Bioinorganic Chemistry of Copper

→ 3rd most abundant and essential trace element in human body Cu 250mg < Zn (3 to 4 gms) < Fe - 4 to 6 gm

→ Widely distributed both in plants & Animals

→ Stored as Cu-thionein in Brain, heart & liver

→ Released as ceruloplasmin or as a complex of serum albumin

→ Lab Test $Cu^{2+} + K_4[Fe^{II}(CN)_6] \rightarrow Cu_2[Fe(CN)_6]$ reddish brown ppt.

In presence of Cd^{2+}

$Cu^{2+} + CN^- \longrightarrow CuCN + (CN)_2\uparrow$ cyanogen gas.
White ppt.

$\downarrow$ Excess

$[Cu(CN)_4]^{3-}$ soluble.

Functions

→ Present as Cu-metalloproteins in many number and conduct various biological processes like —

(a) Electron Transport → Photosynthesis → Name of Protein

Rusticyanin, Plastocyanin, Umecyanin, Stellacyanin, Azurin, cytochrome C-oxidase

(b) Oxidation & Red^n of substrate → Oxygenase Activity in

Laccase, Ascorbate Oxidase, Ceruloplasmin & CuZnSOD

(c) O_2 transport in Mollusca (a large phyllum of vertebrates) → Hemocyanin, ~~SOD etc.~~

↓

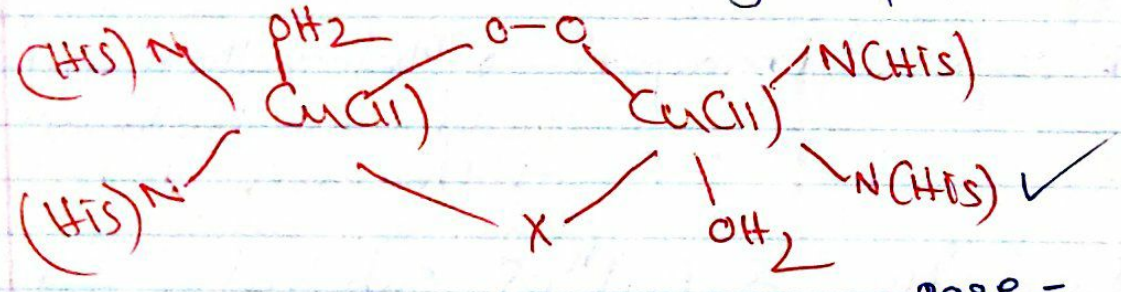
(O_2-Uptake protein like Hb, Mb and Hr)

$[Cu(NH_3)_4]^{2+}$ – dsp^2 – sq. pl. , $[Cu(CN)_4]^{2-}$ – sp^3 – Td

(49)

4 types of Copper Present in Cu-Proteins

Type	Main characteristics
1) Cu(I) d^{10} →	colourless (aq complex), diamag, epr silent.
2) Cu(II) normal →	blue (normal) epr spectra, tetragonally coordinated containing sq. sets of ligands.
3). Cu(II) blue →	very intense absorption band at ~ 600nm ($\epsilon = 5000\ M^{-1}cm^{-1}$), responsible for deep colour due to unusual ligand environment.
4) Coupled $[Cu(II)]_2$ →	Abnormal visible spectrum diamag., epr silent.



One Copper (II) Blue Proteins

→ Extracted from Plastocyanin
→ Present in chloroplast (plant source) spinach, cucumber
→ Structure

By Freeman & Coworkers.

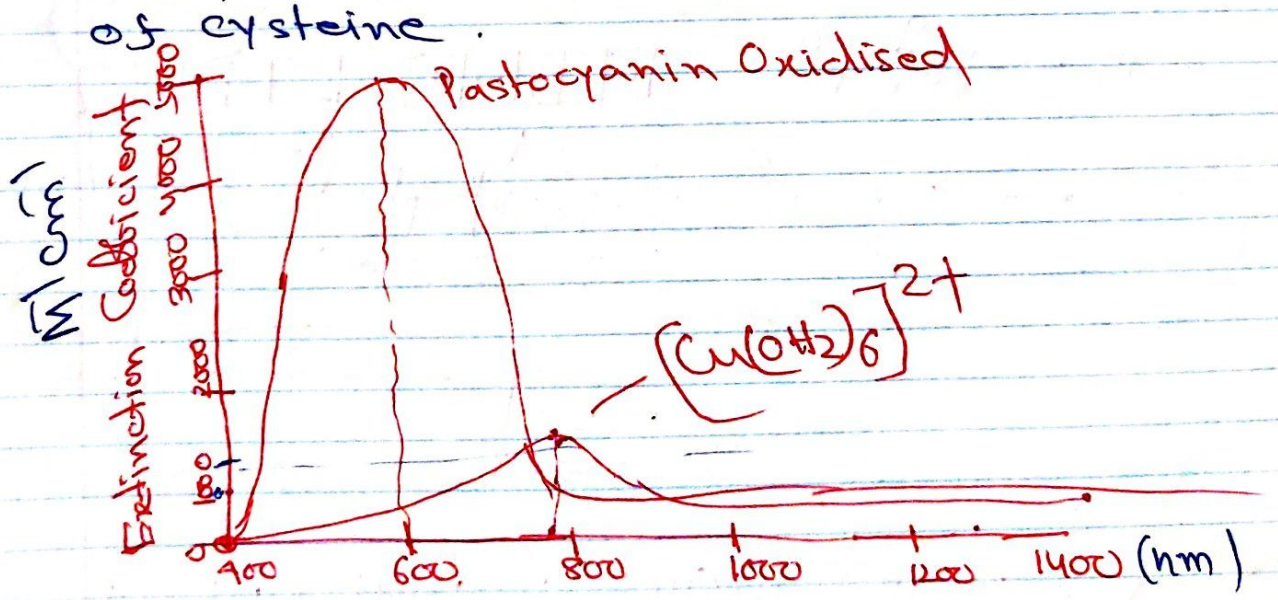
N(Im) His (37)
2.04 A°
(methionine) S (92) —2.13 A°— Cu(II) —2.9 A°— S (cysteine) (84)
2.10 A°
N(Im) His (87)

3D structural information from the X-Ray crystallo.

→ Highly distorted 4-coordinated, neither planar nor tetrahedral, Barrel like str. of eight strands
→ Bond angle varies 85 – 137°, single polypeptide chain
bond length is too high.
→ Intense blue colour at 600 nm due to the charge transfer from

(cyst) S —σ→ Cu

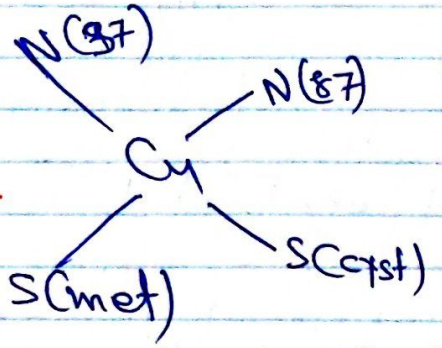
→ Unusual ESR spectra due to more delocalisation of the unpaired electron in Cu(II) atom with the p-π orbital of S atom of cysteine.



Comparison of electronic spectra

→ Barrel like str. of eight strands (staves)
→ single polypeptide chain undergoes a series of loops
→ Cu is at the end of the barrel forms a cylinder.

from Plastocyanin



N – Cu – N = 97°
N(His)87 – Cu – S(met) = 123°
S(met) – Cu – N(37) = 137

→ ligand gr. Hard N(His), soft (S)
→ Differ from Distorted Td 50°

Q 3p orbital of S (cyst) ⟷ $3d_{x^2-y^2}$ (Cu)
Thiolate lone pair
→ Unusual environment is most likely due to the coordination extremely unsymmetrical

CuZn Superoxide Dismutase (CuZnSOD):

$$2O_2^- + 2H^+ \rightleftharpoons H_2O_2 + O_2$$

- heterometallic core enzyme called brass enzyme & cupreins

Source

If RBC, → erythrocuprein Mitochondria liver → heptacuprein	Present in all eukaryotic cells (i.e aerobic forms of life)
Bovine erythrocyte SOD	FeSOD & MnSOD bound in prokaryotic (one) cells)

History

1st reported in 1938 by Mann and Keilin by fractionation of Bovine enzyme and presn of RBC.

Colour — Pale bluish Green.

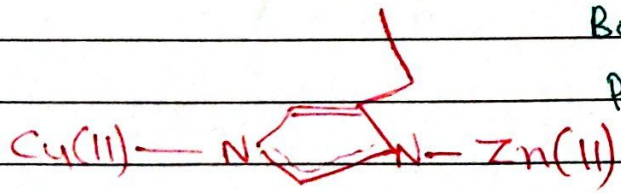
:- Presence of Zn^{2+} not discovered till 1970

:- ~~In 1969~~ by Mc Cord and Fridovich discovered presence of Zn^{2+}.

Structure:- (oxidised form Cu^{2+}, Zn^{2+})

Source — bovine erythrocytes.

(By Richardson and Coworkers)



Both metals are in close proximity bridged by a imidazole ring (unusual) 6.3A° apart

X-Ray diffraction studies:- The polypeptide chain (backbone) is a barrel of eight antiparallel chain of β-sheet (β-barrel).

:- Two chains (units) form a dimer Cu_2Zn_2SOD

:- Cu^{2+} binds 4 Histidine, with fifth axial H_2O

:- Hist-44 & Hist 46 are trans to each other.

CuZn-SOD

(53) (50) (X)

Formation of Superoxide and Peroxide ions in nature

:- Due to the partial reduction of O_2

i.e $O_2 \xrightarrow{4H^+ + 4e^-} 2H_2O$ + Energy

Gain in large energy from the cell is 212 KJ/mol with potential Difference 1.1V

In this process electrons are ~~released~~ (transfered) in stages from the reduced pyridine nucleotides to O_2 with

potential Difference is 1.1V

Gain in free energy from the cell is 212 KJ/mol

nFE = 1.1 × 2 × 96500

So redn can occur in steps

Step-I $O_2 + e^- \rightleftharpoons O_2^-$

Step-II $O_2^- + e^- \rightleftharpoons O_2^=$

Net result is formation of O_2^- and $O_2^=$

Net result formation of O_2^-, $O_2^=$

:- These are toxic to the life processes.

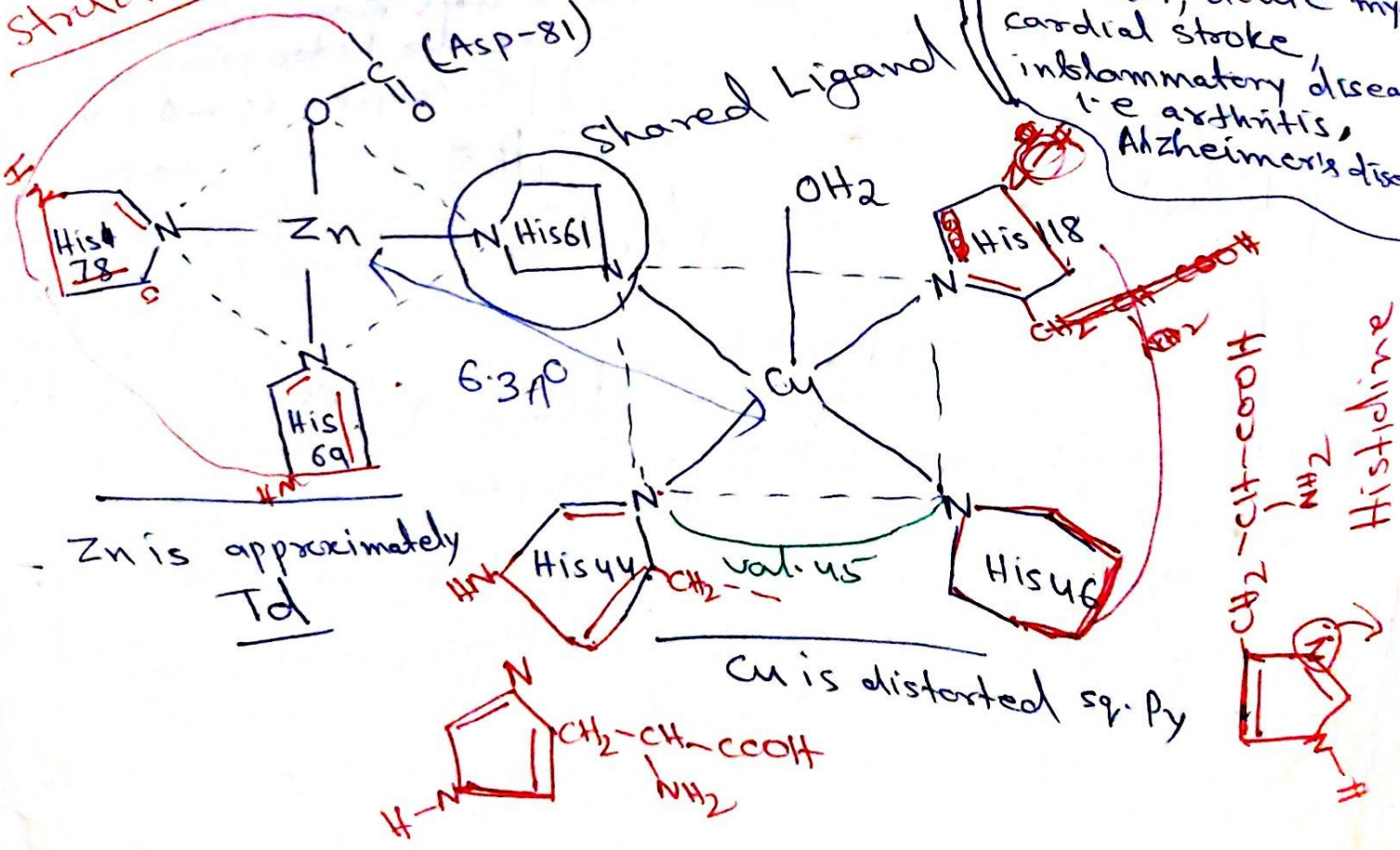
So

(1) $2O_2^- + 2H^+ \xrightarrow{CuZn\ SOD} H_2O_2 + O_2$.

and (2) $2O_2^= + 4H^+ \xrightarrow{Catalases} 2H_2O + O_2$

may cause oxidative stress-induced diseases (Parkison's disease i.e neurological disorder, acute myocardial stroke, inflammatory disease i.e arthritis, Alzheimer's disease

Structure



Zn is approximately Td

Cu is distorted sq. Py

Structural Aspects — Conical structure (5A)

:- Hist 44, Val 45, His 46 completely blocks one side of Cu_4N_4 plane

:- 2) Other side is accessible to solvent/anions with 4A° (wide) conical channel

4) In the lining of the channel there is a sequence of +ively amino acids chain residue ~~such sequence~~

5) Lysine is at 134 at bar end (13A° distance from Cu^{2+})

6) probably O_2^- attracts by this end and diffuse over the protein surface

7) At 131 position glutamic acid partly neutralise the charge and develops electrostatic gradient that

:- drives O_2^- ions towards Cu^{2+}/Zn^{2+}

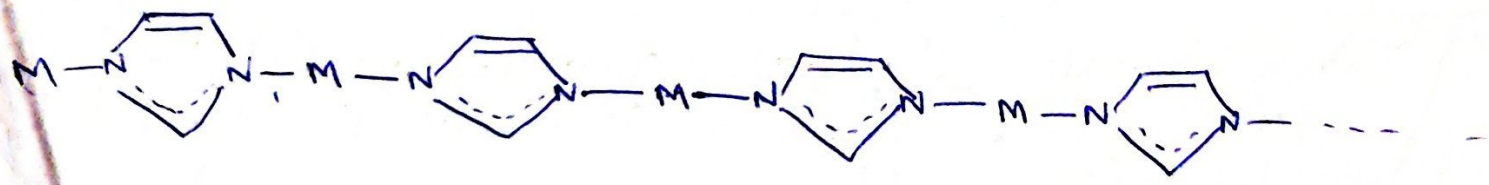
118 - Hist, 119 - Glu, 120 - lys, 130 - glu, 131 - glutamic acid, 133 - glu, 134 - lys, 141 - Arg.

3) Both metals are in close proximity bridged by a imidazole ring of 6.3A° apart. (6)

:- Cu(II) lies at the bottom of that narrow channel (7)

:- Role of Zn is primarily structural, because (8) removal of Zn doesn't change SOD activity but diminised thermal stability (8)

:- SOD sometimes form polymeric imidazole bridged structure which is not bound in other protein yet



Mechanism

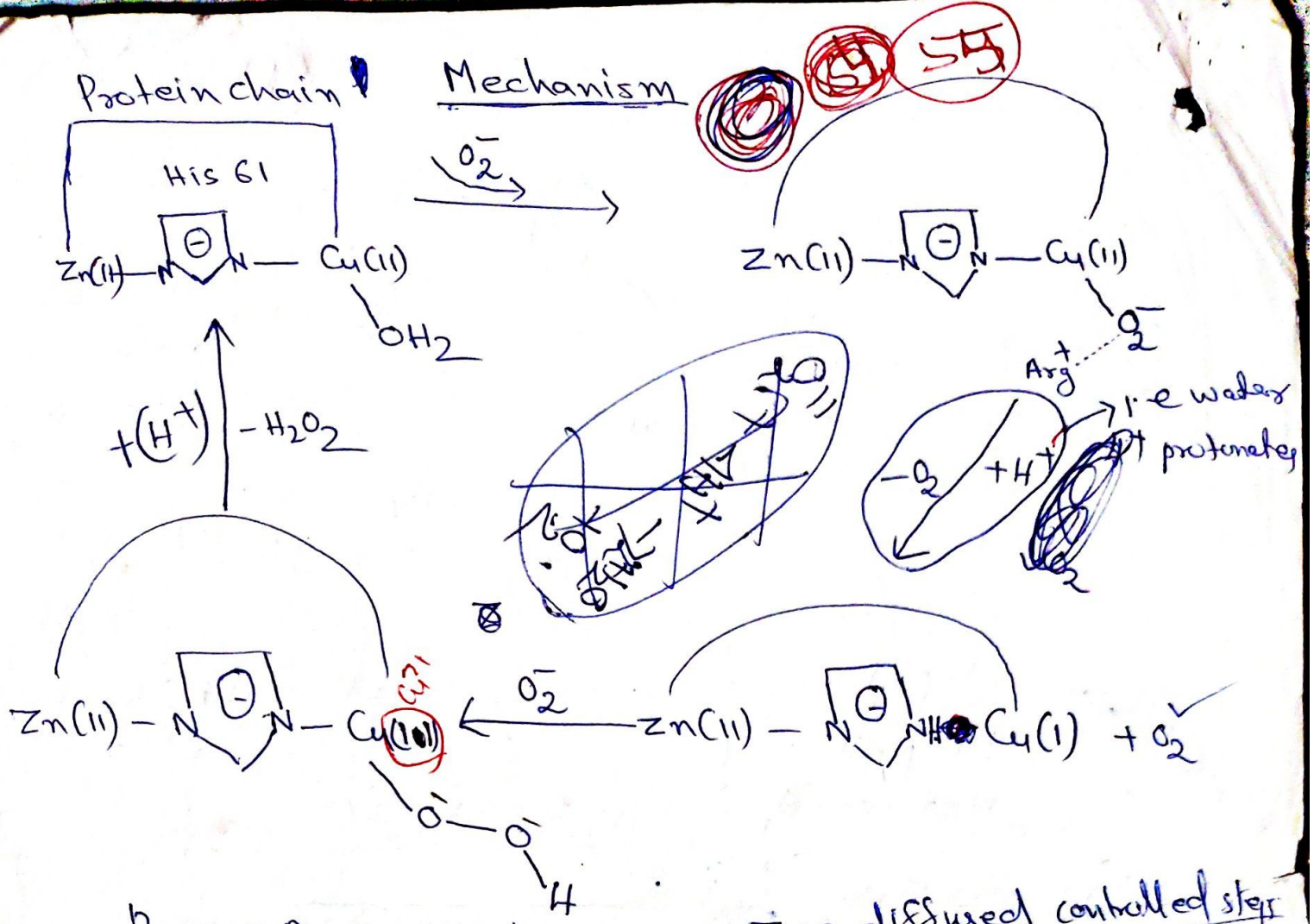
$$ECu^{II} + O_2^- \rightleftharpoons ECu^{I} + O_2$$

$$ECu^{I} + O_2^- + 2H^+ \rightleftharpoons ECu^{II} + H_2O_2$$

Red^n of Cu^{II} to Cu^{I} by 1st superoxide with release of O_2

Reoxidation of Cu^{I} to Cu^{II} by second superoxide ion

Protein chain Mechanism



Ping-Pong Mechanism ⟹ Two diffused controlled step overall mechanism

$O_2^- \longrightarrow O_2 + e^-$

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

$O_2^- + O_2^- + 2H^+ \Longrightarrow H_2O_2 + O_2$

Both steps thermodynamically feasible as the Redox potential

(a) $O_2 | O_2^-$ couple is $-0.33V$

(b) $O_2^- | H_2O_2$ " " $+0.89V$

$ECu^{II} + O_2^- \longrightarrow ECu^{I} + O_2$ (Red^n)

$ECu^{I} + O_2^- + 2H^+ \longrightarrow ECu^{II} + H_2O_2$ (Oxd^n)

Mechanism.

8) → O_2 reaches the bottom of the protein channel where Cu(II) resides. (10)

9) → O_2 reduces Cu(II) to Cu(I) producing O_2 (red^n) (11)

10) → when Cu(I) is produced the bridging imidazole gr (His 61) is dislodged (Knock out) from copper(I) but remain coordinated with Zn(II) and protonated water protonates the partial dislodged gr (12)

($H_2O \rightleftharpoons H^+ + OH^-$)

Only model enzyme capable of one electron redox chemistry between M^{n+1} & M^{n+1}

51

Ceruloplasmin (57, 63, 165, 315)

1- Iron mobilisation (metabolism)
2- present in Human/Animal serum i.e blood plasma of mammals, ~~birds~~ birds, reptiles and amphibians.
3- Contain 8 Cu atom (7 ± 1) (n+b+c)
(2+1+4)
~~4- Synthesized in liver~~ , M.M — 130,000 — 150,000
Human = M.M. ≃ 150,000
2 peptide chain with M.M. ~ 59,000 | EXAFS study
other 2 " " " " ~ 16,000
also contain some saccaride unit.
4- This Cu-protein is synthesised in liver.
$\lambda_{max} \rightarrow 370$ & $605 \Rightarrow \epsilon = 500$ and $1200\ dm^3\ mol^{-1}\ cm^{-1}$

Function Fe transport

(a) $4Fe(II) + 4H^+ + O_2 \xrightarrow{\text{ceruloplasmin}} 4Fe(III) + 2H_2O$
then Fe(III) picked up by the transferrin which good scavenger. ⇒ Synthesis of Hb

(b) Cu transport In the cells of brain Cu present as Cu-thionein but in blood plasma it is as ceruloplasmin is called Cu-transport protein.

Laccase

1- Water soluble enzyme.

2- M. Mass varies with source, ≃ 60,000 to 120,000

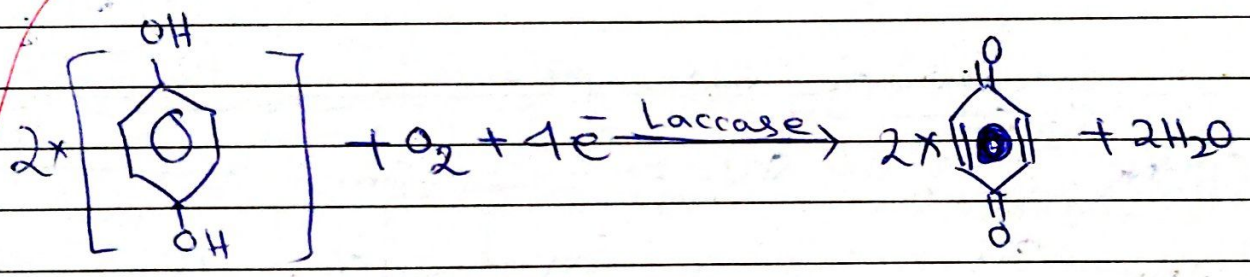
3- Isolated from Fungus has been better studied.

4- Contain 4 Cu atom, one normal Cu^{II}, one Cu^{II} (blue) and ~~1×2~~ 1 unit coupled Cu^{II} which bind O_2.
Absorption bands are at 532, 615, 730 nm
ϵ = 300, 1400, 500 $M^{-1} cm^{-1}$ respectively.

5- Discovered by Yoshida (Japan) from Lac trees

Function

Catalyse the oxidation of several diamines & diphenols.



$Cu(II)$ Enzyme + Substrate ⟶ $Cu(I)$ Enzyme + oxidised substrate

$Cu(I)$ Enzyme + O_2 $\xrightarrow{H^+}$ $Cu(II)$ Enzyme + H_2O

6- Removal of non-blue (normal) Cu^{II} deactivate the enzyme even though other centres are present.

Laccase ⟶ one coupled $Cu(II)$ binds O_2

- one normal $Cu(II)$ — easy detectable
- one blue $Cu(II)$ — not yet understood

Copper ~~Zinc~~ deficiency

→ stored as ~~Cu-thionein~~ Cu-thionein

→ released as ceruloplasmin or as a complex with serum albumin.

→ Found in heart brain and liver.

Deficiency

→ progressive brain disease in infants (Menke' kinky hair syndrome) i.e
↳ due to the defective intestinal absorption of Cu

(i) abnormally spiral curly hair

(ii) the brain tissue largely lack in cyt-c oxidase.

(iii) generates the defects in the structural stabilisation of the fibrous protein of connective tissues in different animals.

(iv) Anaemia – ceruloplasmin

Arises due to

① soil contain high level of Mo

② The Zn induced Cu-deficiency probably arises due to the competition for absorption in gastrointestinal tract.

Advised

$CuSO_4 \cdot 5H_2O$ / Cu(II)-L-Histidine in physiological saline is administered.

Questions :- Outline the possible causes of Menke' and Wilson's disease and discuss their clinical symptoms.