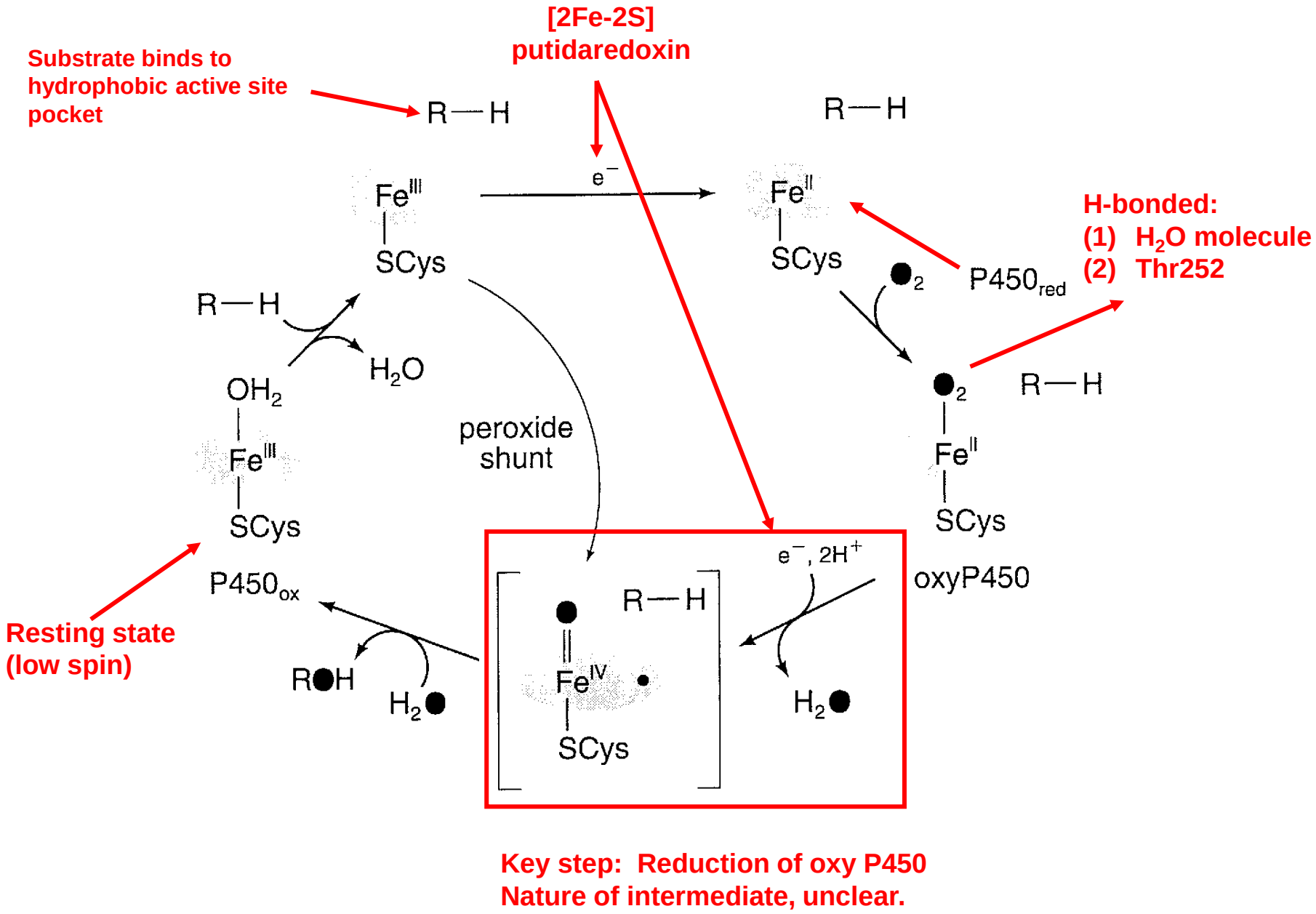
Biological activation of O_2

(B) Monoxygenases

(i) Cytochrome P450

Catalytic mechanism: Deduced from spectroscopic and crystallographic studies of cyt $P450_{cam}$ (*Pseudomonas putida*), which hydroxylates camphor at C5.




Substrate binds to hydrophobic active site pocket
[2Fe-2S] putidaredoxin
R—H
R—H
e⁻
Fe^III
SCys
Fe^II
SCys
H-bonded:
(1) H2O molecule
(2) Thr252
P450red
O2
R—H
H2O
OH2
Fe^III
SCys
peroxide shunt
O2
R—H
Fe^II
SCys
oxyP450
e⁻, 2H⁺
P450ox
Resting state (low spin)
R—H
ROH
H2
Fe^IV
SCys
H2
Key step: Reduction of oxy P450
Nature of intermediate, unclear.

Biological activation of O_2

(B) Monoxygenases

(ii) Monoxygenases with dinuclear active sites

E.g. Tyrosinase and methane monoxygenases, etc.

- Why two metal centers?

(1) The second metal may serve as an additional electron source to reduce O_2 to peroxide without external electron donor (as is needed with cyt P450).

(2) The second metal may serve as a Lewis acid in place of H^+ to help cleave the O—O bond and stabilize oxide ions that form upon O—O bond cleavage.

Biological activation of O_2

(B) Monoxygenases

(ii) Monoxygenases with dinuclear active sites

(a) Tyrosinase

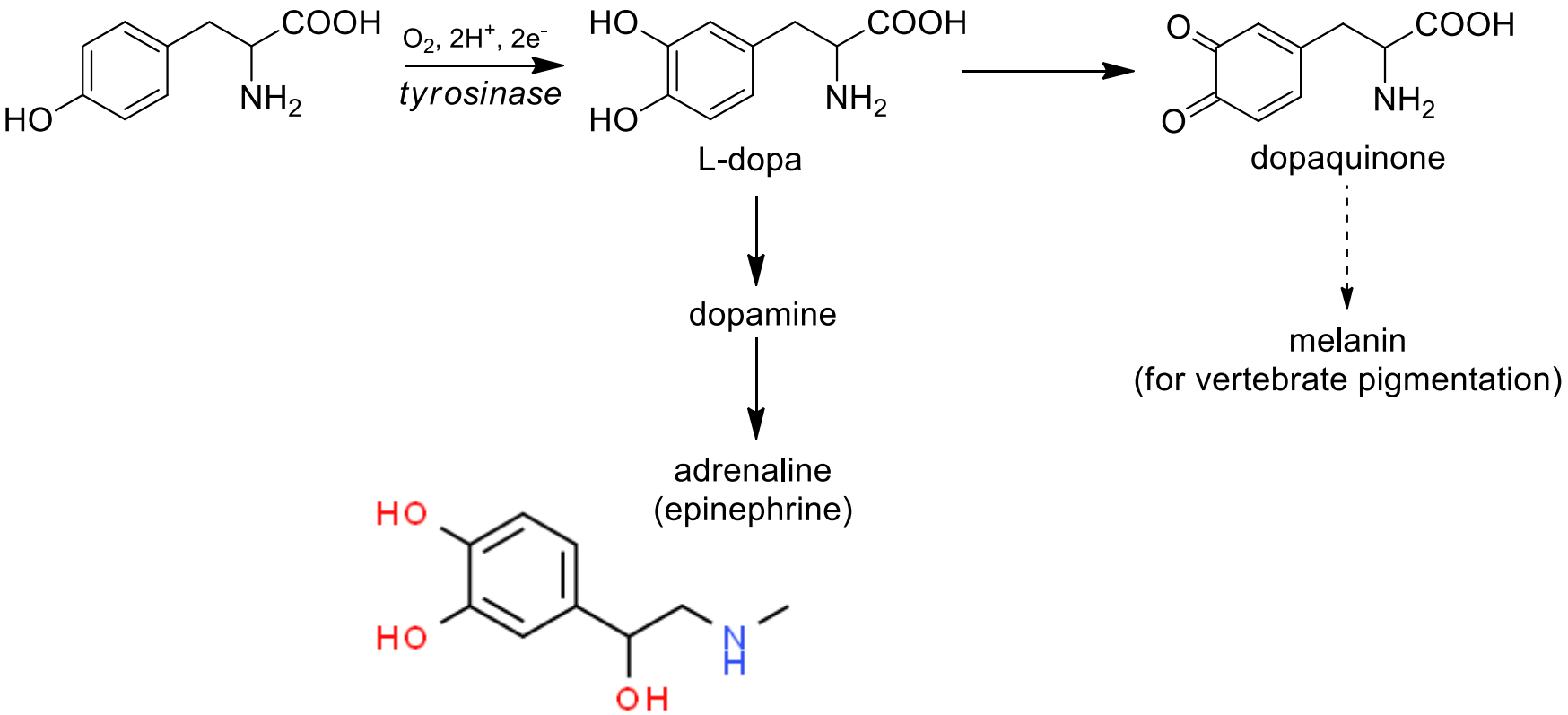
- The active site is thought to be structurally similar to hemocyanin based on spectroscopic data.
- No structural details through X-ray diffraction studies are known for this enzyme.
- Function: Hydroxylation of tyrosine to L-dopa and oxidation of L-dopa to dopaquinone.
- General: Oxidation of phenols to catechols and catechols to orthoquinones.

Biological activation of O_2

(B) Monoxygenases

(ii) Monoxygenases with dinuclear active sites

(a) Tyrosinase



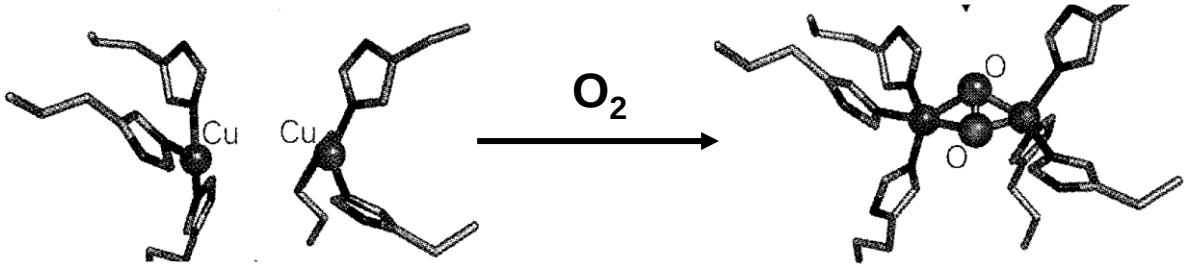
Biological activation of O_2

(B) Monoxygenases

(ii) Monoxygenases with dinuclear active sites

(a) Tyrosinase

Catalytic mechanism & active species:



deoxytyrosinase

• Assumed to feature a Cu-Cu distance of 4.4Å, like hemocyanin.

oxytyrosinase

• 5-coordinate, μ-η^2:η^2-peroxo?

• Cu-Cu distance = 3.6 Å (EXAFS)

• λ_{max} = 590 nm (LMCT)

• νO_2 ~ 755 cm^{-1} (H_2O_2, 880 cm^{-1})

Biological activation of O_2

(B) Monoxygenases

(ii) Monoxygenases with dinuclear active site

(b) Methane monoxygenase (MMO)

- Found in “methanotrophs”, bacteria that metabolize CH_4 as energy source:

$$CH_4 + MMO \rightarrow CH_3OH$$

- Two types: (i) s(soluble)MMO (iron), (ii) p(particulate)MMO (copper)

- “Multi-component” enzyme:
  - (1) hydroxylase (catalytic activity)
  - (2) reductase (e- source)
  - (3) third component regulates activity of (1) & (2)

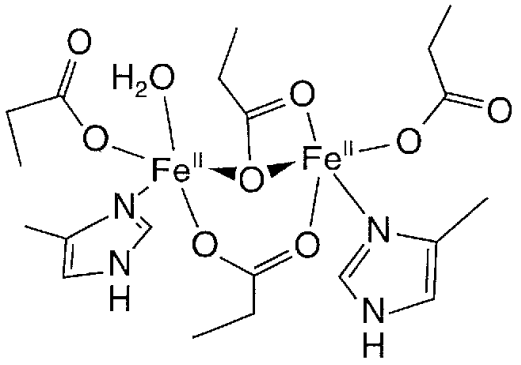
Biological activation of O_2

(B) Monoxygenases

(ii) Monoxygenases with dinuclear active site

(b) Methane monoxygenase (MMO)

- Active site structure is similar to hemerythrin; contains a non-heme bimetallic iron center.
- Core: $Fe_2(\mu\text{-}RCO_2)_2$ + 2 terminal his + 2 terminal carboxylates
- One vacant coordination site on each Fe (this is different from hemerythrin)



Biological activation of O_2

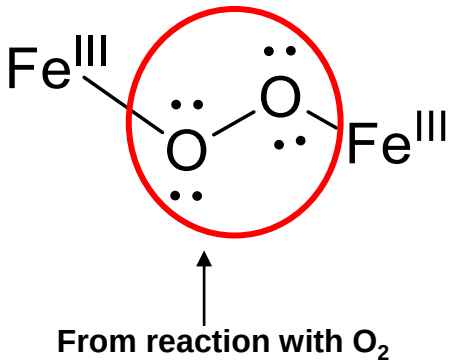
(B) Monoxygenases

(ii) Monoxygenases with dinuclear active site

(b) Methane monoxygenase (MMO)

Catalytic mechanism and active site structure:

- Reaction with O_2 produces two intermediates which have been spectroscopically characterized.
- **P → (μ-1,2-peroxo)diiron(III) species**



λ_{max} = 700 nm (peroxo-to-Fe^{III} CT)

δ = 0.66 mms^{-1}

Biological activation of O_2

(B) Monoxygenases

(ii) Monoxygenases with dinuclear active site

(b) Methane monoxygenase (MMO)

Catalytic mechanism and active site structure:

- Reaction with O_2 produces two intermediates which have been spectroscopically characterized.
- **Q → Responsible for oxidation of CH_4**
- Structure?
- Mössbauer suggests an AFM coupled bimetallic dioxo-bridged Fe^{IV} center, resulting from O-O bond cleavage (δ = 0.17 mms^{-1})
- EXAFS: Fe-Fe distance of 2.5 Å, supports $Fe_2(\mu\text{-}O)_2$ core as do DFT calculations.

Biological activation of O_2

(B) Monoxygenases

(ii) Monoxygenases with dinuclear active site

(b) Methane monoxygenase (MMO)

Catalytic mechanism and active site structure:

- Two important mechanistic questions remain:

(1) What is the nature of the P to Q conversion?

- See Que Jr. *PNAS* **2008**, *105*, 20615-20620.

(2) What is the nature of the reaction between Q with substrate?

- See Que Jr. *Nature Chemistry*, **2010**, *2*, 400-405.

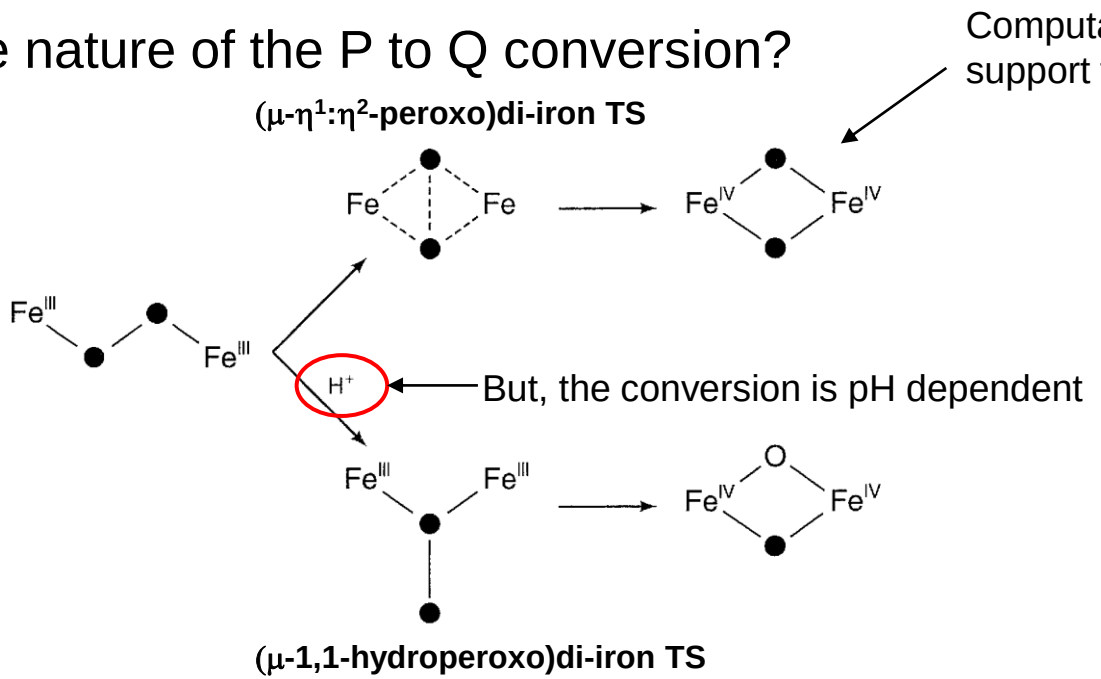
Biological activation of O_2

(B) Monoxygenases

(ii) Monoxygenases with dinuclear active site

(b) Methane monoxygenase (MMO)

(1) What is the nature of the P to Q conversion?



Biological activation of O_2

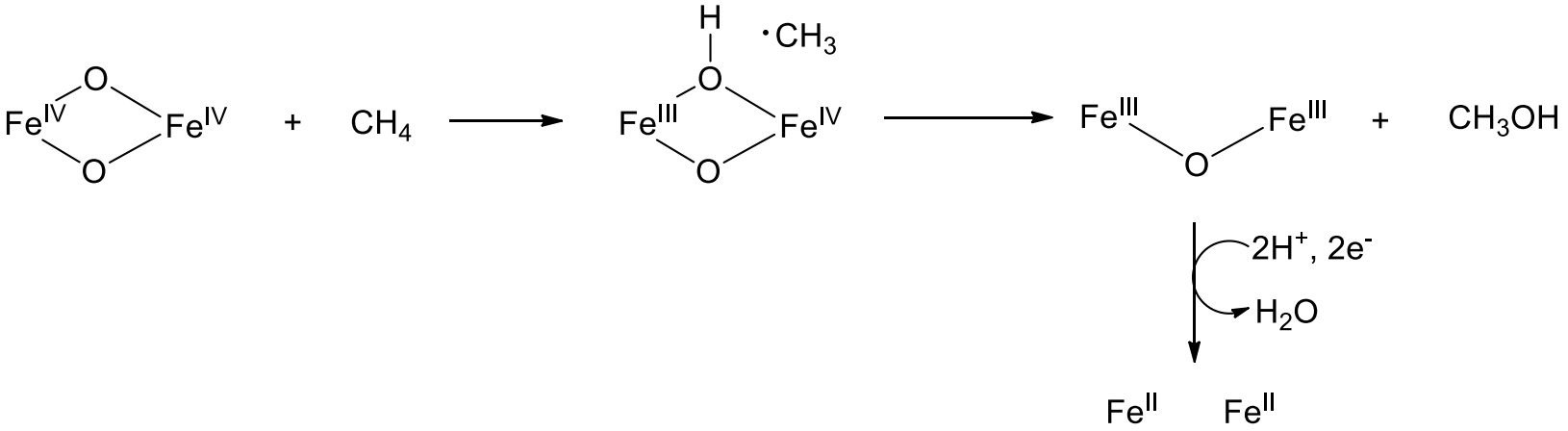
(B) Monoxygenases

(ii) Monoxygenases with dinuclear active site

(b) Methane monoxygenase (MMO)

(2) What is the nature of the reaction between Q with substrate?

Oxygen rebound mechanism?



(9) Biological activation of O_2

(A) Introduction

(B) Monoxygenases

(i) Cytochrome P450

(ii) Monoxygenases with dinuclear active sties

(a) Tyrosinase

(b) Methane monoxygenase

(c) Copper hydroxylases: DBH & PHM

- Bimetallic copper containing enzymes that activate O_2 and stereospecifically hydroxylate substrate (aliphatic C-H bond).

Biological activation of O_2

(A) Introduction

- O_2 activation is carried out by a diverse array of enzymes (active-site structures are very similar to O_2 transporters). Two classes:

1. Monooxygenases: Insert one O in substrate and one O into H_2O.

$$\mathbf{RH + O_2 + \boxed{2H^+ + 2e^-} \rightarrow ROH + \boxed{H_2O}}$$

2. Dioxygenases: Insert both O atoms into substrate.

$$\mathbf{H\text{-}R\text{-}R'\text{-}H + O_2 \rightarrow HO\text{-}R\text{-}R'\text{-}OH}$$

Biological activation of O_2

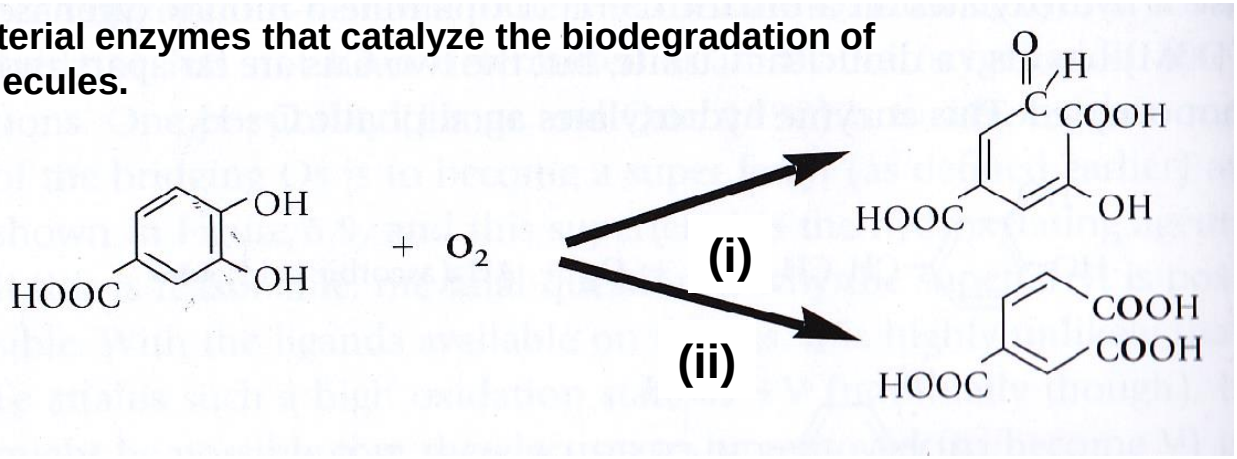
(C) Dioxygenases

- Two general classes:

(i) Fe^{2+} mononuclear, non-heme → "extradiol" dioxygenases (catechol dioxygenases)

(ii) Fe^{3+} mononuclear, non-heme → "intradiol" dioxygenases

Both are bacterial enzymes that catalyze the biodegradation of aromatic molecules.



Biological activation of O_2

(C) Dioxygenases

(a) Extradiol dioxygenases: Transform aromatic compounds into aliphatic compounds by cleavage of “extradiol” C-C bond.

- All extradiol dioxygenases share a common structural motif: 2-His-1-carboxylate facial triad:

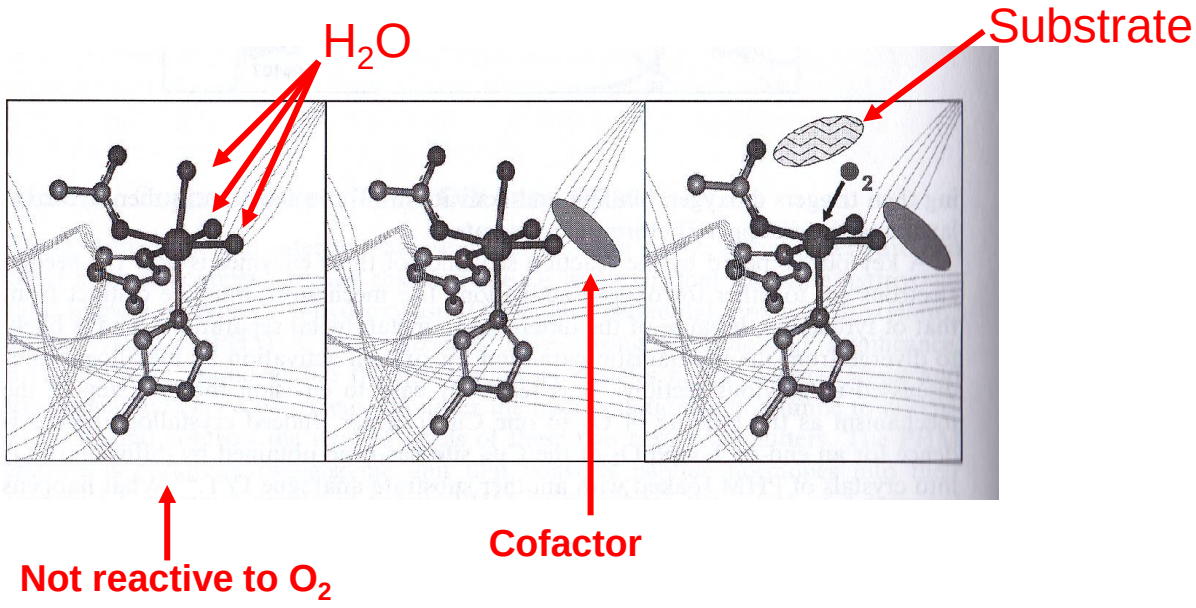


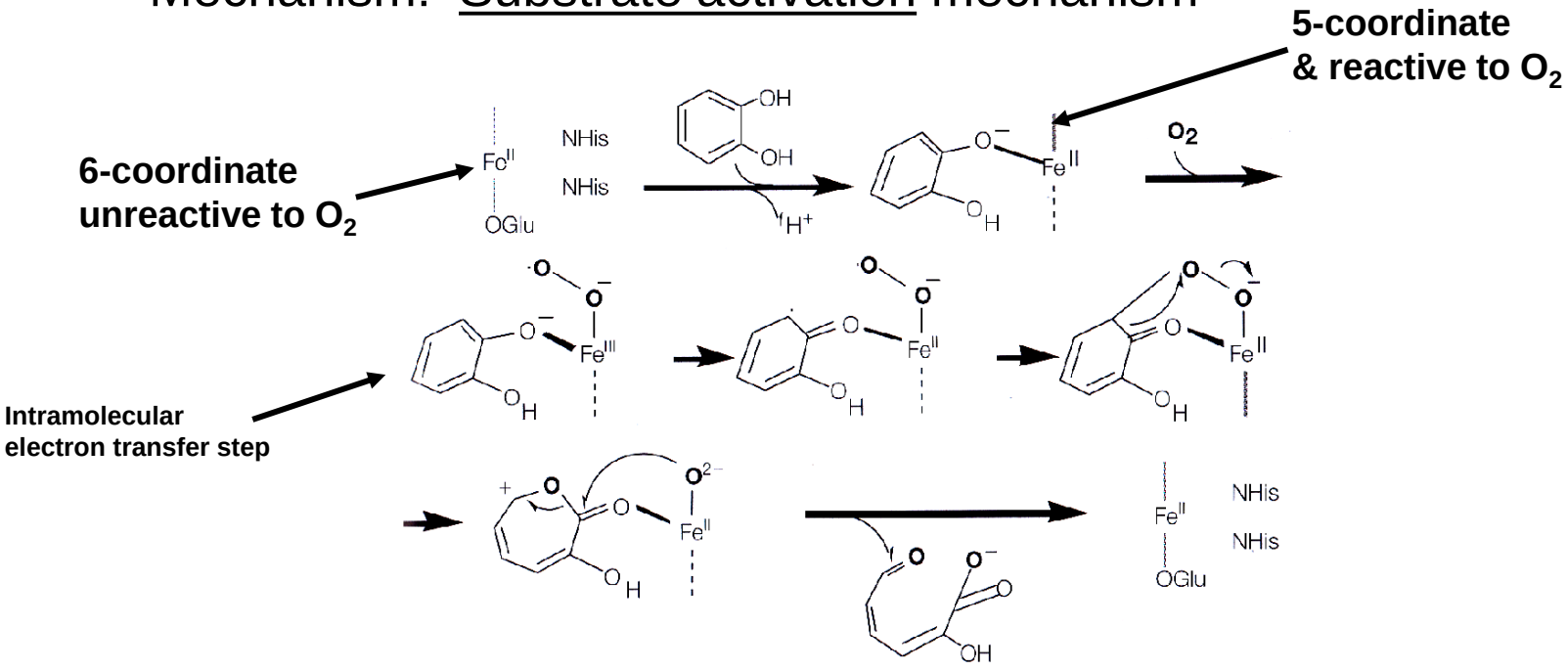
Fig. XI.5.19.
General mechanistic scheme proposed by Solomon et al.[7,8] for a mononuclear Fe(II) enzyme with a 2-His-1-carboxylate facial triad active-site motif. The gray ellipse at the right represents cofactor, while the wavy-lined ellipse represents substrate.

Biological activation of O_2

(C) Dioxygenases

(a) Extradiol dioxygenases: Transform aromatic compounds into aliphatic compounds by cleavage of “extradiol” C-C bond.

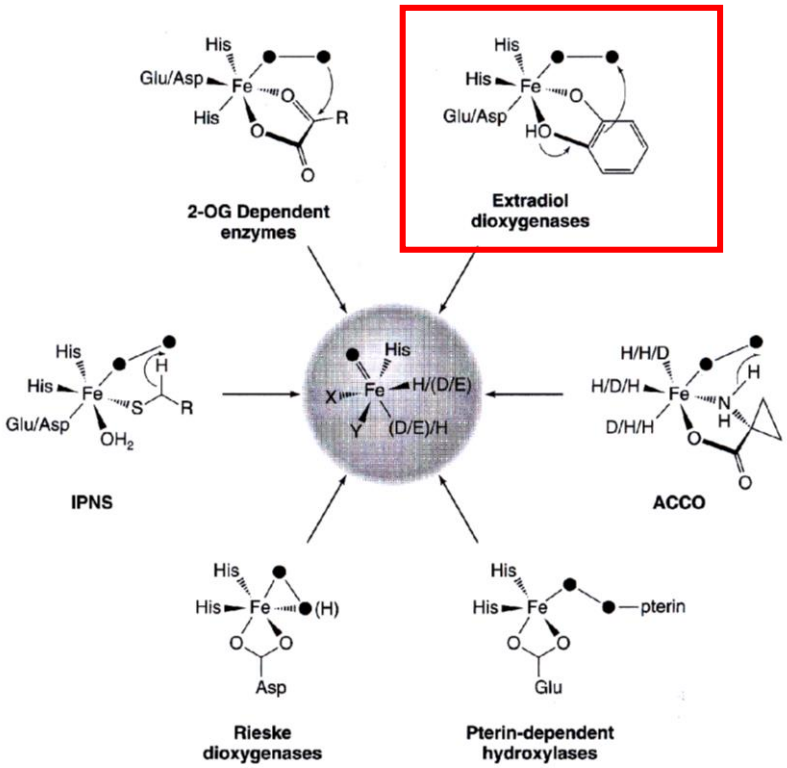
- Mechanism: <u>Substrate activation</u> mechanism



(9) Biological activation of O_2

(C) Dioxygenases

- Extradiol dioxygenases are just one class of a wide variety of Fe^{2+} enzymes featuring the 2-His-1-carboxylate facial triad:

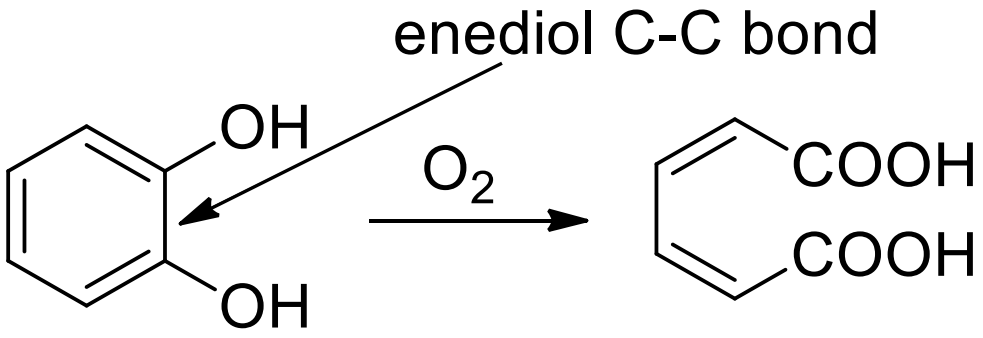


Biological activation of O_2

(C) Dioxygenases

(b) Intradiol dioxygenases

- Very well understood; bacterial enzymes
- Catalyze the cleavage of enediol C-C bonds to form *cis*,*cis*-muconic acid:

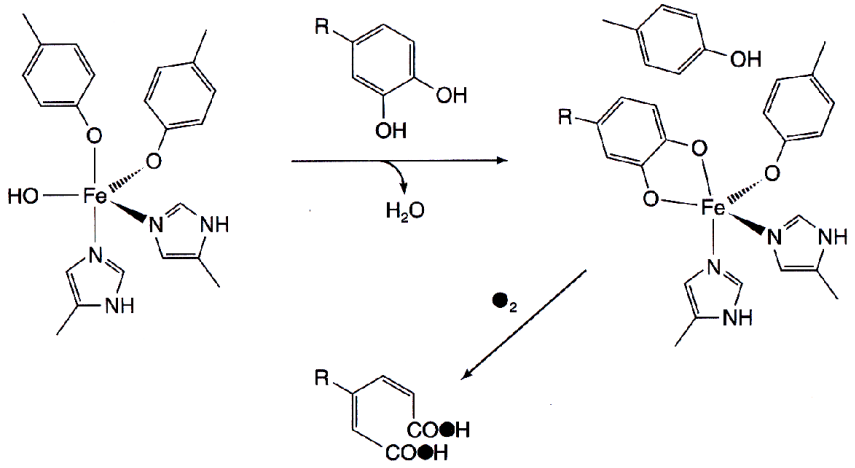


Biological activation of O_2

(C) Dioxygenases

(b) Intradiol dioxygenases

- Active site structure: 5-coordinate, high-spin Fe^{3+} → 2 tyr, 2 his & OH.
- Rich, burgundy color (tyr→Fe^{3+} CT)



Biological activation of O_2

(A) Introduction

(B) Monoxygenases

(C) Dioxygenases

(D) Peroxidases & Catalases

Biological activation of O_2

Preamble: Reactive Oxygen Species (ROS)

- Organisms that live in air have evolved a variety of strategies to cope with oxidative stress:

(1) Small molecule antioxidants: ascorbate, α-tocopherol, coenzyme Q, glutathione, urate.

(2) Antioxidant enzymes: Superoxide dismutases, superoxide reductases, catalase, peroxidases

- ROS: superoxide, hydroxyl radical, H_2O_2, peroxynitrite ($ONOO^-$)
- ROS are produced from superoxide:

(1) A side product of O_2 reduction (respiration)

(2) Immune response: NADPH oxidase system of leukocytes produces superoxide to protect against pathogens

Biological activation of O_2

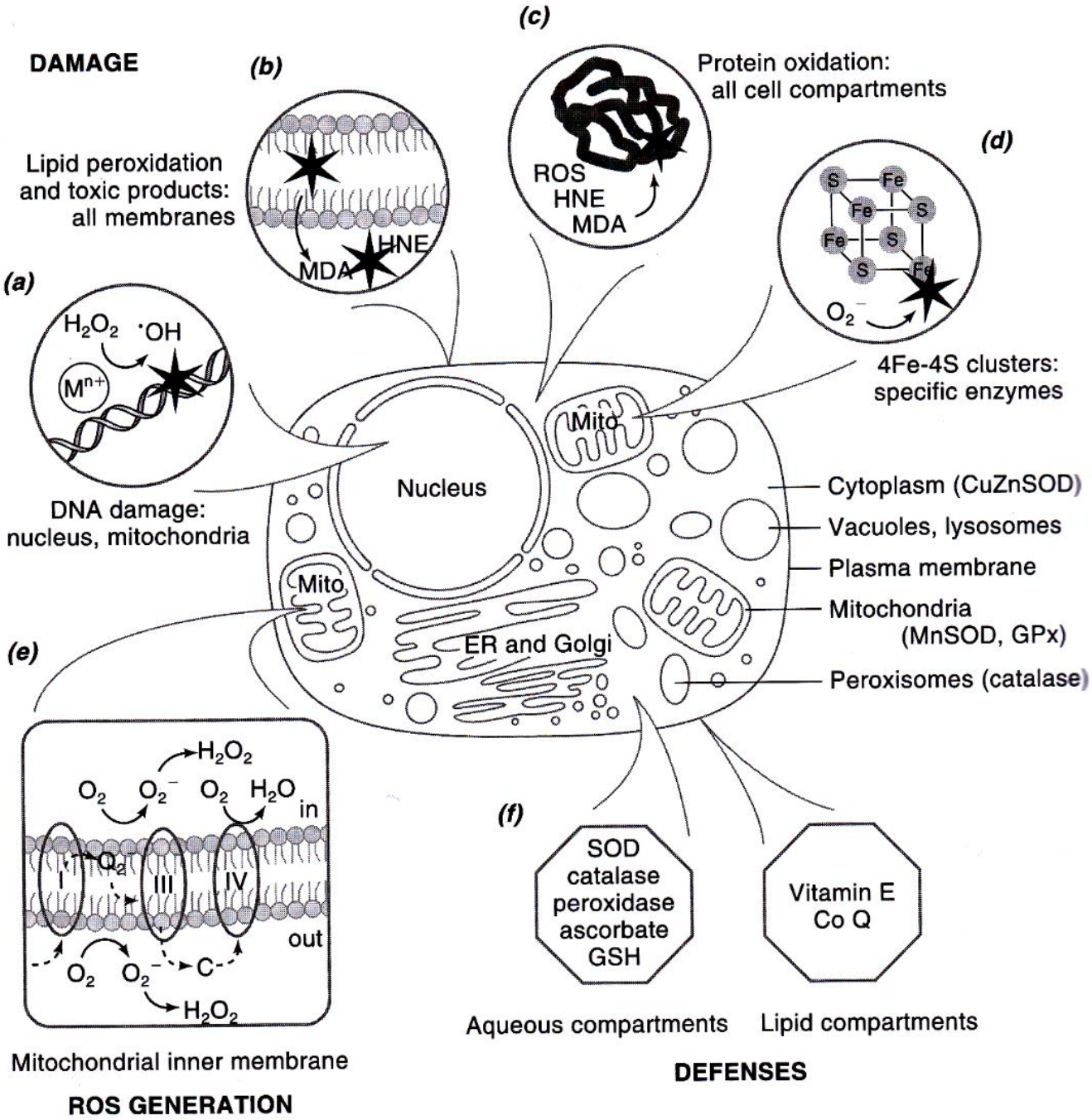
Preamble: Reactive Oxygen Species (ROS)

- Hydroxyl radical is the most reactive ROS.
- Produced by reaction of H_2O_2 with Fe^{2+} or Cu^{+} → Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow [(Fe^{IV}=O)^{2+} + H_2O] \rightarrow Fe^{3+} + H_2O + OH^{\cdot}$$

Agents causing oxidative damage

- initiate free radical auto-oxidation of lipids
- damage proteins, nucleic acids, carbohydrates, etc.


DAMAGE
(a)
(b)
(c)
(d)
(e)
(f)
Lipid peroxidation and toxic products: all membranes
MDA
HNE
Protein oxidation: all cell compartments
ROS
HNE
MDA
4Fe-4S clusters: specific enzymes
DNA damage: nucleus, mitochondria
Nucleus
Mito
Mito
ER and Golgi
Cytoplasm (CuZnSOD)
Vacuoles, lysosomes
Plasma membrane
Mitochondria (MnSOD, GPx)
Peroxisomes (catalase)
in
out
I
III
IV
Mitochondrial inner membrane
ROS GENERATION
SOD
catalase
peroxidase
ascorbate
GSH
Vitamin E
Co Q
Aqueous compartments
Lipid compartments
DEFENSES

Biological activation of O_2

(D) Peroxidases & Catalases

(a) Introduction

- 80% of O_2 taken up by breathing is completely reduced to H_2O (respiration).
- H_2O_2 is also produced as an intermediate of O_2 reduction.
- 2 heme enzymes are used as cellular detoxification agents:

(1) Peroxidases:

$$H_2O_2 + 2\text{Substrate(red)} \rightarrow 2H_2O + 2\text{Substrate (ox)}$$

- Substrates: cytochrome c, Mn^{2+}, Cl-, phenol

E.g. cytochrome c peroxidase; horseradish peroxidase

Biological activation of O_2

(D) Peroxidases & Catalases

(a) Introduction

- 80% of O_2 taken up by breathing is completely reduced to H_2O (respiration).
- H_2O_2 is also produced as an intermediate of O_2 reduction.
- 2 heme enzymes are used as cellular detoxification agents:

(2) **Catalases:**

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

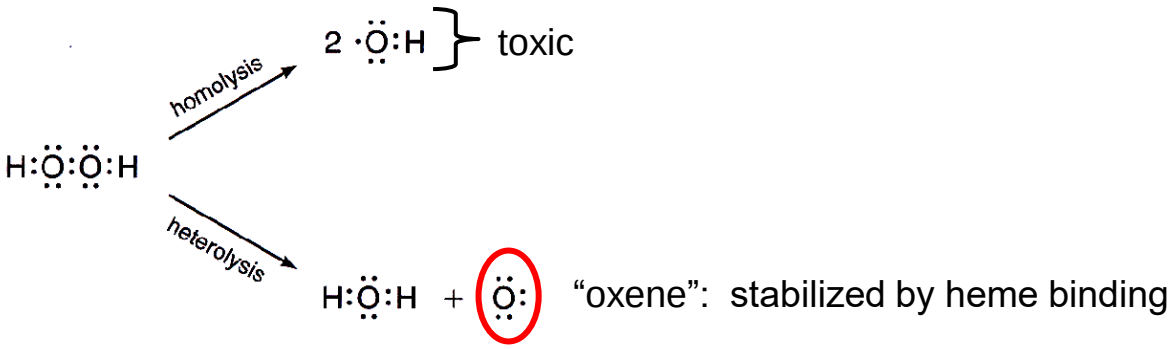
- Catalases simply disproportionate H_2O_2 ("substrate" = H_2O_2)

Biological activation of O_2

(D) Peroxidases & Catalases

$$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O \qquad E_{red} = +1.349\ V$$

- Strength of O-O bond (214 kJ/mol) presents a kinetic barrier to reduction.
- Peroxidases and catalases have evolved a precise catalytic mechanism to overcome this kinetic barrier & break O-O bond heterolytically.



The monatomic diradical -O- derived from oxygen

Biological activation of O_2

(D) Peroxidases & Catalases

(b) Peroxidase

- Overall structure

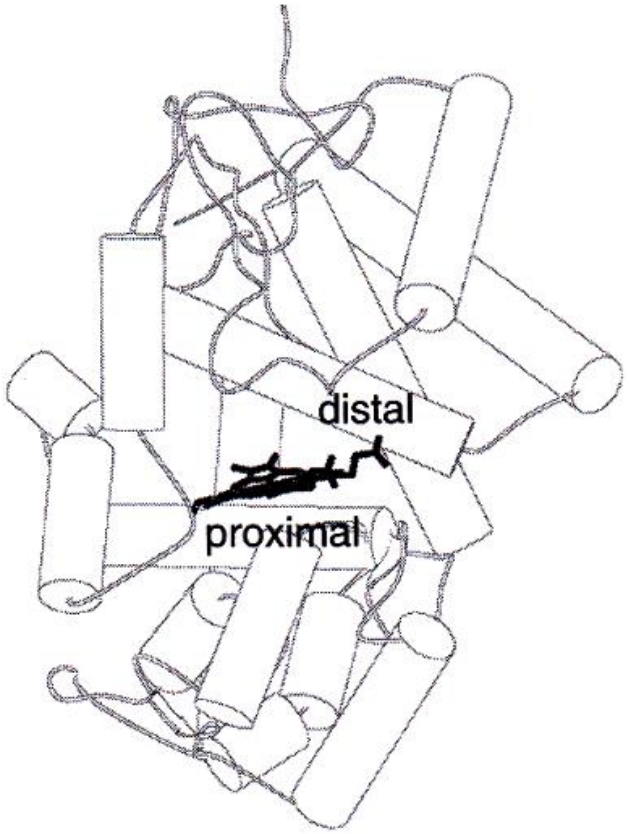


Fig. XI.3.3.
The crystal structure of the most well known peroxidase, horseradish peroxidase or HRP. All non-mammalian heme peroxidases look very similar and consist of a common 10 helical core. The proximal and distal helices provide key residues important for catalysis.

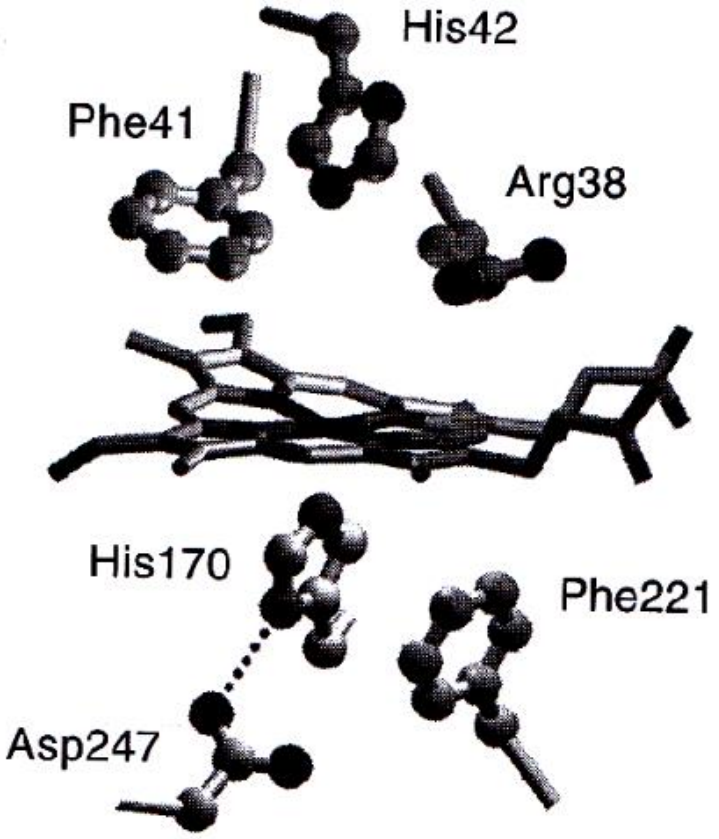
Biological activation of O_2

(D) Peroxidases & Catalases

(b) Peroxidase

- Active site structure

Similar to globin

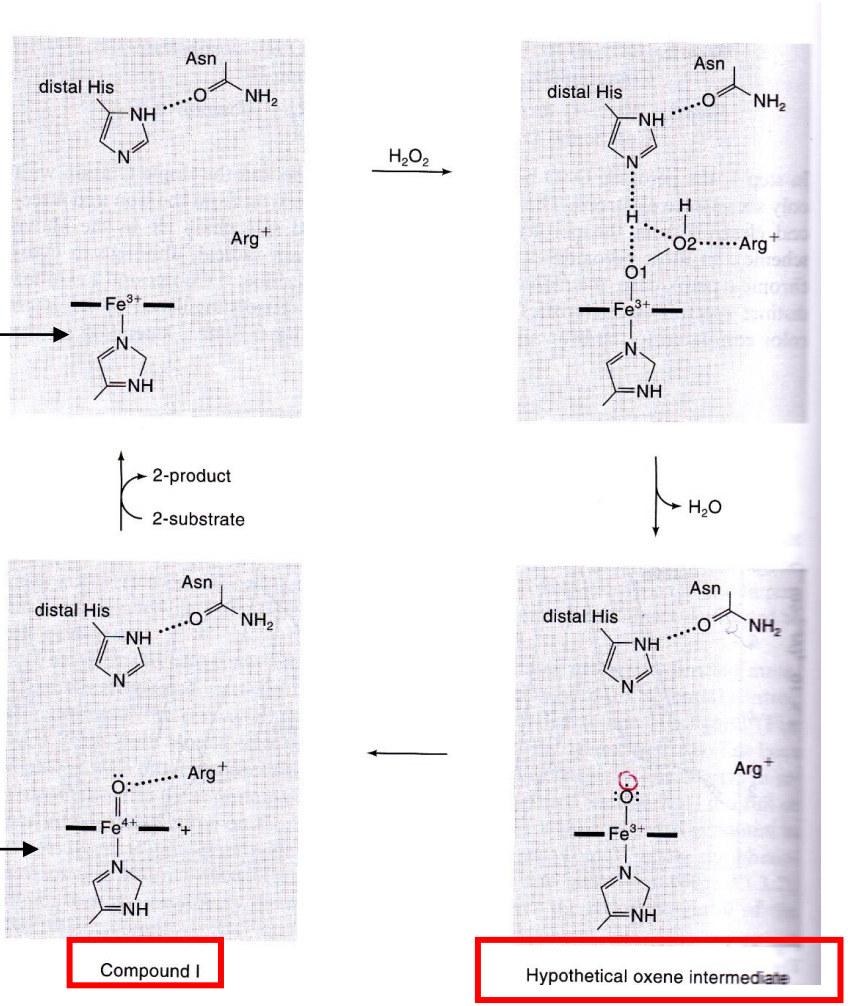


Biological activation of O_2

(D) Peroxidases & Catalases

(b) Peroxidase

- Mechanism



Step 1. $[Fe^{3+}\ R] + H_2O_2 \rightarrow \underset{\text{Compound I}}{[Fe^{4+}{=}O\ R\cdot]} + H_2O$

Step 2. $\underset{\text{Compound I}}{[Fe^{4+}{=}O\ R\cdot]} + \text{Substrate} \rightarrow \underset{\text{Compound II}}{[Fe^{4-}{=}O\ R]} + \text{substrate}\cdot$

Step 3. $\underset{\text{Compound II}}{[Fe^{4+}{=}O\ R]} + \text{Substrate} \rightarrow [Fe^{3+}\ R] + \text{substrate}\cdot + H_2O$

Biological activation of O_2

(D) Peroxidases & Catalases

(b) Peroxidase

- Compounds I & II

-Stable

-Can be spectroscopically characterized

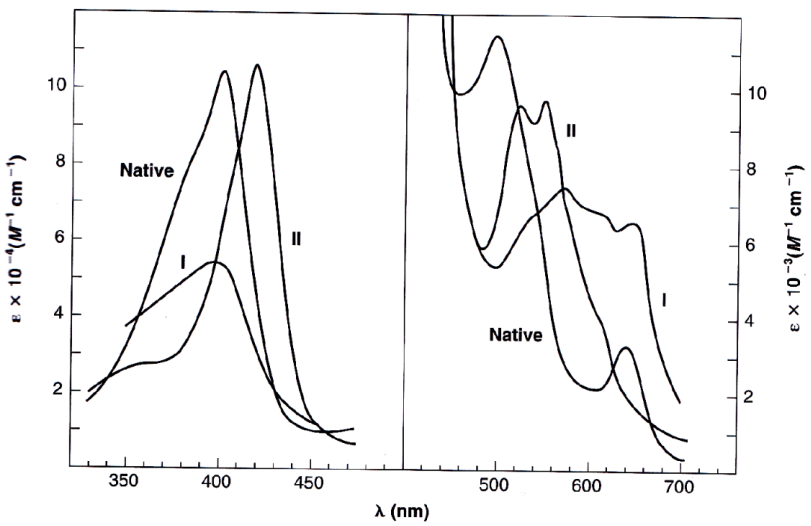


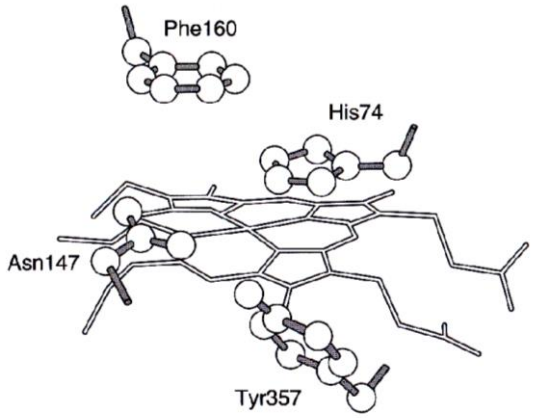
Fig. XI.3.7.
The various spectral intermediates of HRP. The native enzyme in the Fe^{3+} oxidation state gives a typical high-spin spectrum. Upon oxidation with peroxide to give Compound **I**, the main Soret absorbance band decreases to give green Compound **I**. The one-electron reduction of the porphyrin radical in Compound **I** gives red Compound **II** with a Soret band shifted to longer wavelengths relative to the resting native enzyme. These easily distinguished and stable intermediates have enabled a wealth of biophysical tools to be utilized in an attempt to work out the electronic structures of the various intermediates.

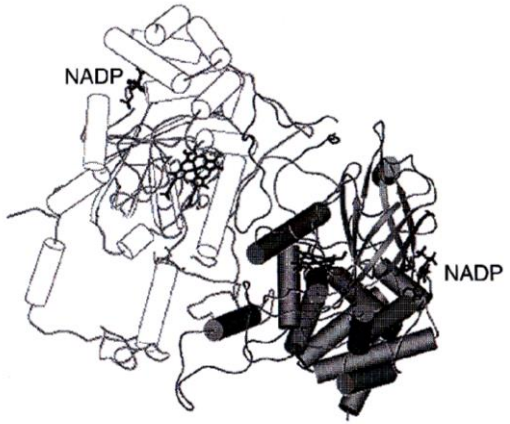
Biological activation of O_2

(D) Peroxidases & Catalases

(c) Catalase

- Active site & overall structure





Biological activation of O_2

(D) Peroxidases & Catalases

(c) Catalase

- Mechanism

-The catalase mechanism is very similar to peroxidase, with the exception of no “step 3” because the substrate in step 2 is another equivalent of H_2O_2.

-Concerted 2 electron reduction.

-No compound II