5. What is nitrogenase? Discuss briefly the mechanism of biological fixation of nitrogen indicating the role of nitrogenase enzyme and functions of Fe and Mo present in it.

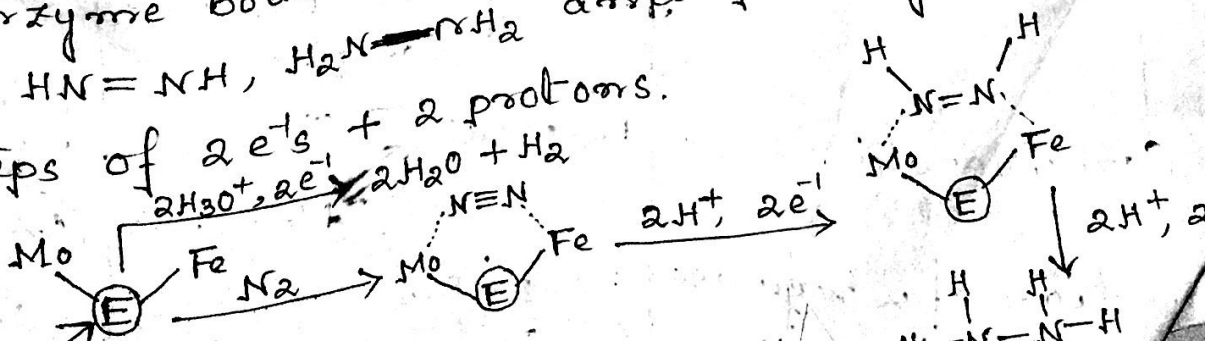
Nitrogenase enzyme is the biological catalyst for reduction of nitrogen. It is an equilibrium mixture of high molecular weight (270×10^3 Da) Fe-Mo protein and a low molecular weight (55×10^3 Da) Fe-protein in 1:2 molar ratio.

Fe-Mo-protein + 2Fe-protein $\rightleftharpoons$ nitrogenase.

Nitrogenase enzyme catalyses reduction reaction. Nitrogen reduction is strongly inhibited by CO, NO, N_2H_4 and O_2. H_2 also inhibits N_2 red^n. In the absence of nitrogen, the active enzyme reduces H_3O^+ ion to evolve H_2 gas. This H_2 evolut lowers the catalytic efficiency of nitrogenase to about 75%. The overall stoichiometry of N_2 reduction is, therefore,

$$N_2 + 8H^+ + 8e \longrightarrow 2NH_3 + H_2.$$

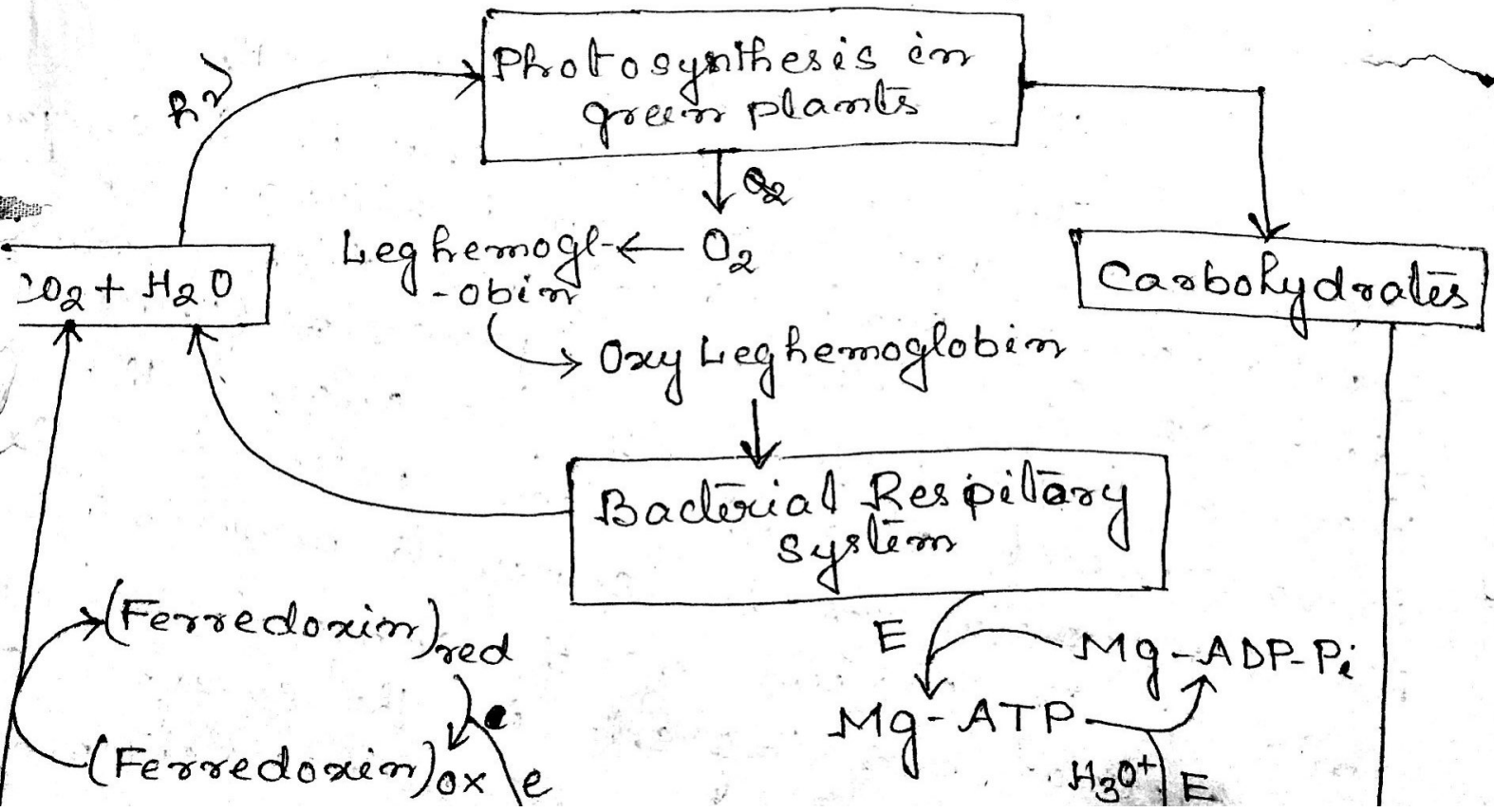
There are separate sites in the enzym for electron activation and nitrogen red^n. Isolation of symmetrical reduction product diimides ($HN=NH$) and hydrazine (H_2N-NH_2 from nitrogen fixing systems suggest that th involvement of a binuclear centre, i.e., the H Fe-Mo protein as the substrate binding site and the Fe-protein as the ~~substrate binding~~ site electron binding site in the enzyme. The enzyme bound nitrogen is successively reduce to $HN=NH$, H_2N-NH_2 and finally to NH_3 in Steps of 2 e$^-$s + 2 protons.



In addition to the enzyme nitrogenase, the nitrogen fixing system requires a source of reducing power and a source of energy for activation, of N_2 and electrons. Oxidation of carbohydrates acts as a source of reducing power. The activation energy is supplied by hydrolysis of ATP, catalysed by kinase enzyme

$$ATP^{4-} + H_2O \rightleftharpoons HADP^{2-} + HPO_4^{2-}$$

The reaction helps to maintain anhydrous atmosphere and appropiate pH at which the enzyme is most active. ATP is supplied by the metabolic oxidation of carbohydrates. Leghemoglobin, a Fe(II)-heme protein present in the nitrogen fixing system, binds any O_2 that may be present. Oxy-Leghemoglobin serves as the source of metabolic energy that is required for the fixation process.



8) What is Carboxy peptidase A (CPA)? what is its function. what are the requirement for function describle the active site structure of CPA. Give a view regarding the mechanism of action of CPA. what happens when the central metal is replaced by Co^{2+}.

Carboxy peptidase A is a Zn(II) containing metalloenzyme. It consists of 307 amino acid residues in a single polypeptide chain. Its molecular weight is 34,600 Da.

CPA catalyse the hydrolysis of the C-terminal amino acid residue in a peptide or a protein chain.

CPA is highly specific. There are two absolute requirements for CPA enzyme, the C-terminal end must have L-configuration and its carboxyl group must be free. Substrates with C-terminal end having an aromatic group (Rs) are favoured. Peptides ~~having~~ with any amino acid except proline at the C-terminal end are hydrolysed.

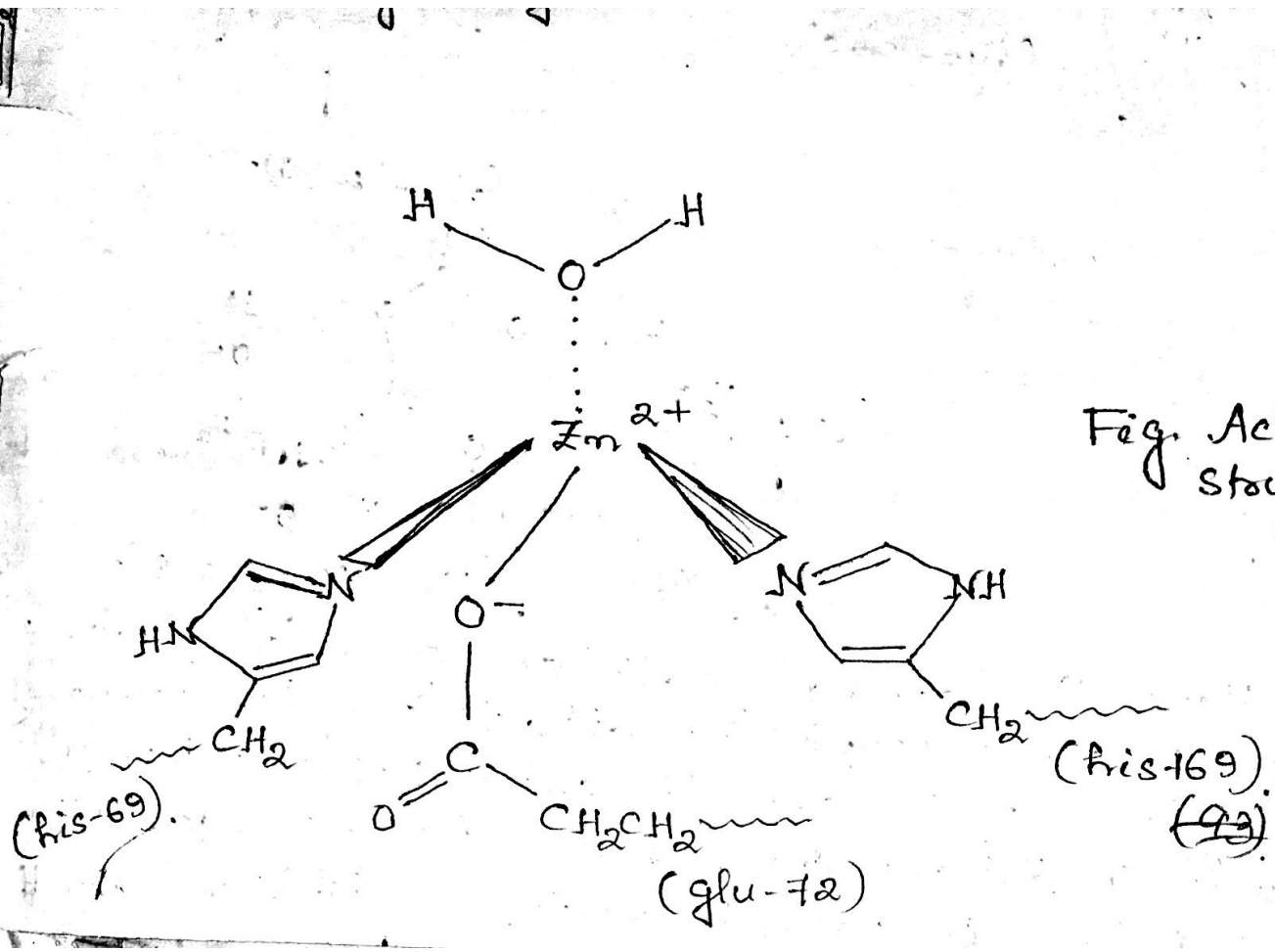
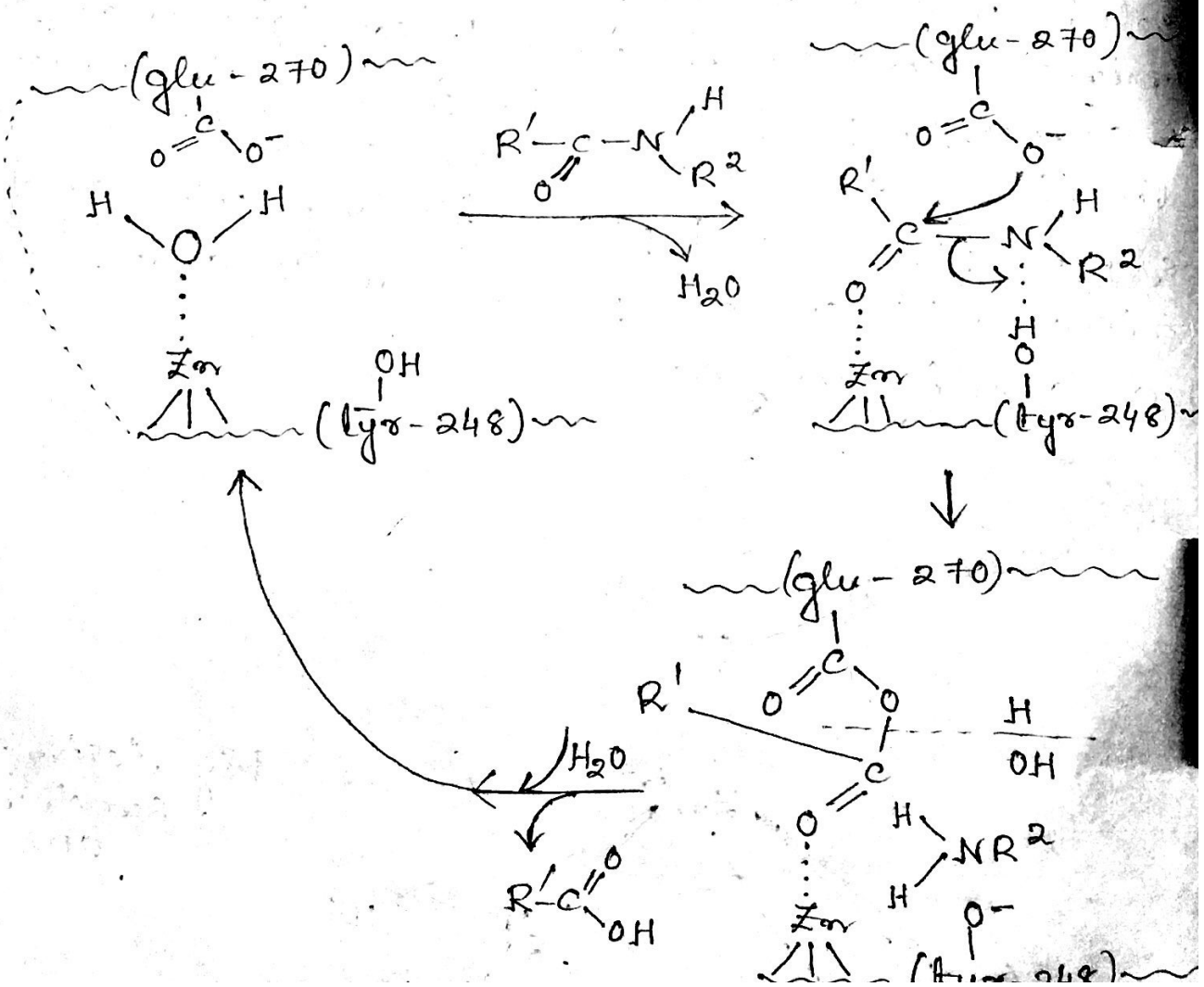
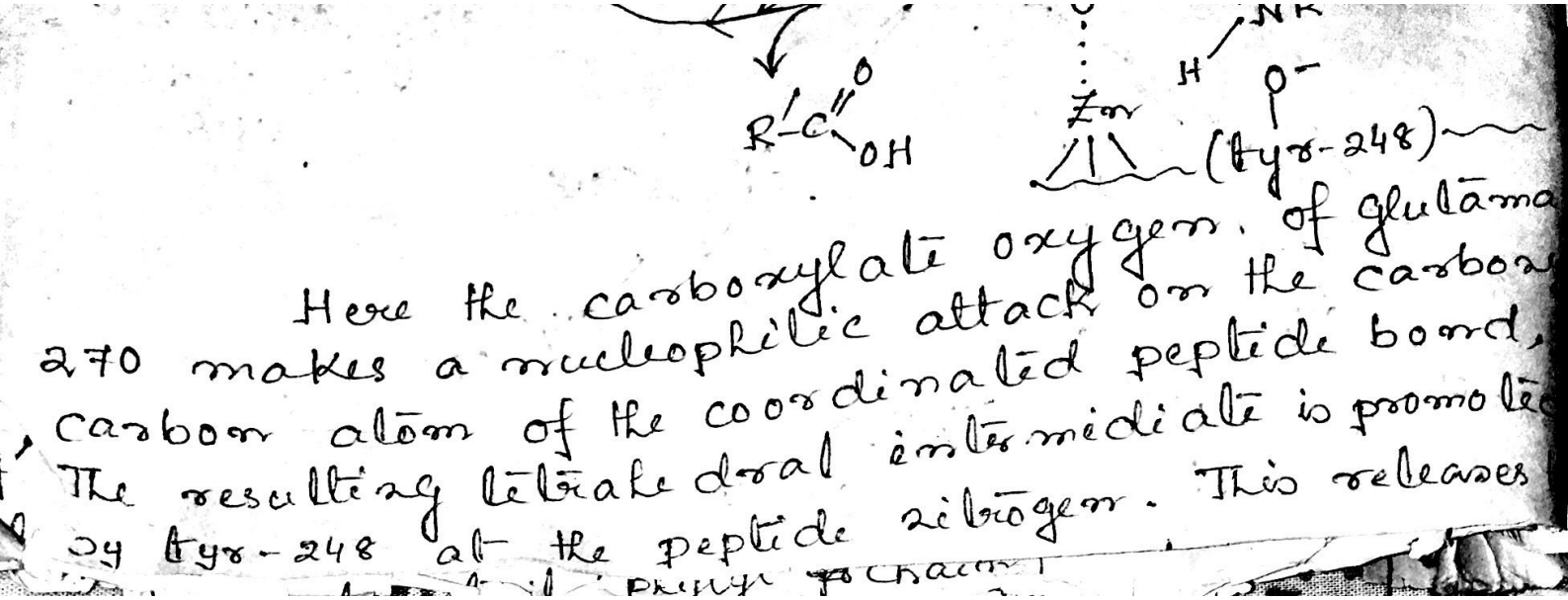


Fig. Active site structure of CPA.

The active site containing Zn^{2+} comprises about a quarter of the ellipsoidal molecule and is situated in a cleft in protein structure. Zn^{2+} is coordinated by two histidine N atoms at positions 69 and 169 and by a carboxylate oxygen of the glutamate residue at position 72 in the chain. The fourth position is occupied by water molecule or a $\bar{O}H$ ion, giving a distorted tetrahedral geometry around

There are two alternative views regarding the mechanism of action of C
Breaking of protein molecules may place as follows -





Here the carboxylate oxygen of glutama
270 makes a nucleophilic attack on the carbon
carbon atom of the coordinated peptide bond,
The resulting tetrahedral intermediate is promote
by tyr-248 at the peptide nitrogen. This releases

c-terminal amino acid with accompanying formation of a mixed anhydride, which on subsequent hydrolysis regenerates the enzyme.

** When the central metal Zn^{2+} ion is replaced by Co^{2+} ion, the enzymatic activity of CPA will be restored. Only the difference will be in the spectral and magnetic property. Zn^{2+} ion is a d^{10} electron system and hence the complex containing Zn^{2+} ion will absorbs light in the UV region and also the complex will be diamagnetic. Hence the spectral & magnetic properties of CPA enzyme can not be studied properly.

But Co^{2+} is a d^7 electron system and hence strong d-d transition occur in the complex containing Co^{2+} ion. So the complex will absorb light in the visible region and shows coloured complex. Moreover, the complex will be paramagnetic under any field. So the spectral and magnetic property can be studied properly.] **