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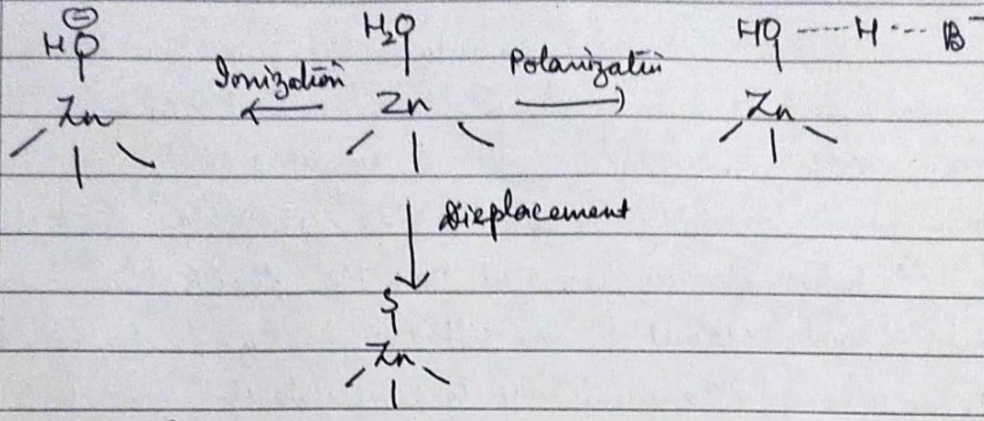
Enzymes containing Zn^{2+}

Zn^{2+} occurs in the biochemical systems mainly in the form of metalloenzymes. It is a very common Lewis acid in biochemical system & it is of some interest to explore why Zn^{2+} occurs rather than Mg^{2+} & Cu^{2+} which are also small di+ve ions. A feature that distinguishes Zn^{2+} from Mg^{2+} is that EA of Zn^{2+} > EA of Mg^{2+} ($EA \text{ of } M^{2+} = \frac{1}{2}(\text{IE}_1 + \text{IE}_2 \text{ of } M)$) This indicates Zn^{2+} to be a stronger Lewis acid than Mg^{2+}. The EA of Cu^{2+} is similar, so another feature must account for Zn's difference from Cu. Zn does not have variable oxid^n state so redox reac^{ns} are unlikely to complicate its behaviour & there is no risk of generating radicals.

A further point is that catalysis, in general, requires reorganization of atom. Fast reac^{ns} will be more characteristic of a metal ion that, while affecting ligands structurally, can take up & release ligands easily. Zn^{2+} is a d^{10} system having no LFSE and no preferred C.N. It forms labile complexes & geometrical rearrangements are facile. It can take up C.N. 4, 5 or 6. The stability of a Zn(II) complex is determined by the Zn–L distance & repulsion b/w the ligands. The Zn–L distance is short in a Td complex & the interligand repulsion high. As C.N. increases the M–L distance becomes larger & interligand repulsion decreases. Due to the cumulative effect of these 2 factors, complexes of CN 4, 5 & 6 have small difference in stability & can change from one form to another. Zn^{2+} is larger than H^+ but has double +ve charge. The +ve charge density of Zn^{2+} is high & it attracts $e^{\ominus}$s strongly. It does not hydrolyse at pH 8. Hence it acts as a super acid ie. it can act as a Lewis acid in higher pH where proton catalysis fails.

The unique feature of all structurally characterized active mononuclear Zn sites is a H_2O molecule, which can be activated by ionization, by polarization or be poised for displacement by a substrate. Two general mechanistic pathways have been suggested for the catalytic action of Zn enzymes. In the first the coordinated H_2O molecule is deprotonated to leave a bound OH^- which can attack the C atom of a $>C=O$ gp. or any +ve centre.



In the 2nd the substrate directly attaches to Zn through a carbonyl gp. Zn has several chemical features which benefit its presence at the catalytic site. The ready formⁿ of low CN sites which have more acidity than high CN sites, the easy deformation of the coordⁿ site geometry & the accessible change in CN from 4 to 5 to 6 are all of assistance at a catalytic site where there must be flexibility throughout the catalytic cycle. Zn^{2+} can exchange its ligands readily but is not itself lost readily from the protein.

- More available than Cd, Ni, Cu
- Faster ligand exchange than Ni^{2+}, Mg^{2+}
- More flexible coordⁿ geom. than Ni^{2+}, Mg^{2+}
- More strongly complexed than Mn^{2+}, Fe^{2+}
- Not redox active like Cu^{2+}, Fe^{2+}, Mn^{2+}
- Good Lewis acid among M^{2+} (only Cu^{2+} stronger)

Carbonic anhydrase

It is a Zn(II) protein, which catalyses the reversible hydration of CO_2. It is present in animals, plants & certain microorganisms. The presence of Zn(II) was established in 1939. With its +vely charged metal centre Zn(II) acts as a Lewis ~~catalyst~~ acid for activating a substrate molecule. It is a redox inactive metal ion & the +ve charge on it is a key factor of significance in metalloenzymes. It lowers the pK_a of coordinated water & provides a very high local conc^n of OH^- ions which otherwise are not available to the cytosol

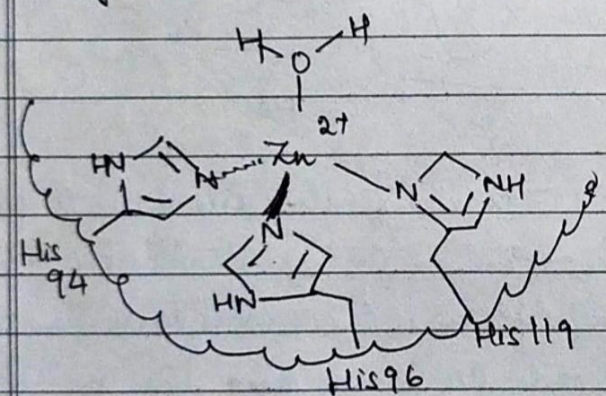
$$Zn^{2+} \cdots :\ddot{O}H_2 \rightleftharpoons Zn^{2+} \cdots :\ddot{O}H^- + H^+$$

The hydration reacⁿ catalysed by the enzyme is

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

In the absence of the enzyme this reacⁿ is slow and the rate at physiological pH ~ $10^{-2} s^{-1}$. In the presence of enzyme it rises to a turnover as high as $10^6 s^{-1}$. CO_2 generated by oxidative processes must be converted to carbonic acid in the blood & carried by deoxy Hb to the lungs. The dehydration of HCO_3^- in lungs is catalysed by CA for CO_2 to be exhaled. The nomenclature of the enzyme is based on the reacⁿ involving dehydration of H_2CO_3.

There are 3 types of CA – A, B & C. The str of B from occurring in humans has been determined most exhaustively. It is monomeric containing 1 atom of Zn(II) bound to protein having 256 – 265 amino acid residues. The protein has an ellipsoidal str divided into 2 halves. The Zn(II) is present at the bottom of the large hydrophobic cavity almost at the centre of the ellipse ~ 1800 pm from the surface



The Zn(II) is bound to 3 histidine residues (His 119, His 96 and His 94) in a distorted Td geometry, with the 4th coordⁿ position occupied by a H_2O molecule. The coordinated H_2O molecule is H bonded to other non-coordinated H_2O mols. & amino acids.

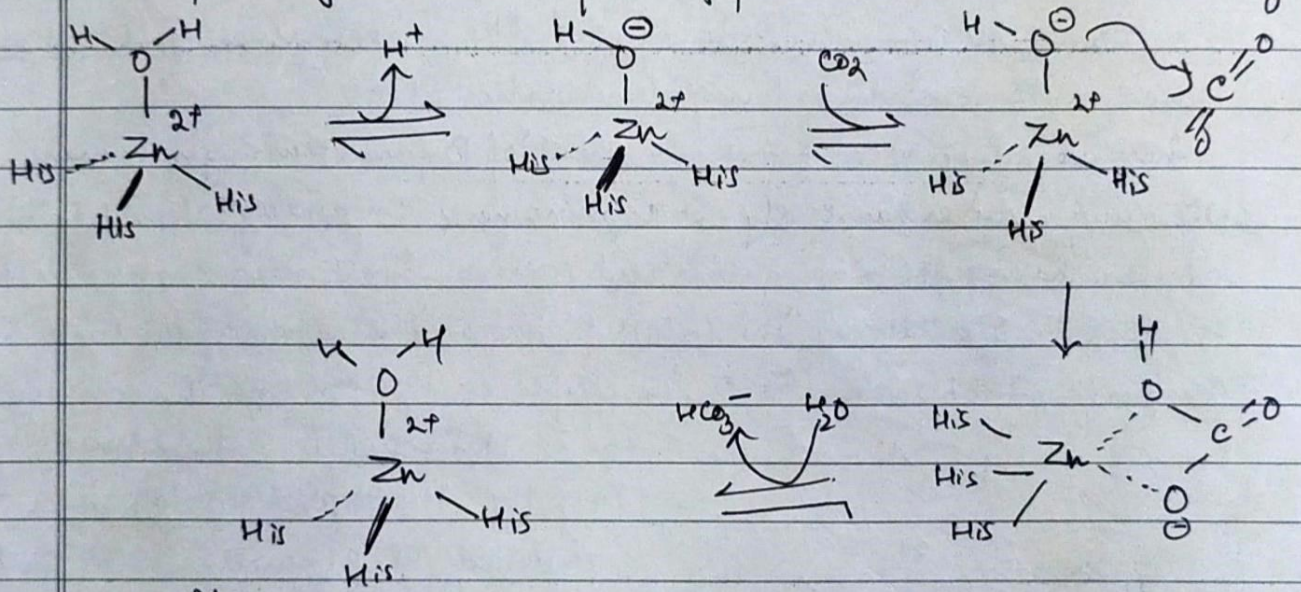
Zn(II) can be removed from the enzyme by adding 1,10 – phenanthroline or 8-hydroxyquinoline. There is no change in str of the apo protein on demetallation. The catalytic activity of ~~Zn(II) in~~ the enzyme is revived on adding Zn(II).

Mechanism of action

The key to the accepted mechanism is that the kinetics of the CA reacⁿ depend on pH (faster at high pH & when under the influence of a gp with an

apparent pKa value around 7.0)

- The pKa value for Zn coordinated water is ~7.0 which is lower than the value of 14.0 for free H_2O.
- The mechanism starts with the form^n of zinc bound hydroxide ion since water bound to the zinc ion is rapidly converted into hydroxide ion. The nucleophilic hydroxide ion is precisely positioned to attack the C atom of CO_2.

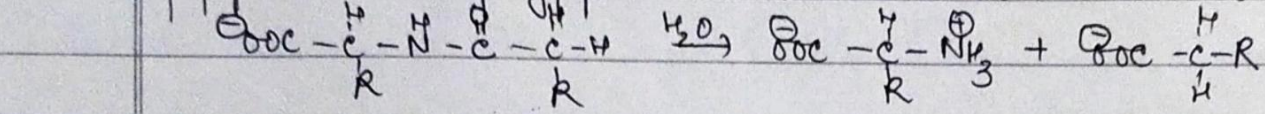


- The Zn^{2+} ion also helps to orient the CO_2 at the active site so as to provide a high conc^n of OH^-. Thus the role of Zn^{2+} in CA is central to bring the substrate into close proximity which optimizes their orientation for reac^n
- After the form^n of metal-hydroxide, CO_2 binds and the nucleophilic attack by OH^- yields a metal bound bicarbonate. HCO_3^- is subsequently liberated & the enzyme is regenerated for the second cycle.

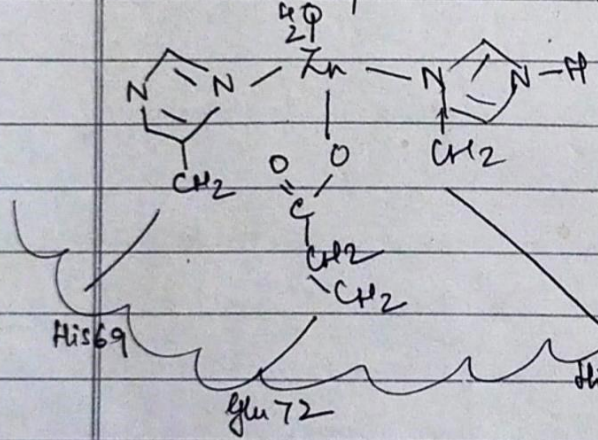
(Cl^- inhibits enzymatic activity binding to Zn^{2+} replacing H_2O; proton transfer to solvent is mediated by buffer if conc^n of buffer > 10 mol dm^{-3}. Deuterium isotope effect has been observed suggesting H^+ transfer b/w catalytic gp. & proton transfer gp. is rate determining step).

Carboxypeptidases

These are hydrolytic enzymes containing Zn(II) which catalyses the hydrolysis of peptide bonds in polypeptides at the C-terminal amino acid residue



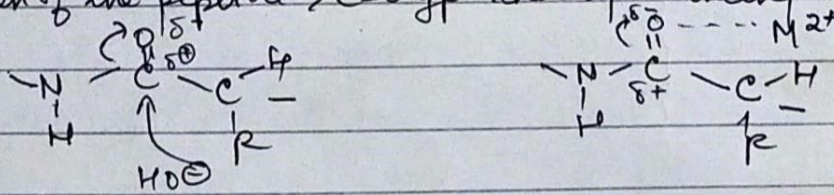
CPA catalyzes the hydrolysis of protein having a large hydrophobic, preferably aromatic or aliphatic side chain at the C-terminal amino acid residue whereas CPB requires the presence of a +vely charged side chain at the C terminal. It was the first Zn enzyme to be characterized crystallographically. The enzyme consists of a protein chain of 307 amino acid residues in a single polypeptide chain plus one Zn^{2+} ion for a molar mass of ~ 34,600. The molecule is roughly egg shaped with max. & minimum dimensions of ~5000 pm & ~ 3800 pm respectively. There is a cleft on one side containing the Zn^{2+} ion which is coordinated approximately Td ally to 2 N atoms & 1 O atom from 3 amino acids in a protein chain (2 histidine residues & 1 glutamic acid residue). The 4th coord^{n} site has a loosely bound H_2O molecule when the enzyme is not involved in active catalysis. When the enzyme is involved in catalysis the site can accept an $e^{\ominus}$ pair from a donor atom in the substrate to be cleaved. Arg-145 & Tyr-248 seem to be involved in H-bonding to the substrate causing conformational changes in the protein. A 2nd H_2O mol. occupies the pocket at ~350 pm from the metal ion. On demetallation the enzyme loses its catalytic activity, but retains the same overall str. The catalytic activity is revived on addition of Zn(II), Co(II) or other bivalent metal ions of similar size.



Peptide hydrolysis is a rather difficult reaction despite being thermodynamically

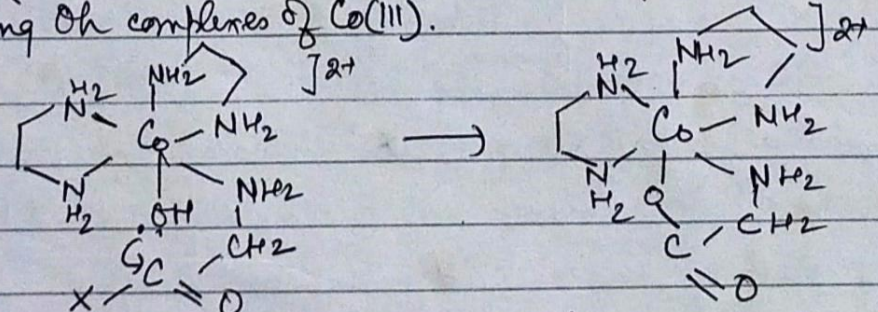
favourable in aq. medium. At neutral pH the uncatalyzed hydrolysis is a slow process with rate consts. as low as 10^{-11} s^{-1}. In presence of CPA it increases upto 10^{4} s^{-1}. In order to catalyze this reacⁿ, the enzyme must accomplish several things —

- It must facilitate nucleophilic attack on the peptide carbonyl gp. by a nucleophile. This may be achieved by producing a highly reactive nucleophile or by activating the carbonyl for attack by polarization. The metal ion may promote loss of H^+ from H_2O converting it to the more forceful nucleophile or act as a Lewis acid catalyst by binding to the oxygen of the peptide >C=O gp. thereby reducing $e^{\ominus}$ density on C



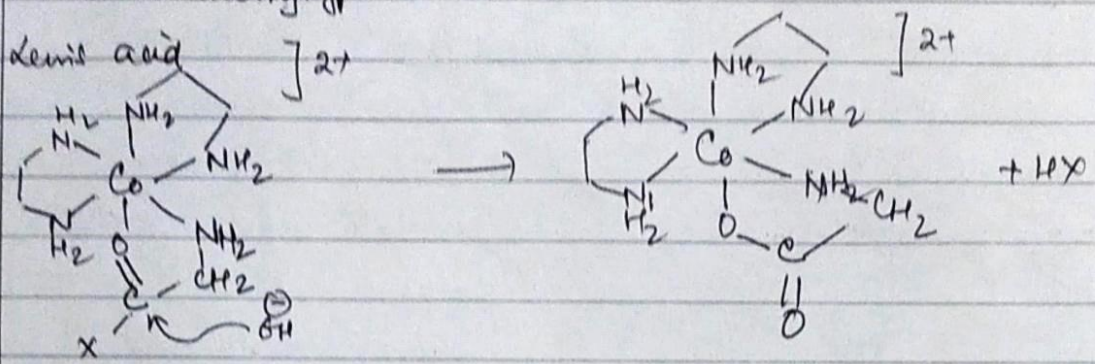
- It must stabilize the Td intermediate or TS generated by nucleophilic attack on >C=O carbon.
- It must stabilize the amide N atom to make it a suitable leaving gp. so that the Td intermediate can collapse upon C–N bond cleavage.

In principle a metal ion eg. Zn^{2+} might play a role in any or all of these processes. Either the hydroxo or Lewis acid mechanism may operate but to know the path we have to know the detailed enzyme str. We consider the model system using Oh complexes of Co(III).



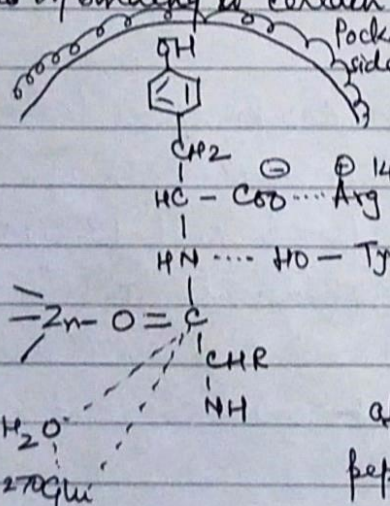
The aqua ligand cis to the >C=O loses a proton forming hydroxo (OH) the

attacks the carbonyl gp.



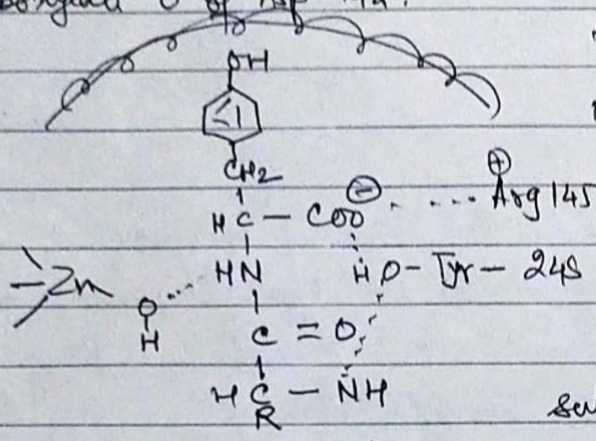
The carbonyl gp. is coordinated to Co(III) & the latter is a source of Lewis acid catalysis for attack of H_2O mol. or OH^- from the sol^n. The Co(III) model system has been developed as a useful lab method for both hydrolytic breakdown analysis of peptides & the reverse, their synthesis.

2 possible binding schemes are zinc-carbonyl binding & zinc-hydroxide binding. In the former, the substrate is bound to zinc by carbonyl gp. The potential catalytic gps. in the enzyme are: (i) Tyr-248 – probable donor to NH gp. of susceptible peptide bond; (ii) Zn(II) ion binding to O of peptide bond, (iii) Glu-270 which suitably positions a H_2O mol. & polarizes it by H-bonding to facilitate attack of the amide linkage. Stabilization of the substrate for hydrolysis is affected by binding to certain amino acid residues.



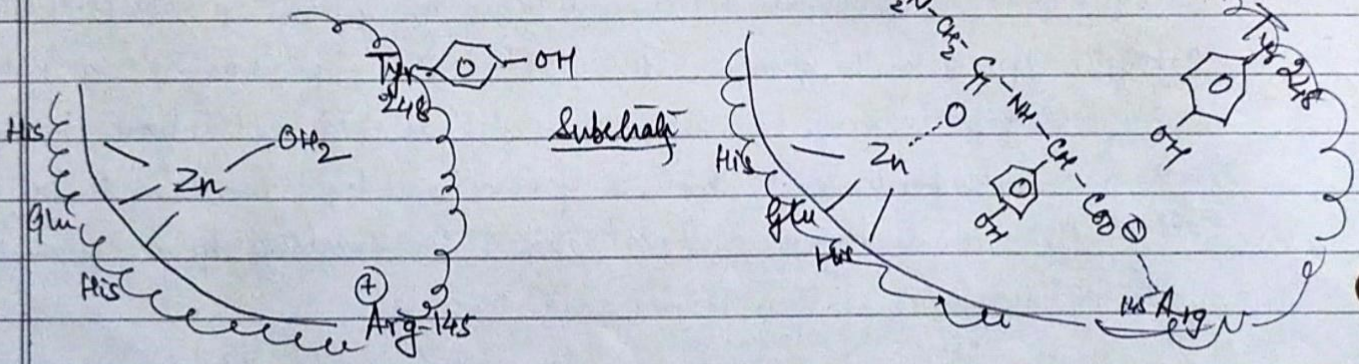
The catalyst is initiated by nucleophilic attack on the C atom of polarized >C=O gp. The H_2O mol. is H-bonded to Glu-270. This assists the form^n of OH^- by transferring the proton to the COO^- gp. of glutamate 270 forming glutamic acid. This OH^-, a more powerful nucleophile than H_2O effects a nucleophilic attack on the carbonyl gp. The peptide >C=O may be activated to nucleophilic attack possibly but not necessarily due to coord^n of >C=O oxygen with Zn(II).

The protonation of the amide N due to H bonding with Tyr 248 also makes the $C^{\delta\oplus}$ deficient & prone to attack by coordinated $^{\oplus}OH^{\ominus}$. In addn the coordinating amino acid residues form H bonded st^s with other residues, eg. one N of his-69 is H-bonded to carboxylate O of Asp-142.

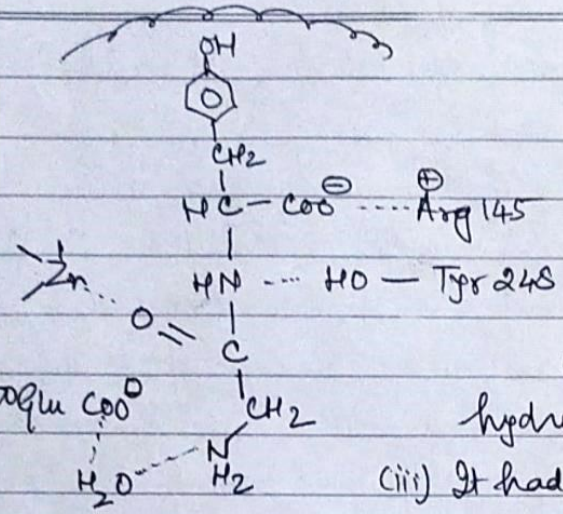


An alternative mechanism in accordance with X-ray data involves a mode of binding with the susceptible $>C=O$ being directed away from the zinc while the zinc is linked to H_2O or OH^-

Studies were done with a model pseudo-substrate - glycyltyrosine bound to the active site. The conformational changes induced in CPA on binding to substrate are shown:



The guanidinium gp. of Arg-145 moves ~ 200 pm closer to the COO^- gp. of the substrate, while the phenolic -OH of Tyr 248 moves ~ 1200 pm with a twisting of the C-C chain so that -OH gp. lies close to the peptide N. These movements lead to the plausible fact that Tyr 248 donates an H^+ of the NH gp. to the hydrolyzable peptide link. The closeness of Glu-270 to the carboxyl C suggests its role as base catalysis attack of H_2O or in direct nucleophilic attack of the C. This is shown as:



(i) The C terminal carboxylate gp. of the substrate interacts electrostatically with the +ve guanidine gp. of Arg-145

(ii) As a result of the positioning of COO^- at Arg-145, the C terminal hydrophobic aromatic gp $-OH-C_6H_4-CH_2$ inserts itself in the hydrophobic pocket of the enzyme displacing several H_2O mole.

(iii) It had been suggested earlier that the substrate dipeptide gets bound to the Zn(II) from the peptide O, displacing a H_2O mol. from the coord^n sphere of Zn(II). Binding with the metal ion is thought to polarize the peptide $>\overset{\delta+}{C}=\overset{\delta-}{O}$, thereby facilitating nucleophilic attack on C. However, it has now been shown that coord^n of $>C=O$ with Zn(II) is not necessary though it has not been ruled out. It is known that flexibility of coord^n types of Zn(II) is allowed so alongwith peptide coord^n, H_2O coord^n may occur simultaneously resulting in coord^n of 5-coord. intermediate.

(iv) Phenolate $-O^-H$ of Tyr-248 migrates through 1200 pm for form H bond with HN gp. of the peptide to be cleaved. The fact that Tyr 248 moves considerably towards the active site suggests that it is likely to be involved in the mechanism. However studies indicate that role of Tyr is in binding to substrate & not in cleavage.

(v) As a result of the nucleophilic attack by $-O^-H$ on peptide C, the C–N bond breaks & the peptide $-NH$ gets converted to $-NH_2$ $\left(-\overset{O}{\overset{\|}{C}}-NH-\right)$ by acquiring a H^+ from glu-acid 270 (proton previously transferred from coordinated H_2O) The C-terminal amino acid, Tyr is released. Glu-acid 270 gets converted to glutamate & temporarily coordinates to Zn(II)

(vi) The N terminal amino acid, glycine forms due to hydrolysis of the dipeptide leaves the coord^n site of the metal ion, & one H_2O mol. gets bound to Zn(II). The

Zn(II) glutamate bond breaks & the coordinated H_2O forms H bond with glutamate-270. The enzyme is regenerated for the fresh cycle.

Metal Subs^n:- Replacement of Zn^{2+} by Co^{2+} displays similar activity. Co(II) & Zn(II) have similar ionic radii. The CFSE in high spin Co(II) in Oh ($-0.8\,\Delta_o$) and Td ($-1.2\,\Delta_o$) fields is not very different & one form can change into another as in Zn(II) complexes. Co(II) forms colored complexes in contrast to Zn(II); Co(II) is also paramagnetic & both NMR & ESR as well as visible spectra can be obtd. which can give an idea of the str^r of the enzymes. The Co-CPA complex has a very high value of molar extinction coeff. indicating that regular Td str^r is not present. The distortion helps the metal to effect the reac^n. The metal is peculiarly poised for the reac^n as it lowers the energy of the T.S. The term entactic has been coined for this.

The entactic state :- Structural studies on metal enzymes have shown that coord^n geometries around the metal ion can be distorted. This can be related to the catalytic efficiency of the enzyme & the protein can fold to generate special stereochemistries which help the metal to adopt a geometry closer to the T.S. of the reac^n being catalysed rather than to a more normal regular state geometry. There is a 'preselting' of the geometry such that there is a catalytically poised state intrinsic to the active site - This is called "entactic state" (entasis — stretched, under tension) eg. Zn^{2+} in CPA.

The mechanisms available in enzymes are much more elaborate than in simple metal complexes in solution. The substrate is bound to a favourable place by hydrophobic & ionic interactions. Various features operate together.