Metallo Enzymes

A metalloenzyme is an enzymatic protein in which a metal ion is embedded in the cavity of the enzyme and forms strong bond with the donor atoms of the protein.

Donor atoms are soft bases like C & H Sulphur or hard bases as O & N

Metal may be either soft metals as Cu^{+}, Hg^{+}, Cd^{+2}.
or hard metals as Fe^{+3} / Zn^{+2}.

Protein part - Apoenzyme
Metal ion / complex Metal - prosthetic group.

Coenzyme:- The ~~protein~~ reversible group that combines with an enzyme for a particular rxn and then is released to combine with another is called co-enzyme.

Co-factors :- Both prosthetic grp & coenzyme are some-times called cofactors.

Peptide hydrolysis

$$R-CO-NH-R' + H_2O \rightleftharpoons RCO_2^- + R'NH_3^+$$

The enzyme catalysis arises due to:-

1) polarizatn of C=O bond through metal - oxygen coordinatn
2) Generation of metal hydroxo (M-OH) species at the biological pH and the metal bound -OH acts as powerful nucleophile.

Peptidases:- biological catalysts degrades proteins to A. Acids by cleaving peptide linkage.

Peptidases can be classified → Endopeptidase → non terminal
→ Exopeptidases → hydrolyse terminal peptide bond.

Exopeptidase → Aminopeptidase → N-terminal peptide bond hydrolyze.
→ Carboxypeptidase → C- terminal peptide bond.

$H_2NCH(R^1)CO$ ___ $NHCH(R^2)CO-NH$ ..... $CO-NHCH(R^n)COOH$

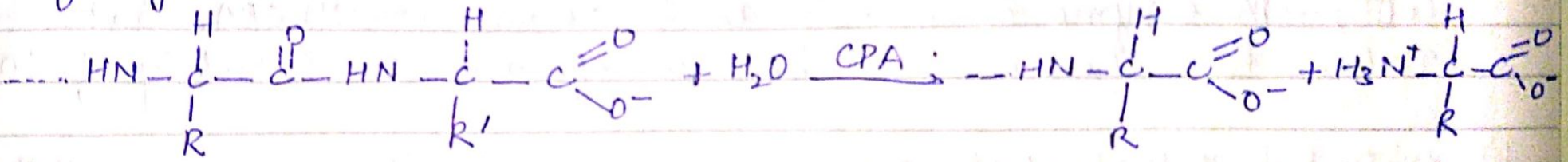
Amino pepti ↑ Endopepti ↑ Carboxypeptidase.

CARBOXYPEPTIDASES

- → Carboxypeptidase A (307 Amino Acid Residue)
- → Carboxypeptidase B (308 " " ")

Both require one Zn(II) site per molecule.

Carboxypeptidase A is a pancreatic metalloenzyme which catalyse the hydrolysis of peptide bonds in protein during the process of digestion.



I:- Structural feature:-

Diff forms :- CPA-α , CPA-β , CPA-γ etc

Commonly CPA-α is CPA

CPA looks egg-shaped. Zn(II) is coordinated by two N-sites (His-69 , His-196), one carboxylate oxygen of glutamate Glu-72 and a H_2O molecule. This provides distorted tet. geometry around Zn(II).

Near the vicinity of active site three A.A residues are present.

a) protonated guanidyl moiety of Arg-145
b) phenolic OH of Tyr-248
c) carboxylate end of of Glu-270.

CPA Reactivity:-

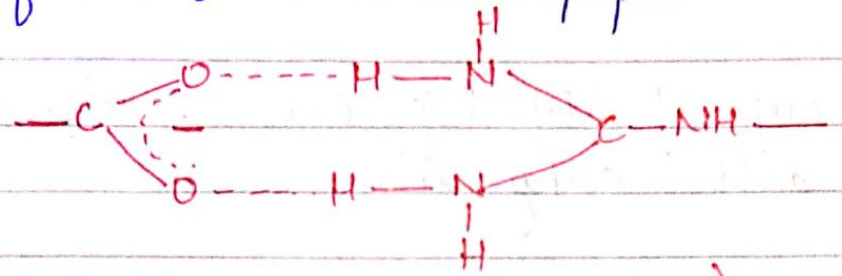
a) **Metal substitution:-** Zn^{+2} can be removed by using chelating agents. The apoenzyme itself is inactive. The activity can be restored by adding Zn^{+2} again.
Co(II)-CPA enzyme is more active than Zn(II) CPA but nature has chosen Zn(II) because of non avalability of Co.

b) **Distorted tetrahedral geometry around Zn(II) = entatic State**
The electronic spectra using Co(II) as spectral probe indicates distorted td. geometry. This distorted condition as the "entatic state" which is believed to lower the activation energy to attain the transition state.

c) **Hydrophobic pocket:-** This pocket created by polypeptide chain resides in the vicinity of the active site to house the hydrophobic group of the terminal residue of the substrate.

d) Role of Arg-145 in substrate recognition:- The terminal carboxyl grp of the substrate forms a salt bridge (ionic interactive) with the protonated guanidyl group of Arg-145.

It keeps the substrate in a proper position and orientation as required in the process. That is why enzyme is specific for the terminal peptide linkage at the carbonyl end.



Substrate. (Arg-145).

e) Generation of carbocationic character at the terminal peptide Carbonyl carbon:-

The carbonyl oxygen replaces H_2O molecule at the active site of Zn(II).

The Hydrogen bonding interaction between the -NH- group and the phenolic -OH group of Tyr-248 also enhances the cationic character of the carbonyl carbon & it also helps in the rupture of N-C bond. With the increase of the charge on the carbonyl carbon centre, the nucleophilic attack on it is facilitated.

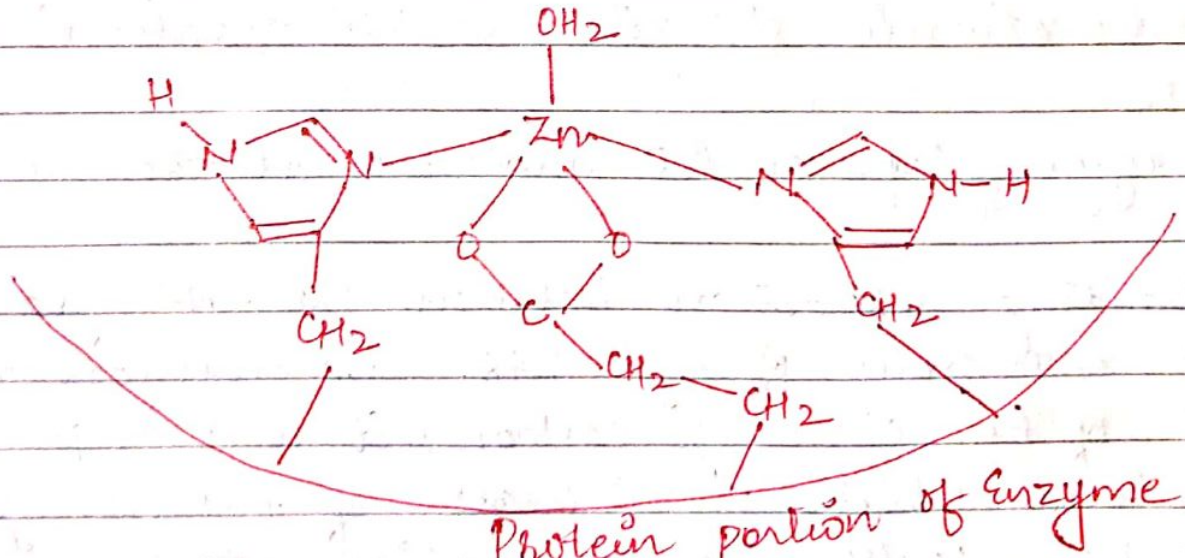
(f) Role of Glu-270 to generate a potential Nucleophile:-

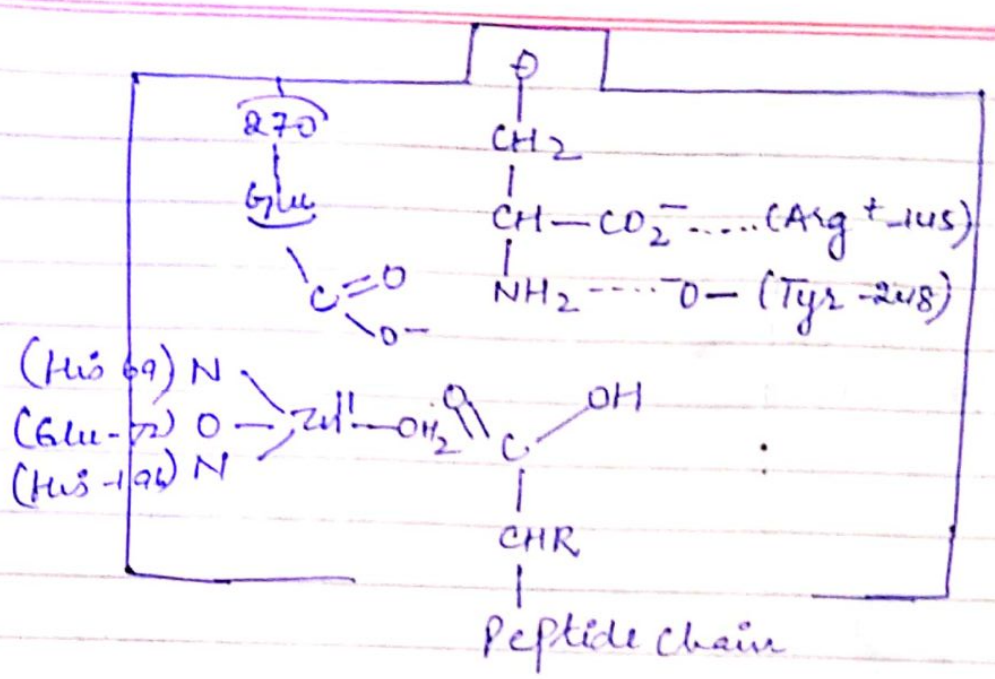
1) It keeps the nucleophile (H_2O) in position by H-Bond which in a way help the attack on the target Carbonyl Carbon

2) It may interact with H_2O bound to Zn(II) to generate metal bound hydroxide group which is a powerful Nu^- to attack the peptide linkage.

3) The carboxylate group of Glu-270 can itself act a good Nu^- to produce an acid anhydride.

Structure of CPA





Carbonic-Anhydrase (CA): Structure and Reactivity

→ Zn (II) containing metalloenzyme CA is responsible for reversible hydration of CO_2 in blood.

The hydration of CO_2 and dehydration of H_2CO_3 is imp. in animals, plants and several bacteria.

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \quad (K \simeq 10^{-1} s^{-1})$$
(at physiological pH)

$$CO_2 + H_2O \overset{CA}{\rightleftharpoons} HCO_3^- + H^+ \quad (K_{cat} \simeq 10^6 s^{-1})$$
(at physiological pH)