Cytochrome P450

- R.T. Williams - in vivo, 1947. Brodie – in vitro, from late 40s till the 60s.
- Cytochrome P450 enzymes (hemoproteins) play an important role in the intra-cellular metabolism.
- Exist in prokaryotic and eukaryotic (plants insects fish and mammal, as well as microorganisms)
- Different P450 enzymes can be found in almost any tissue: liver, kidney, lungs and even brain.
- Plays important role in drugs metabolism and xenobiotics.

P450 Reactions

- Cytochrome P450 enzymes catalyze thousands of different reaction.
- Oxidative reactions.

  $SH + O_2 + NADPH + H^+ \quad SOH + H_2O + NADPH^+$

- The protein structure is believed ~~to~~ → determines the catalytic specificity through complementarity to the transition state.

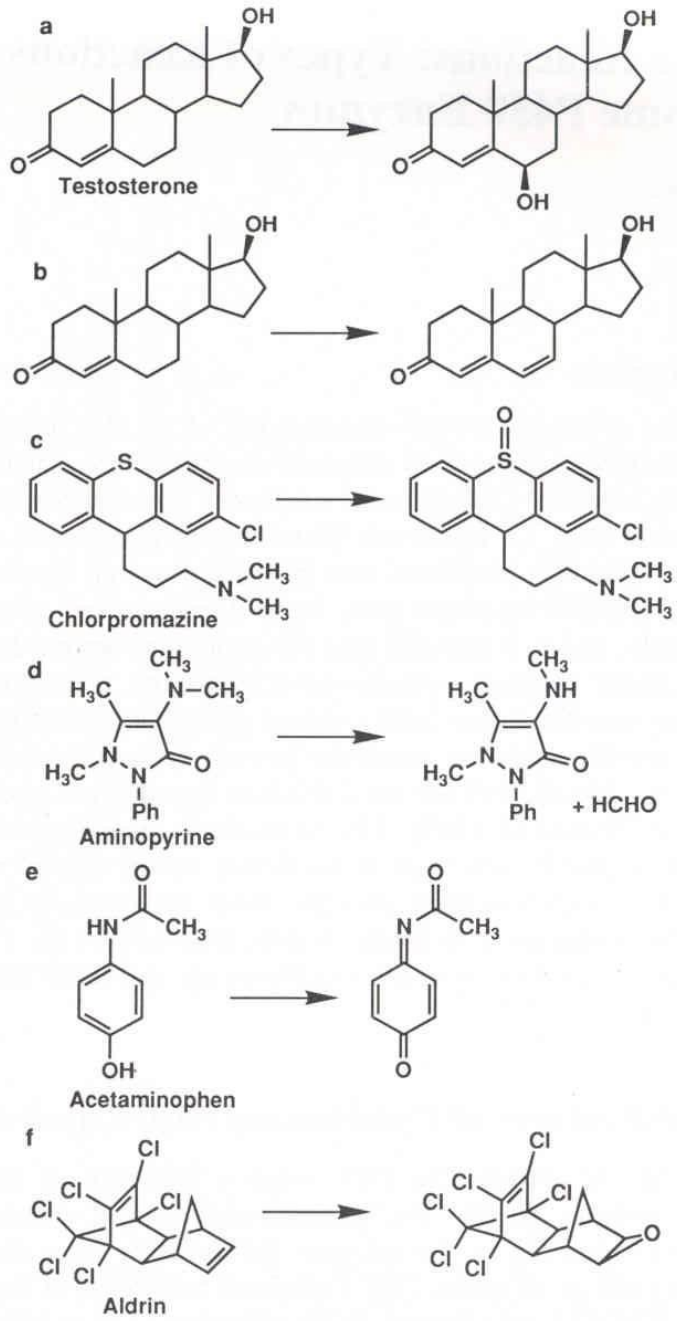


Fig. 1. Oxidations of drugs and pesticides catalyzed by cytochrome P450 enzymes. See text for discussion

General Features of Cytochrome P450 Catalysis

1. Substrate binding (presumably near the site of the heme ligand)
2. 1-electorn reduction of the iron by flavprotein NADPH cytochrome P450 reductase
3. Reaction of ferrous iron with O_2 to yield an unstable FeO_2 complex
4. Addition of the second electron from NADPH or cytochrome b_5
5. Heterolytic scission of the FeO-O(H) bond to generate a formal (FeO)3+
6. Oxidation of the substrate.
    1. Formal abstraction of hydrogen atom or electron
    2. Radical recombination
7. Release of the product.

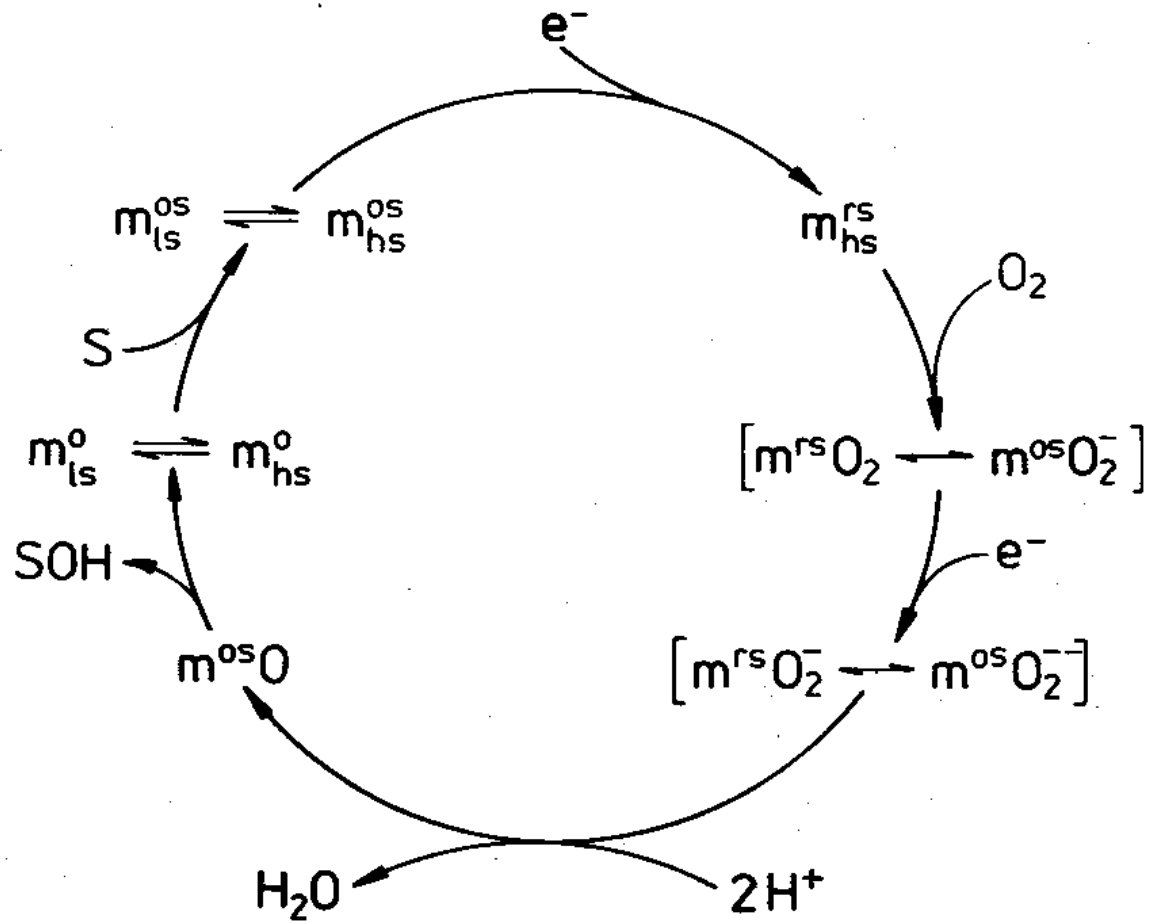
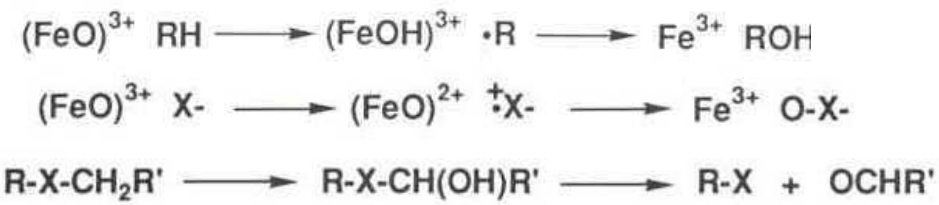
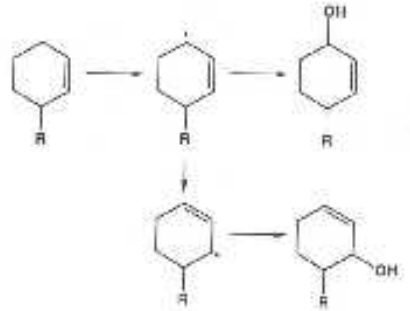


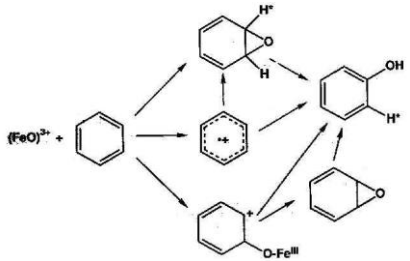
Fig. 1. Reaction cycle of cytochrome P450 considering the spin equilibrium of the ferric state and the resonance structures of oxy-cytochrome P450. *m*, monooxygenase; *o*, oxidized (Fe^{3+}) state; *r*, reduced (Fe^{2+}) state; *s*, substrate; *ls*, low spin; *hs*, high spin

- Oxidative Reactions
  - Carbon Hydroxylation
  - Heteroatom Hydroxylation
  - Heteroatom Release
  - Rearangement Related to Heteroatom Oxidations

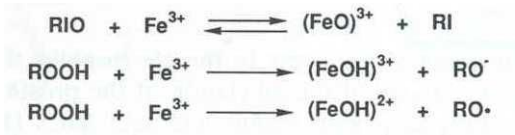


  - Oxidation of π-System

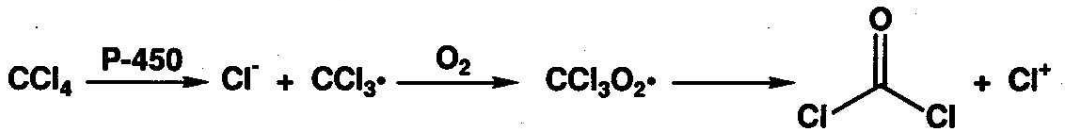




  - Hypervalent Oxygen substrate



- Reductive Reactions

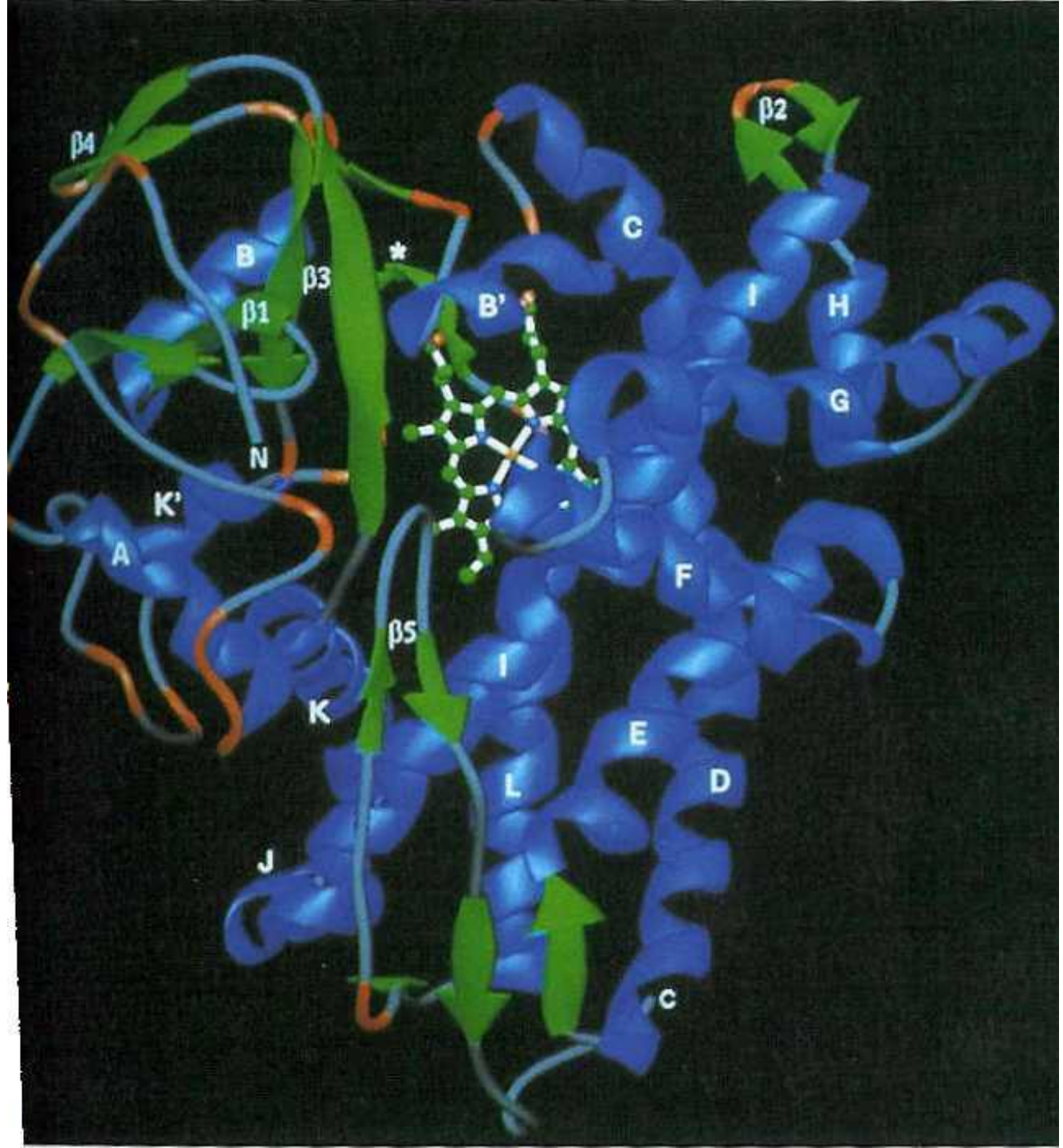


Humans CYP450 -18 families, 43 subfamilies

- CYP1 drug metabolism (3 subfamilies, 3 genes, 1 pseudogene)
- CYP2 drug and steroid metabolism (13 subfamilies, 16 genes, 16 pseudogenes)
- CYP3 drug metabolism (1 subfamily, 4 genes, 2 pseudogenes)
- CYP4 arachidonic acid or fatty acid metabolism (5 subfamilies, 11 genes, 10
- pseudogenes)
- CYP5 Thromboxane A2 synthase (1 subfamily, 1 gene)
- CYP7A bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus (1
- subfamily member)
- CYP7B brain specific form of 7-alpha hydroxylase (1 subfamily member)
- CYP8A prostacyclin synthase (1 subfamily member)
- CYP8B bile acid biosynthesis (1 subfamily member)
- CYP11 steroid biosynthesis (2 subfamilies, 3 genes)
- CYP17 steroid biosynthesis (1 subfamily, 1 gene) 17-alpha hydroxylase
- CYP19 steroid biosynthesis (1 subfamily, 1 gene) aromatase forms estrogen
- CYP20 Unknown function (1 subfamily, 1 gene)
- CYP21 steroid biosynthesis (1 subfamily, 1 gene, 1 pseudogene)
- CYP24 vitamin D degradation (1 subfamily, 1 gene)
- CYP26A retinoic acid hydroxylase important in development (1 subfamily member)
- CYP26B probable retinoic acid hydroxylase (1 subfamily member)
- CYP26C probabwle retinoic acid hydroxylase (1 subfamily member)
- CYP27A bile acid biosynthesis (1 subfamily member)
- CYP27B Vitamin D3 1-alpha hydroxylase activates vitamin D3 (1 subfamily member)
- CYP27C Unknown function (1 subfamily member)
- CYP39 unknown function (1 subfamily member)
- CYP46 cholesterol 24-hydroxylase (1 subfamily member)
- CYP51 cholesterol biosynthesis (1 subfamily, 1 gene, 3 pseudogenes) lanosterol
- 14-alpha demethylase

Structure

- Till 2001 there was no mammal CYP.
- P450cam structure was solved in 1987
- x-ray structure of P450cam with different substrate and inhibitors.


β4
β2
B
β3
*
C
β1
B'
I
H
G
N
K'
A
F
β5
I
K
E
L
D
J
C

- Heme exists in hydrophobic environment, oriented nearly parallel to the surfaces between the L and I helices. Heme-ligating Cys-357 (beginning of L)
- Helix-rich on the right side
- Beta-sheet-rich on the left side
- 14 alpha helices, 5 anti parallel beta-sheets
- Compact structure, especially the helical region.

- Closed structure, conformational dynamic is essential.
- No obvious substrate channel.
- The area bounded by B’ F/G and beta 5 identified as the channel.
- 6 water molecule fill the substrate active site
- Substrate binding loop residues 80-103
- Binding free energy is most likely due to hydrophobic interactions of the substrate and the heme, Leu-244 and Val-295

Structural Model for CYP450 Substrates and inhibitors

- Large number of drugs chemical are already known
- Systematic attempts to explore substrate and inhibitor specificity of individual cytochrome P40 species

Motivation

- Chemical toxicity studies
- Predict whether therapeutic effect may be subjected to individual variations.
- Predict drugs inhibition.

Elucidate Specificity approaches

- Determination of three dimensional structure of the active site.
- Design of pharmacophor:
  - Molecular modeling
  - Quantitative structure activity relationship

Three dimensional Structure of the Active Site

- In P450cam substrate binding, there are three regions of AA flexibility.
  - One at the substrate binding site
  - Two are at the assumed substrate access channel
- Backbone flexibility of P450cam in case of inhibitor binding.

Peroxidases

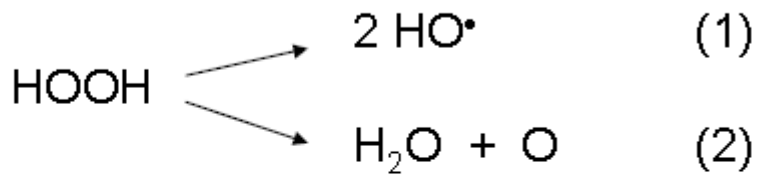
XI.3. Peroxidases and Catalases

XI.3.1. Introduction

Substrate: hydrogen peroxide (IUPACese: dioxidane)

peroxidases: oxidised by H_2O_2, then reduced by "SH", a substrate
catalase: catalyses dismutation into O_2 and H_2O

Electrode potentials, $E°'(H_2O_2/H_2O)$ and $E°'(O_2/H_2O_2)$:
see earlier Table with values at pH 7.



O. Loew. Catalase, A new enzyme of general occurrence with special reference to the tobacco plant, *U.S.Department of Agriculture* Report No. 68:7-47 (1901)

Fig. XI.3.2. protoporphyrin IX
proximal ligand

peroxidase:	histidine (H-bonded to an Asp)
catalase:	tyrosinate

Reaction of Fe(III)porphyrin with H_2O_2 results in Compound I and H_2O

What is Compound I?

a Fe(IV)Opor(•+), possibly formed via a Fe(V)por intermediate

XI.3.2. Overall structure of peroxidases, from fungi to plants – *conserved*, structural identity, but not necessarily sequence similarity.
Fig. XI.3.3., non-mammalian haem peroxidases, 10 helical bundle motive

XI.3.3. Active site structure

Fig. XI.3.4., active site structure, overhead

His170 – Asp247 H-bond

Fig. XI.3.5., catalase, structure, tetramer,

different from peroxidases

Fig. XI.3.6., active site of catalase

Tyr357, tyrosinate as ligand to Fe(III)

Peroxidases:

distal and proximal helix, close to haem;

distal: H_2O_2 binding,

proximal: His ligand to Fe

His ligand forms H-bond with Asp (conserved) $\rightarrow$ anionic character to His, stabilises Fe^{3+} - and FeO^{2+}

Contrast: His in globins H-bonds with peptide C=O, his is correspondingly less negative, electrode potential is higher.

Catalase: fold is different from peroxidases. $TyrO^-$ stabilises Fe^{3+}.

XI.3.4. Mechanisms

Fig. IX.3.7., Spectral intermediates, overhead
Compound I is green, except that of cytochrome *c* peroxidase

Fig. IX.3.8., Peroxidase Compound I formation, overhead
Intermediate Fe(V)? Importance of distal His, T. XI.3.1.
His→Leu, Compound I formed 5 orders of magnitude slower.
Proximal? Appears less important!?
(problem with amino acid changes)

XI.3.5. Reduction of Compounds I and II

Peroxidase: a phenol, $\times 2 \Rightarrow$ 2 phenoxyl radicals;
Compound I $\rightarrow$ Compound II $\rightarrow$ Fe(III) resting state

Binding site near the solvent-accessible haem edge; based on NMR, suicide inhibitors, *crystal structures;* binding site may depend on the substrate (lignin peroxidase)

Cytochrome *c* peroxidase, substrate is cytochrome *c*, a one-electron donor.

Complex between c*c*p and *c*: Fig. XI.3.12 (Problem?)
Radical centred on trp191. Haem of cytochrome c is near residue 194, electron transfer by tunnelling. FeO2+ oxidises trp 191 again, see Fig. XI.3.13.

XI.5. Dioxygen activating enzymes

XI.5.1. Converting carriers to activators

Fig. XI.5.1. (next ppt)

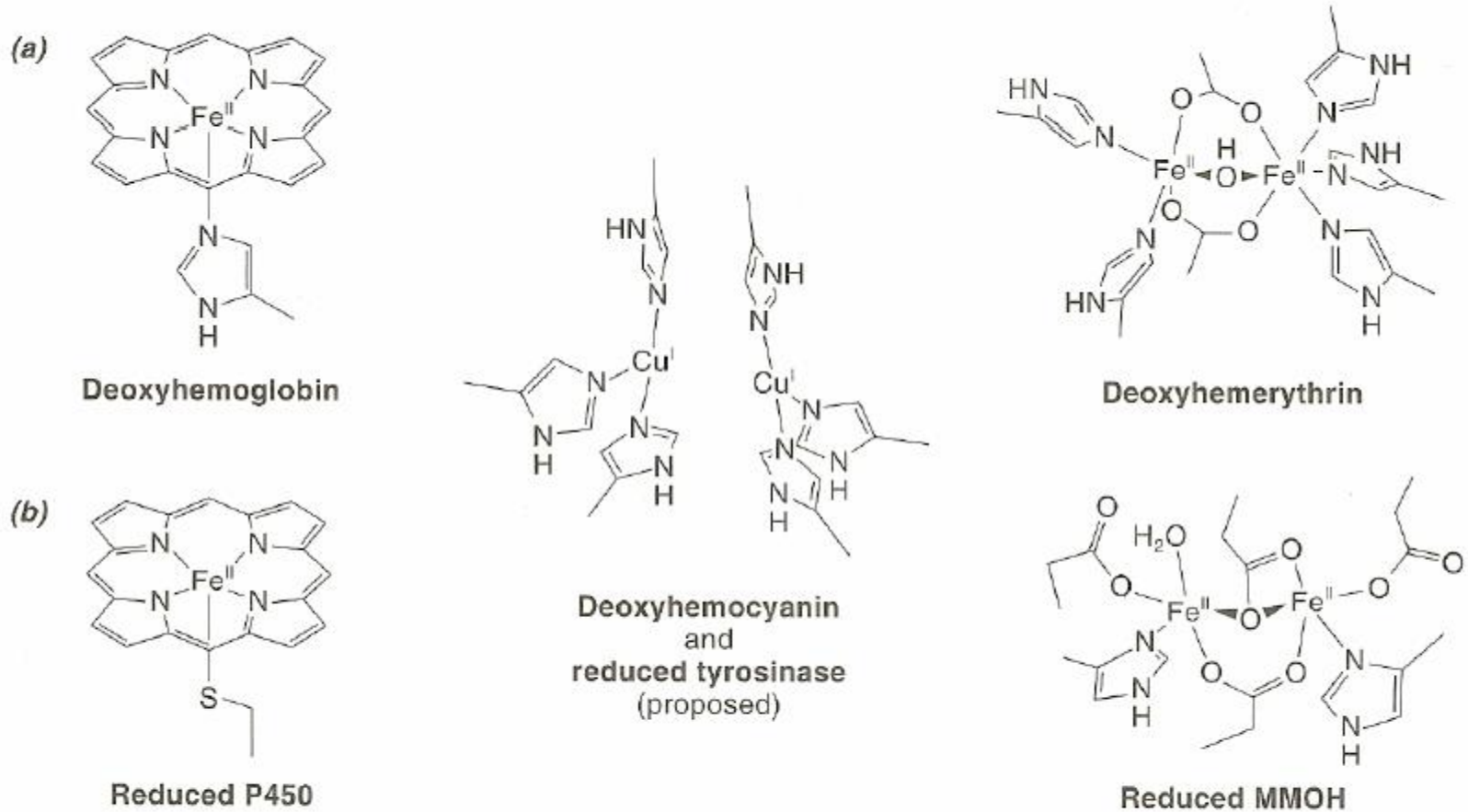


Fig. XI.5.1.
Active sites of the three known O_2 carriers (*a*) and their counterparts in oxygen activation (*b*). (MMOH = methane monooxygenase hydroxylase)

Some Naturally Occurring Antioxidant Systems

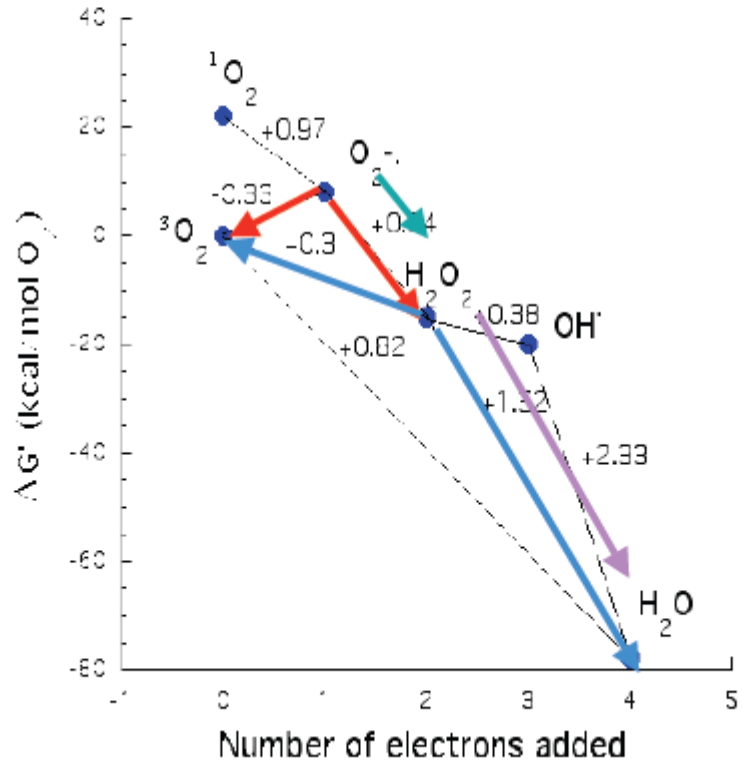
Antioxidant enzyme systems

- SODs and SOR
- Catalase and heme peroxidases
- Glutathione peroxidase and the glutathione system

Small molecule antioxidants

- Ascorbic acid (vitamin C)
- Tocopherol (vitamin E)
- Glutathione

- SOD: $2\ O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2$
- SOR: $O_2^- + e^- + 2\ H^+ \rightarrow H_2O_2$
- Catalase (hydroperoxidase): $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$
- Peroxidases: $H_2O_2 + AH_2 \rightarrow 2\ H_2O + A$



SOD and SOR Mechanistic Principles

A. Oxidation of superoxide to give dioxygen

B. Reduction of superoxide to give hydrogen peroxide

SOD and SOR Enzymes

A. CuZnSOD
B. FeSOD and MnSOD
C. NiSOD
D. SOR

SOD mechanisms

Oxidation of superoxide:

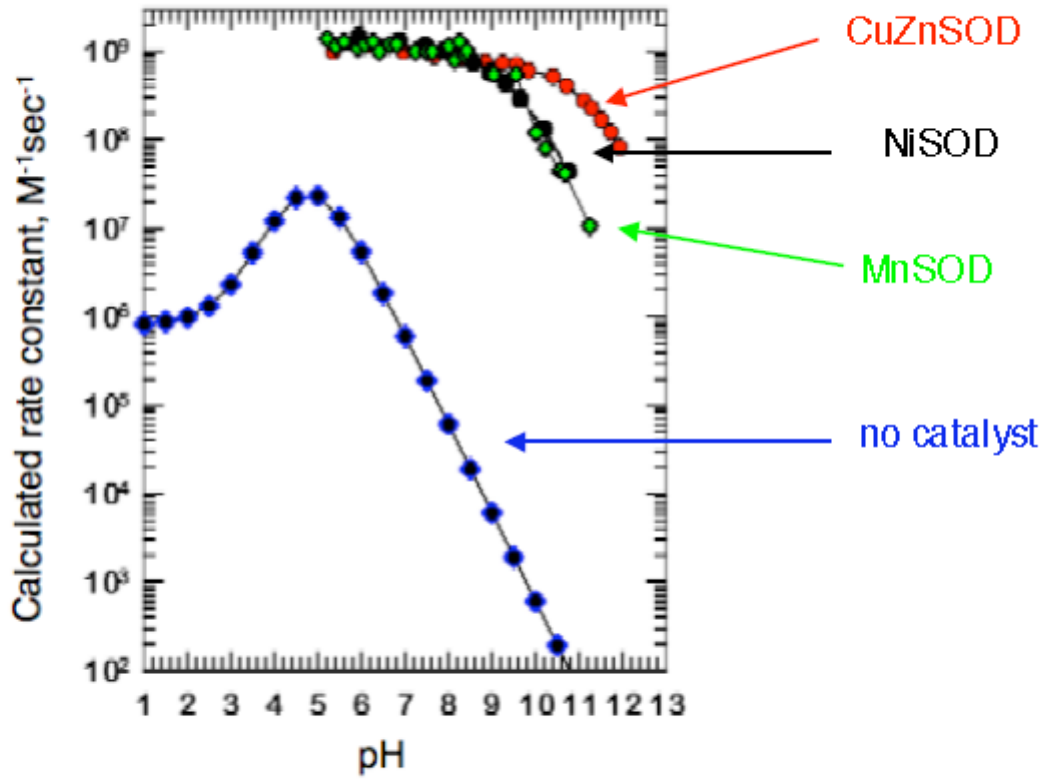
$$O_2^- + Enzyme_{Ox} \rightarrow O_2 + Enzyme_{RED}$$

Reduction of superoxide:

$$O_2^- + 2\ H^+ + Enzyme_{RED} \rightarrow H_2O_2 + Enzyme_{Ox}$$

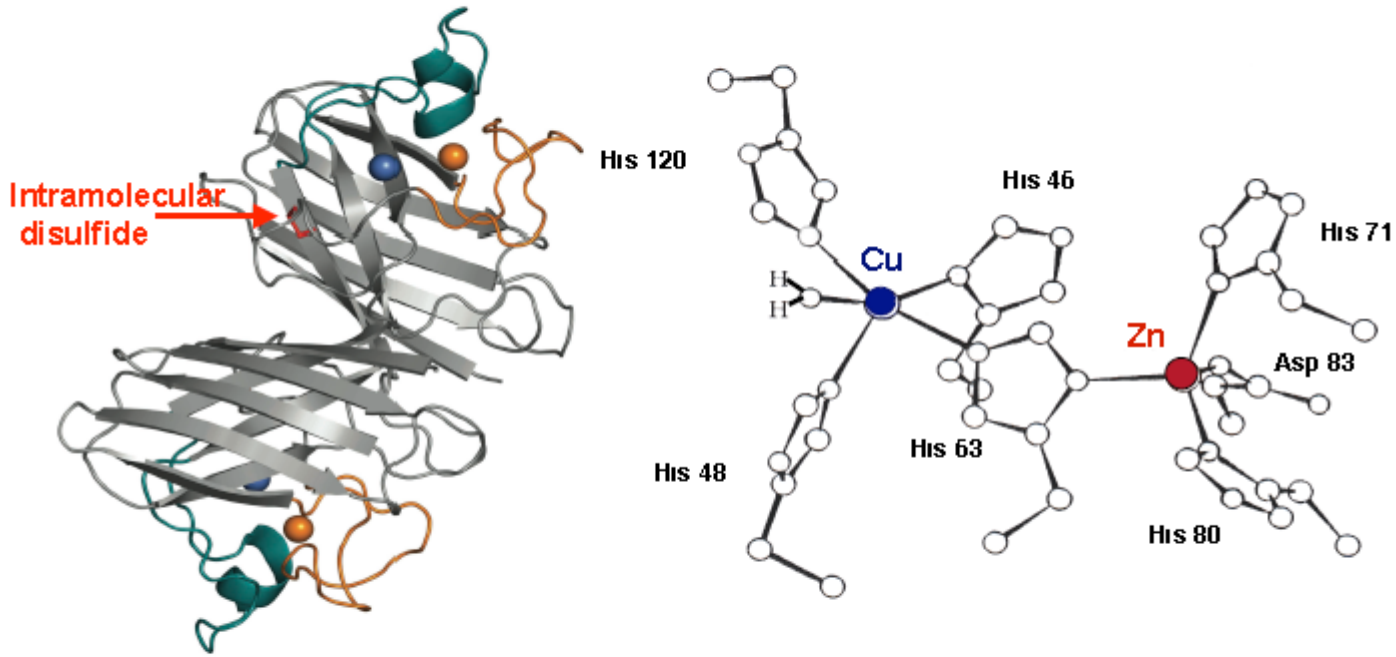
The SOR mechanism uses the second reaction.
The oxidized enzyme is then returned to the reduced state by reducing agents other than superoxide.

Rate Constants for Superoxide Dismutation



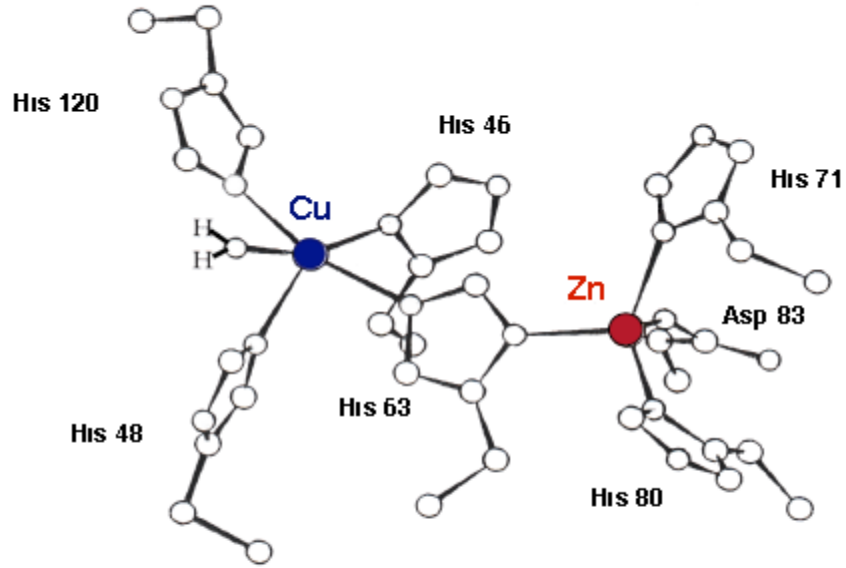
Rate constants for the SODs are per concentration Cu, Mn, and Ni, respectively. (Figure courtesy of Dr. Diane E. Cabelli.)

CuZnSOD



$$2\,O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

Metal Binding Sites and SOD Mechanism

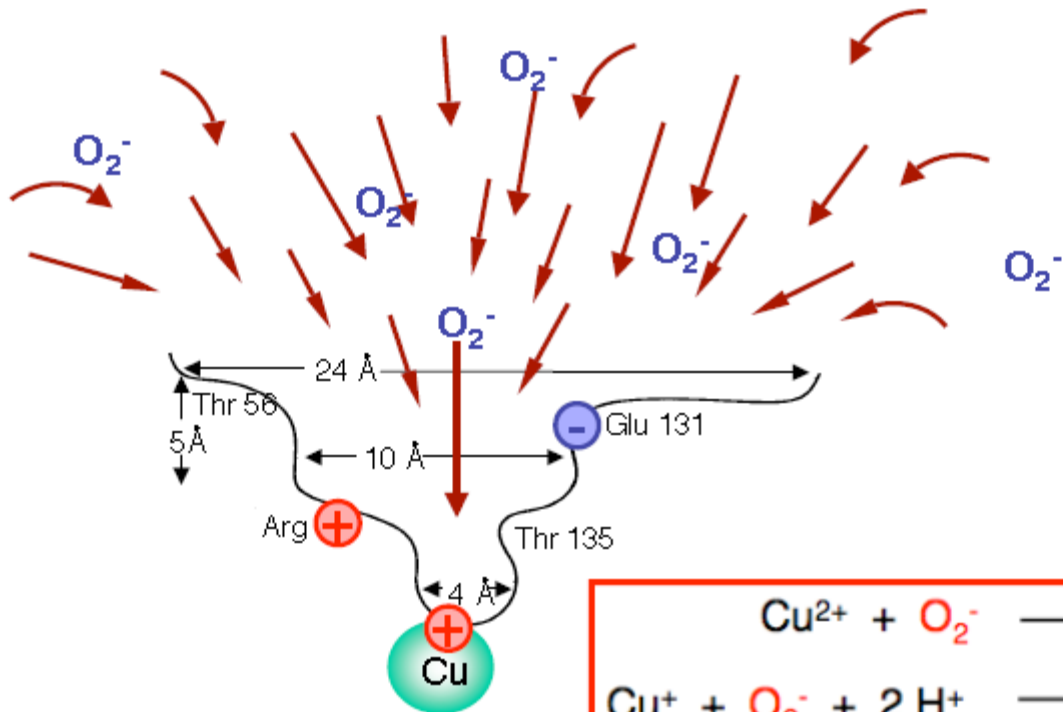


Metal binding properties of the two sites are coupled

Enzymatic reaction:

$$2\,O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

Active Site Channel and Superoxide Specificity

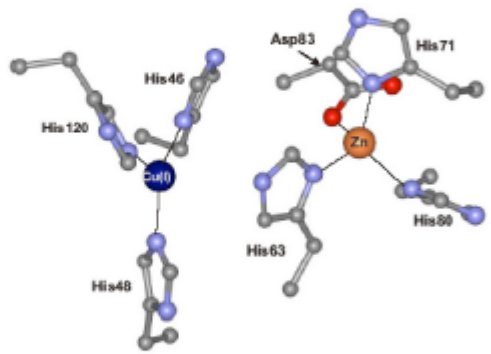


$$Cu^{2+} + O_2^- \longrightarrow Cu^+ + O_2$$

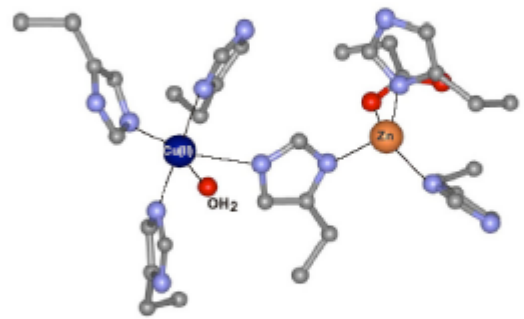
$$Cu^+ + O_2^- + 2\,H^+ \longrightarrow Cu^{2+} + H_2O_2$$

$$2\,O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

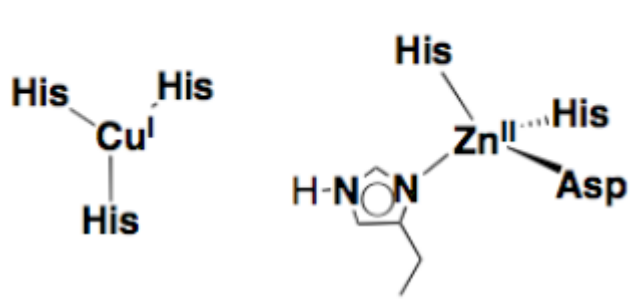
Metal binding site regions of reduced and oxidized CuZnSOD



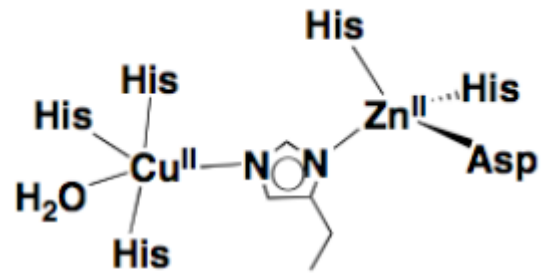
Reduced (Cu^{+}) yeast CuZnSOD



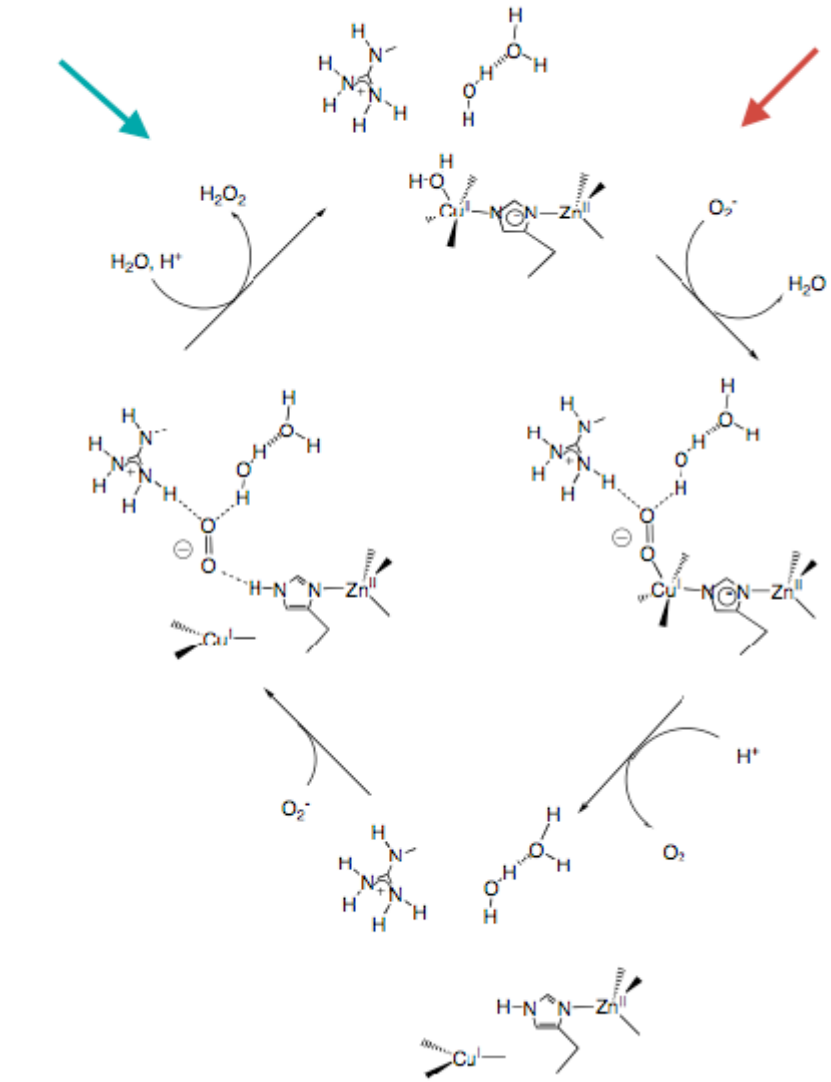
Oxidized (Cu^{2+}) human CuZnSOD



reduced



oxidized

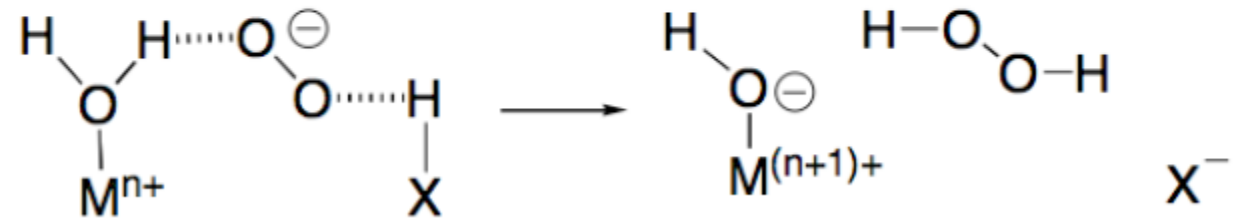


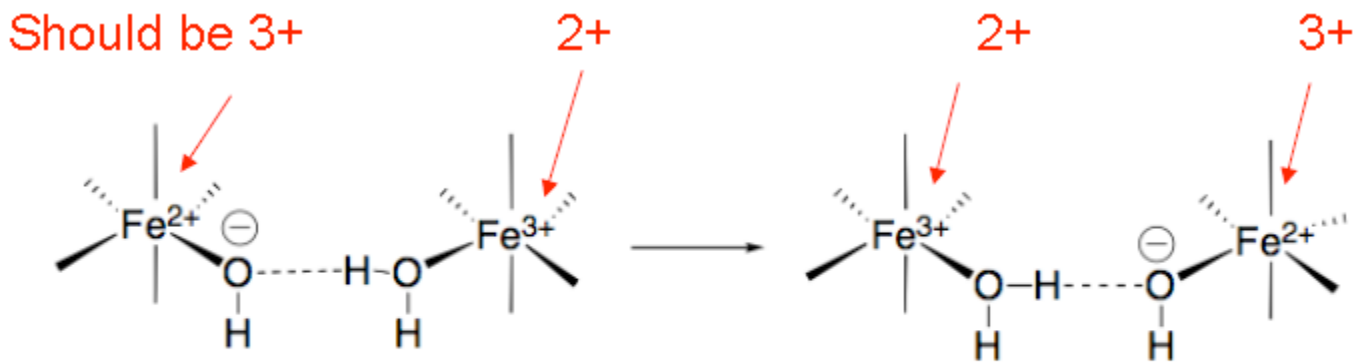
Schematic diagram of the catalytic cycle for CuZnSOD.

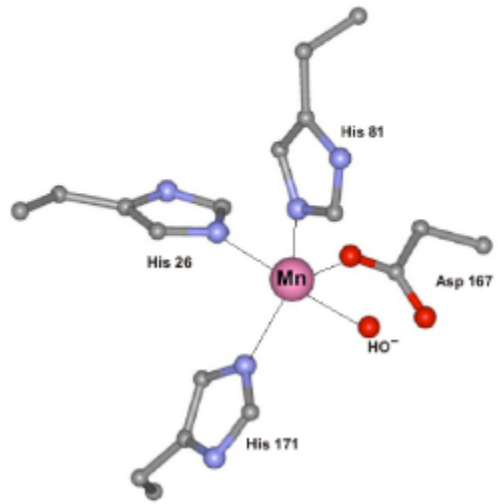
The right hand side describes the oxidation of superoxide by Cu(II)ZnSOD to give dioxygen.

The left side describes the reduction of superoxide by Cu(I)ZnSOD to give hydrogen peroxide.

The solution may be proton-coupled electron transfer

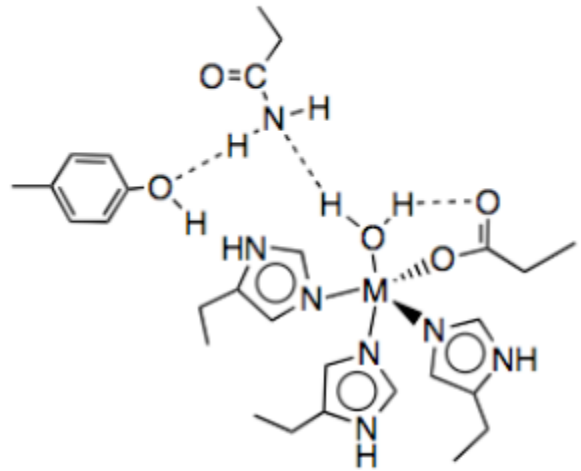






Metal site in *E. coli* MnSOD

Three equatorial ligands (His81, Asp167 and His 171) and two axial ligands (His26 and a solvent molecule, modeled as hydroxide ion) form a trigonal bipyramidal arrangement.

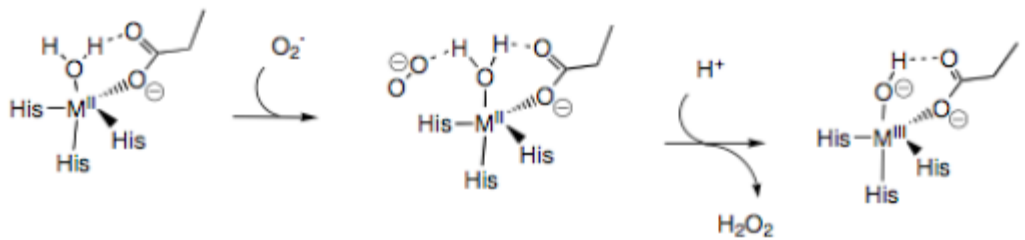


Active site configuration of Mn(**II**)SOD and Fe(**II**)SOD

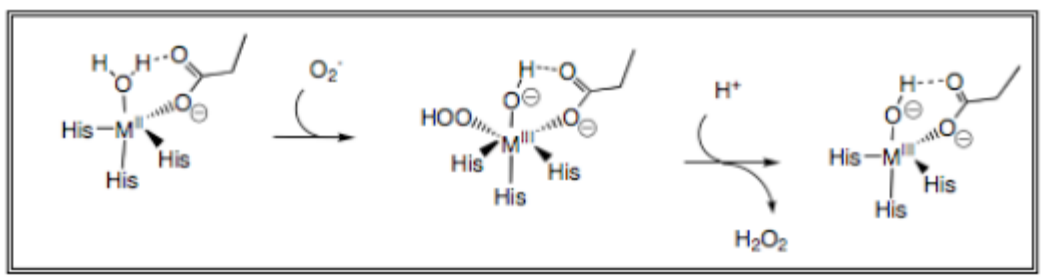
Oxidation of superoxide by Mn(III)SOD or Fe(III)SOD

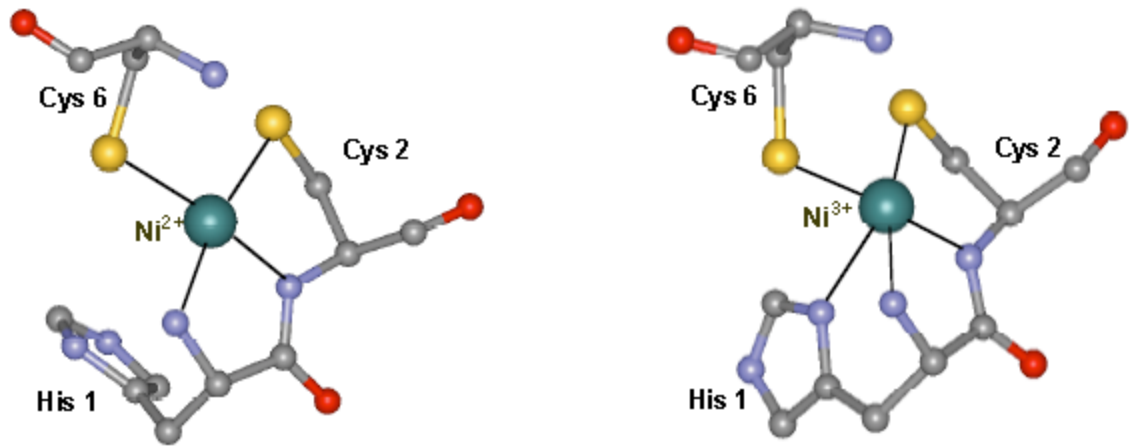
$$O_2^- + M(III)SOD \rightarrow (O_2^-)M(III)SOD \rightarrow M(II)SOD + O_2$$

Reduction of superoxide by Mn(II)SOD or Fe(II)SOD:

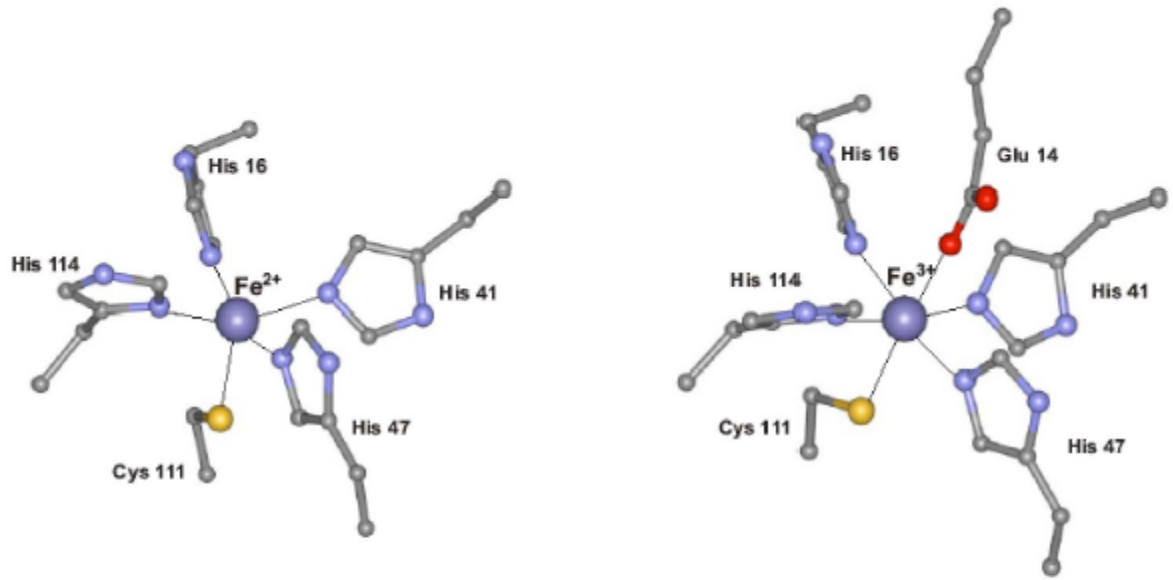


Minor pathway for reduction of superoxide by Mn(II)SOD

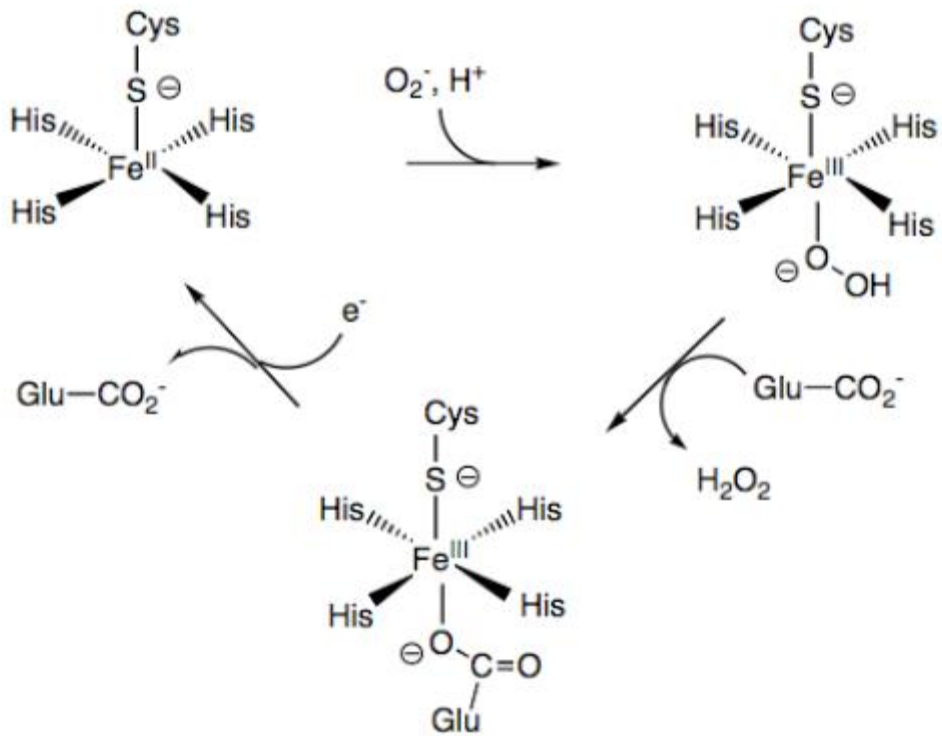




Nickel binding site of Streptomyces seoulensis superoxide dismutase in oxidized, Ni^{3+} and thiosulfate reduced, Ni^{2+} forms

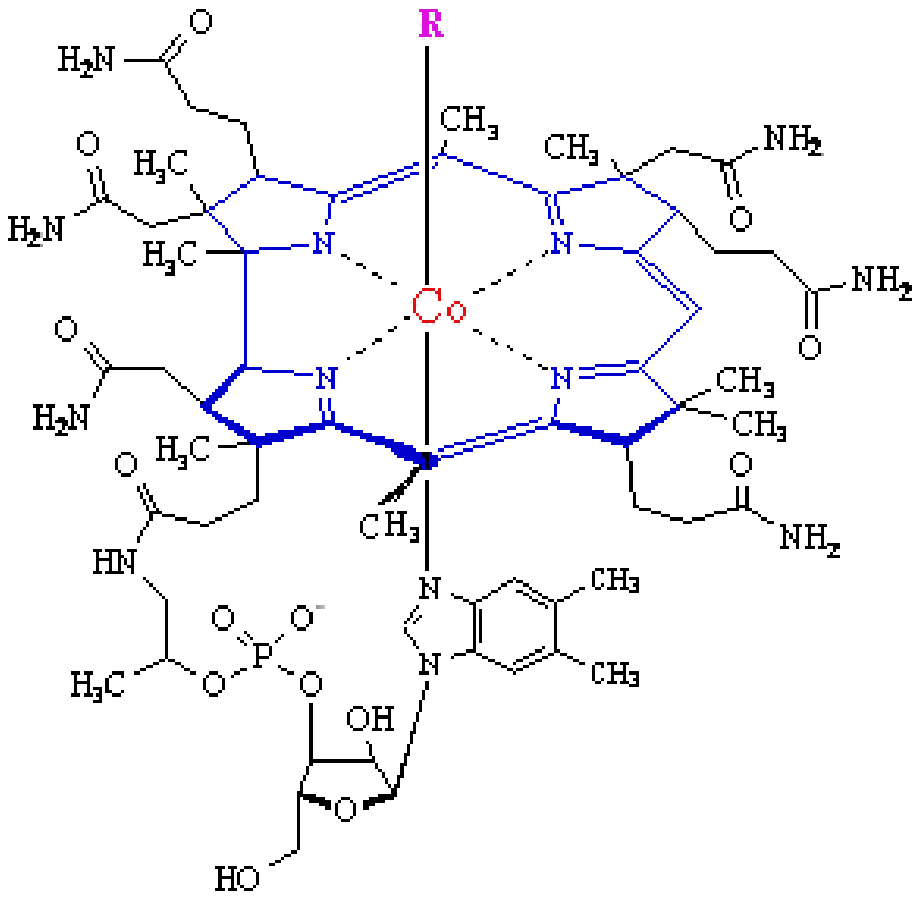


Iron center of *Pyrococcus furiosus* superoxide reductase in ferric (left) and ferrous (right) states.


Cys
S ⊖
His
His
Fe II
His
His
O2-, H+
Cys
S ⊖
His
His
Fe III
His
His
⊖ O
OH
Glu—CO2-
H2O2
e-
Glu—CO2-
Cys
S ⊖
His
His
Fe III
His
His
⊖ O
C=O
Glu

VITAMIN B12 (cobalamin)

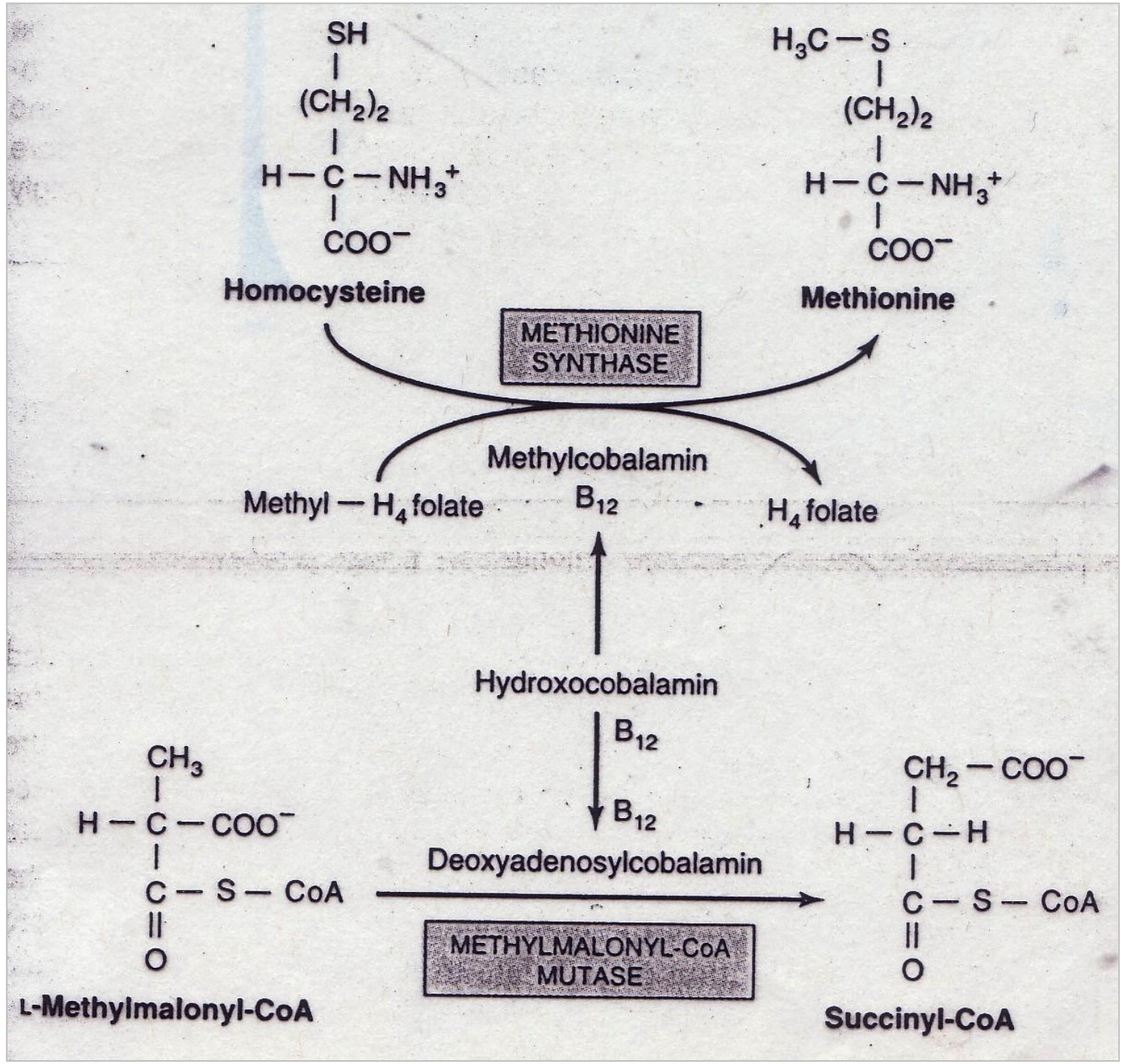
- Vitamin B12, is also called cobalamin, cyanocobalamin and hydroxycobalamin.
- It is built from :
  1. A nucleotide and
  2. A complex tetrapyrrol ring structure (corrin ring)
  3. A cobalt ion in the center.
  4. A R- group
- When R is cyanide (CN), vitamin B12 takes the form of cyanocobalamin.
- In hydroxycobalamin, R equals the hydroxyl group (-OH).
- In the coenzyme forms of vitamin B12,
  - R equals an adenosyl group in adenosylcobalamin.
  - R equals a methyl (-CH3) group in methylcobalamin.
- Vitamin B12 is synthesized exclusively by microorganisms (bacteria, fungi and algae) and not by animals and is found in the liver of animals bound to protein as methycobalamin or 5'-deoxyadenosylcobalamin.



- Known as the "red" vitamin because it exists as a dark red crystalline compound, Vitamin B12 is unique in that it is the only vitamin to contain cobalt (Co^{3+}) metal ion, which, gives it the red color.
- The vitamin must be hydrolyzed from protein in order to be active.
- Intrinsic factor, a protein secreted by parietal cells of the stomach, carries it to the ileum where it is absorbed.
- It is transported to the liver and other tissues in the blood bound to transcobalamin II.
- It is stored in the liver attached to transcobalamin I.
  - It is released into the cell as Hydroxocobalamin *(see the next slide)*
    - In the cytosol it is converted to methylcobalamin.
    - Or it can enter mitochondria and be converted to 5'-deoxyadenosyl cobalamin.

Dorothy Crowfoot Hodgkin (1910-1994)

Dr. Stadtman in her lab

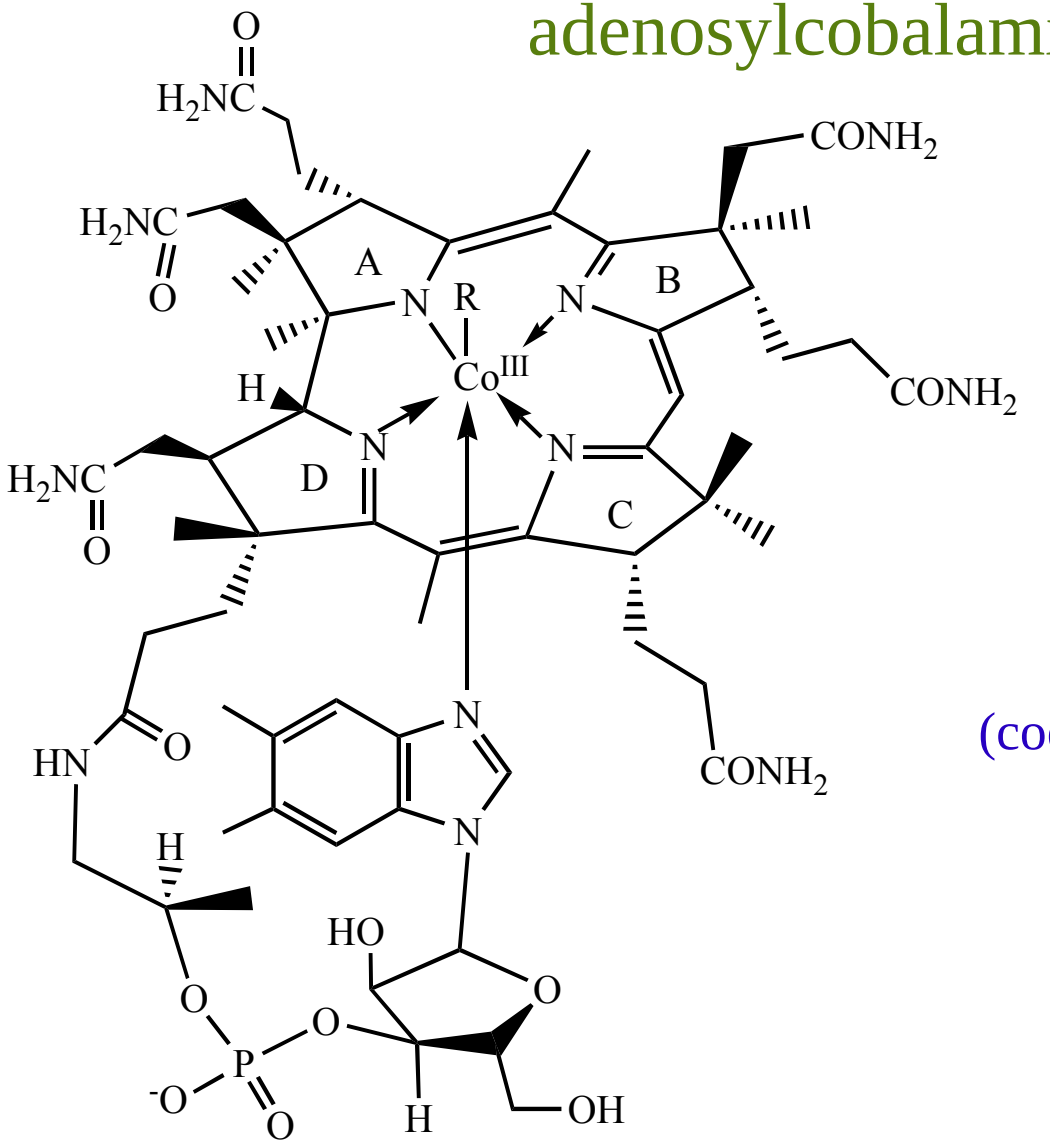

SH
(CH2)2
H — C — NH3+
COO−
Homocysteine
H3C — S
(CH2)2
H — C — NH3+
COO−
Methionine
METHIONINE SYNTHASE
Methylcobalamin
B12
Methyl — H4 folate
H4 folate
Hydroxocobalamin
B12
B12
Deoxyadenosylcobalamin
CH3
H — C — COO−
C — S — CoA
O
L-Methylmalonyl-CoA
METHYLMALONYL-CoA MUTASE
CH2 — COO−
H — C — H
C — S — CoA
O
Succinyl-CoA

Only two reactions in the body require vitamin B12 as a cofactor:

1. During the catabolism of fatty acids with an odd number of carbon atoms and the amino acids valine, isoleucine and threonine the resultant propionyl-CoA is converted to succinyl-CoA for oxidation in the TCA cycle.
   methylmalonyl-CoA mutase, requires vitamin B12 as a cofactor in the conversion of methylmalonyl-CoA to succinyl-CoA.
   5'-deoxyadenosine derivative of cobalamin is required for this reaction
2. The second reaction catalyzed by **methionine synthase**
   converts homocysteine to methionine
   This reaction results in the transfer of the methyl group from N5-methyltetrahydrofolate to hydroxycobalamin generating tetrahydrofolate and methylcobalamin during the process of the conversion.

Coenzyme B_{12} Rearrangements

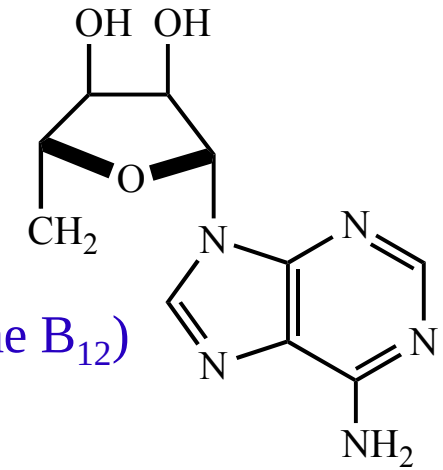
adenosylcobalamin



13.73

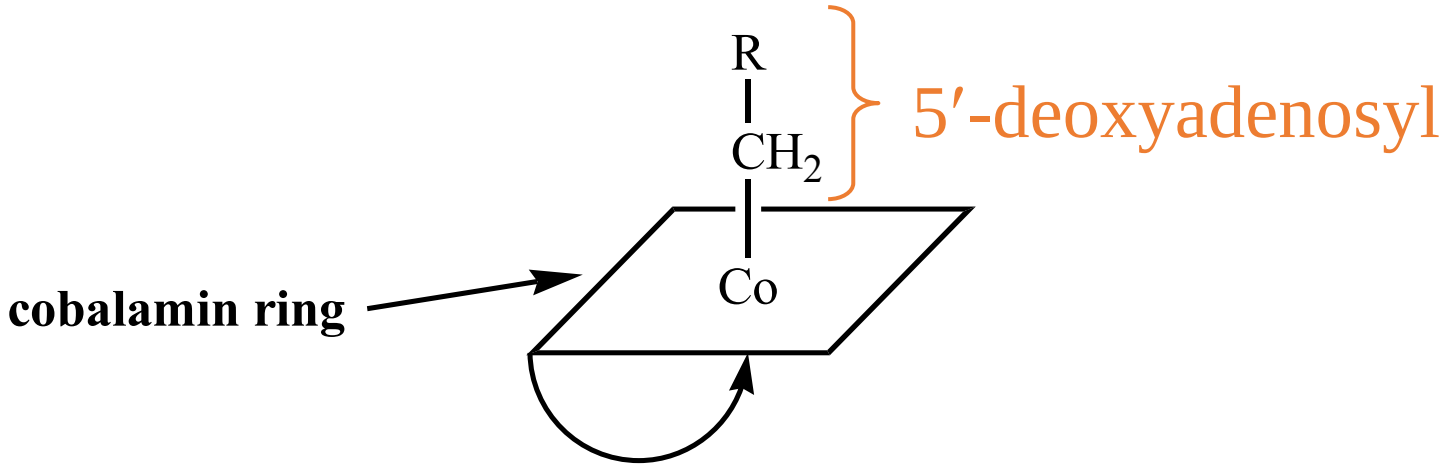
R

a



(coenzyme B_{12})

b H_2O (vitamin B_{12})

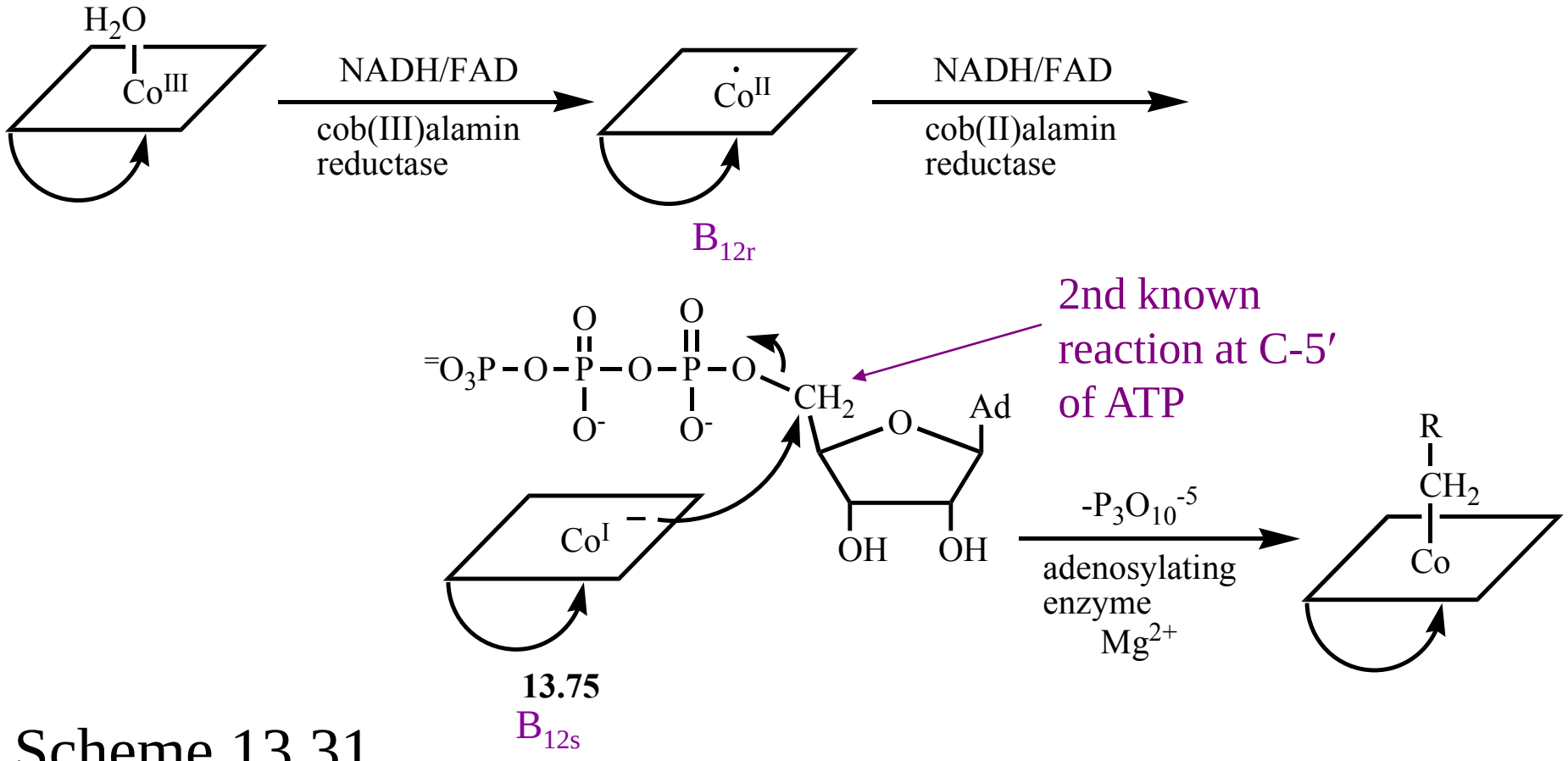


13.74

abbreviation for coenzyme B_{12}

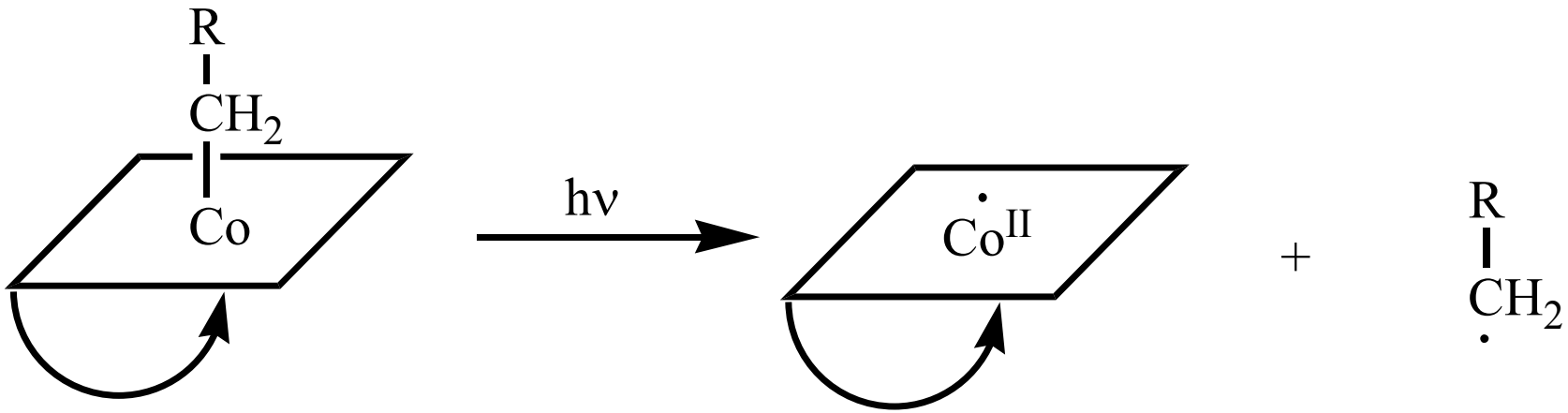
Conversion of Vitamin B_{12} to Coenzyme B_{12}

Bioynthesis of coenzyme B_{12}



Scheme 13.31

Light Sensitivity of the Co-C Bond of Coenzyme B_{12}



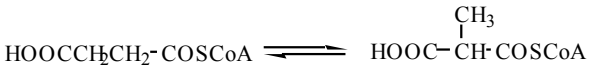
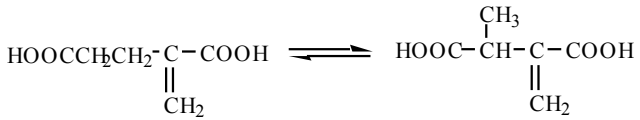
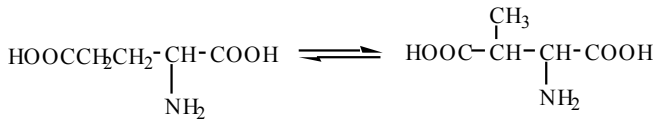
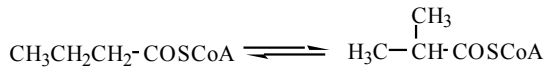
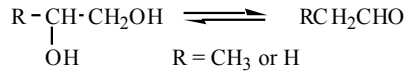
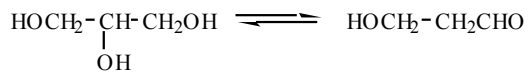
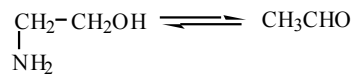
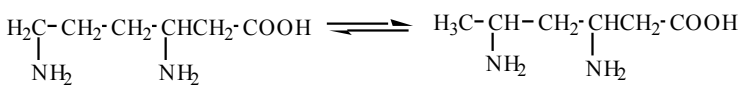
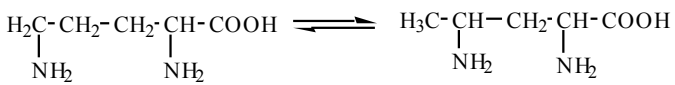
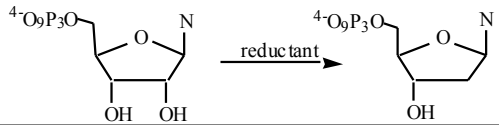
RCH_2 is 5'-deoxyadenosyl

13.76

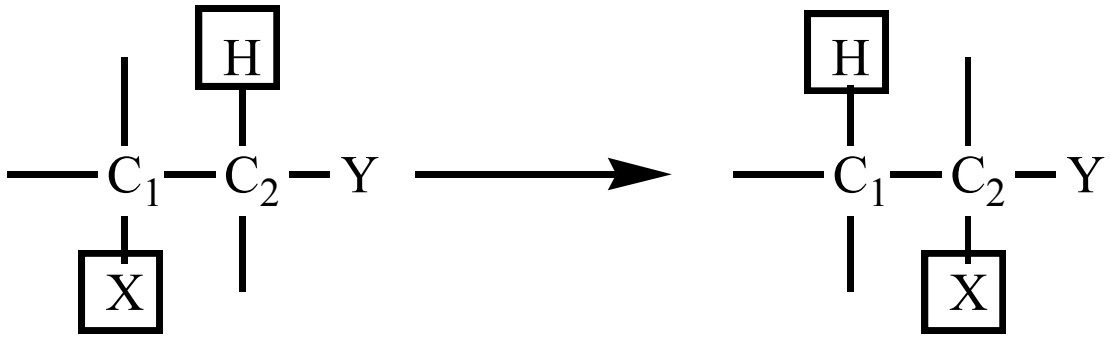
13.77

Scheme 13.32

Table 13.1. Coenzyme B_{12}-Dependent Enzyme-Catalyzed Reactions

Enzyme	Reaction Catalyzed
CARBON SKELETAL REARRANGEMENTS	
Methylmalonyl-CoA mutase	
2-Methyleneglutarate mutase	
Glutamate mutase	
Isobutyryl-CoA mutase	
ELIMINATIONS	
Diol dehydratase	
Glycerol dehydratase	
Ethanolamine ammonia lyase	
ISOMERIZATIONS	
L-b-Lysine-5,6-aminomutase	
D-Ornithine-4,5-aminomutase	
REDUCTION	
Ribonucleotide reductase	

General Form of Coenzyme B_{12}-Dependent Rearrangements



X is alkyl, acyl, or electronegative group

Scheme 13.33

Three Examples of Coenzyme B_{12} Rearrangements

Figure 13.2