Metal Ions in Enzymes

Introduction

Many enzymes contain one or more metal ions, usually held by coordinate covalent bonds from amino acid side chains, but sometimes bound by a prosthetic group such as heme. These enzymes are called **metalloenzymes**. The bound metal ion acts similarly to a coenzyme, conferring on the enzyme catalytic properties that it would not possess in its absence. These metal ions perform diverse biochemical roles in catalysis, structure, and regulation.

For example, the **zinc ion** in *carboxypeptidase A* binds the water molecule that attacks the carbonyl group of the peptide bond and also acts as an electrostatic catalyst. The zinc ion stabilizes the tetrahedral oxyanion in the transition state, just as the oxyanion hole does in chymotrypsin.

In other cases, the metal in a metalloenzyme serves as a **redox reagent**. For instance, the **heme-iron enzyme catalyzes** the breakdown of hydrogen peroxide, a potentially destructive compound in cells. Such redox activity requires metals like **Fe** or **Cu**, which can exist in multiple oxidation states and facilitate reversible electron transfer.

In many enzymatic reactions, certain metal ions are necessary for catalytic efficiency even though they may not remain permanently bound to the enzyme. For example, enzymes that couple ATP hydrolysis to other reactions require $\mathbf{Mg^{2+}}$, because the Mg–ATP complex is a better substrate than ATP itself.

The **types and biological roles** of these essential metals and trace elements are summarized in **Table 11.9**, which lists key examples of metalloenzymes and the catalytic or structural function of their metal cofactors.

Table 11.9. Metals and Trace Elements Important as Enzymatic Cofactors

Metal	Example of Enzyme	Role of Metal
Fe	Cytochrome oxidase	Oxidation–reduction
Cu	Ascorbic acid oxidase	Oxidation–reduction
Zn	Alcohol dehydrogenase	Helps bind NAD^+
Mn	Histidine ammonia lyase	Aids in catalysis by electron withdrawal
Co	Glutamate mutase	Co is part of cobalamin coenzyme
Ni	Urease	Catalytic site
Mo	Xanthine oxidase	Oxidation–reduction
V	Nitrate reductase	Oxidation–reduction
Se	Glutathione peroxidase	Replaces sulfur in cysteine in active site

As illustrated in Table 11.9, iron (Fe) and copper (Cu) play essential roles in redox reactions, zinc (Zn) assists in cofactor binding, and manganese (Mn) aids in catalytic electron withdrawal. Selenium (Se), meanwhile, enhances antioxidant defense by replacing sulfur in cysteine residues.

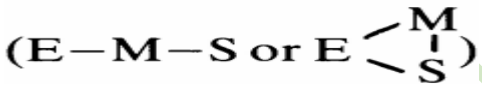
Metal Ions in Enzyme Catalysis

More than a quarter of all known enzymes require metal ions for full catalytic activity. These ions usually exist as **cations** and often have more than one oxidation state, such as **Fe^{2+}/Fe^{3+}**. Their positive charge helps stabilize transition states by electrostatic interactions, one of the key mechanisms of metal-assisted catalysis.

Metal ions can contribute to catalysis in several ways:

1. Accepting or donating electrons to activate electrophiles or nucleophiles.
2. Masking nucleophiles to prevent side reactions.
3. Orienting substrates or groups within the enzyme in a specific three-dimensional configuration.

Metalloenzymes contain tightly bound metal ions, while **metal-activated enzymes** bind metal ions more loosely during catalysis. Complexes formed among the enzyme (E), metal ion (M), and substrate (S) include:

1. Enzyme bridge complexes (M–E–S)
2. Substrate bridge complexes (E–S–M)
3. Metal bridge complexes 

Metalloenzymes, however, typically exist as E–M complexes after purification and cannot form substrate bridge complexes.

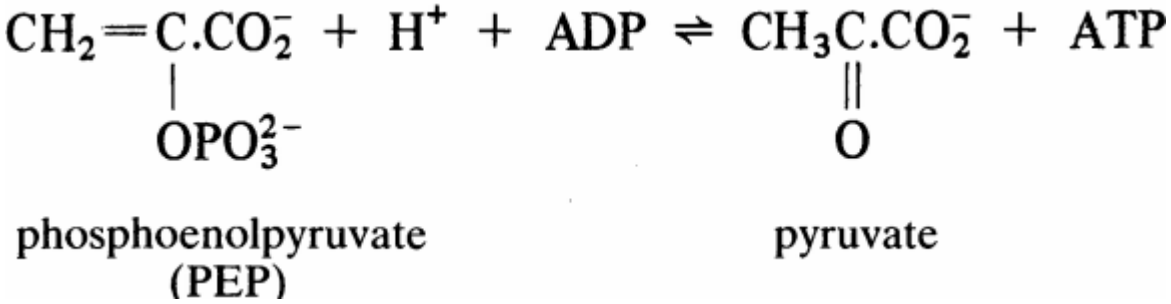
Role of Metal Ions in Enzyme Catalysis:

1-Activation of enzymes by alkali metal cations (Sodium (Na^+) and Potassium(K^+).

Alkali metal cations **bind weakly** to form complexes with enzymes (sodium (Na^+) and potassium (K^+).

Potassium ion (K^+) is the most abundant intracellular cation. It is known to activate many enzymes, particularly those that catalyze phosphoryl-transfer or elimination reactions. K^+ appears to **bind to negatively charged groups** on an inactive form of the enzyme and thus causes a change in conformation to a more active form. However, in some cases, $\mathbf{K^+}$ may also aid substrate binding.

For example, muscle **pyruvate kinase,** a tetrameric enzyme that catalyzes the reaction:



Pyruvate kinase requires alkali metal cations (K^+) and Mn^{2+} (or Mg^{2+}); these bind in the active site. The carboxyl group of PEP binds to the enzyme-bound **K^+**. Thus, a conformational change takes place, facilitating the progress of the reaction via an **E-Mn^{2+}- PEP** complex.

2-Activation of enzymes by alkaline earth metal cations (Ca^{2+} and Mg^{2+})

Oxygen atoms are often involved in the bonds of alkali metals and alkaline earth metals.

The cation bonds of alkaline earth metal oxides are stronger than those of alkali metal oxides. These cations, Ca^{2+} and Mg^{2+} can form six coordinate bonds to produce octahedral complexes.

Mg^{2+} is accumulated by cells in exchange for the transport of Ca^{2+} in the opposite Direction. As might be expected, therefore, the enzymes requiring Ca^{2+} for activation They are mainly extracellular, for example, the **salivary and pancreatic α-amylases**: the Ca^{2+}.

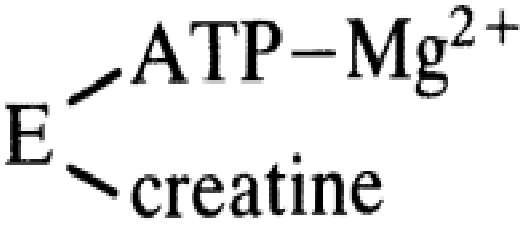
Appears to play a role in maintaining the structure required for catalytic activity.

In contrast, a variety of intracellular enzymes require Mg^{2+} for activity, and in most cases, this requirement can be replaced in vitro by one for Mn^{2+}. Mn^{2+} is paramagnetic, which makes the system easier to investigate. It has been shown that all possible types of ternary bridge complexes involving divalent cations can exist. Most kinases form **E-S-M** complexes, where S (substrate) is the reacting nucleotide.

Example: muscle creatine kinase, the reaction catalyzed:

$$\text{creatine} + \text{MgATP} \rightleftharpoons \text{MgADP} + \text{phosphocreatine} + \text{H}^+$$

The true substrate is **Mg-ATP, and the reaction proceeds via the formation of the complex**.



The divalent cation binds to the α- and β-phosphates of the nucleotide but not to the terminal (γ) phosphate transferred to creatine. Therefore, the cation helps orient the complex and may also assist in breaking the pyrophosphate bond by withdrawing electrons from the β-phosphate.

3- Activation of enzymes by transition metal cations (Cu, Zn, Mo, Fe, and Co cations).

Transition metal ions such as **Cu, Zn, Mo, Fe, and Co** bind to enzymes much more strongly than alkali and alkaline earth metal ions, and they usually form **metalloenzymes.**

Transition metal cations are found in only trace amounts in living organisms, as higher levels can be toxic. The trace metals Mo and Fe are found in **nitric oxide reductase; Fe is also** a component of hemoglobin.

Another trace metal, Co, is found in vitamin B_{12}.

Example: In a little more detail, we will now consider an example of a Cu- and a Zn-metalloenzyme.

1- Superoxide dismutase:

Superoxide dismutase is a copper-metalloenzyme that catalyzes the removal of the highly reactive O_2 produced. The superoxide dismutase reaction is as follows:

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

Bovine erythrocyte **superoxide dismutase is a dimeric protein** containing two. Cu^{2+} ions and two Zn^{2+} ions.

The Zn^{2+} ions appear to have a structural rather than a catalytic role, while the Cu^{2+} ions are involved in the reaction sequence:

$$E - Cu^{2+} + O_2^- \rightarrow E - Cu^+ + O_2$$

$$E - Cu^+ + O_2^- \xrightarrow{+2H^+} E - Cu^{2+} + H_2O_2$$

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

2-Carboxypeptidase-A:

Carboxypeptidase-A zinc metalloenzyme is in contrast to the **Superoxide dismutase,**

Were,

The zinc ion in **carboxypeptidase A** has a catalytic role in the reaction catalyzed by **Carboxypeptidase A**. Carboxypeptidase A from the bovine pancreas is a monomeric enzyme containing one zinc atom.

Carboxypeptidase-A shows that: The active site of carboxypeptidase-A contains the Zn^{2+} ion that is attached to histidine-69, glutamate-72, histidine-196, and H_2O, as well as a channel for the polypeptide substrate and a

hydrophobic region for binding the side chain of the C-terminal amino acid. **The terminal carboxyl group** of **the substrate** forms an **electrostatic** interaction with arginine-145 (Fig. 11.39).

The mechanism of the **carboxypeptidase A** included:

1- The zinc ion (orange circle) binds a water molecule (blue).

2- Zinc ion serves as **an electrostatic catalyst** to promote the hydrolysis of **the C-terminal amino acid** from **a peptide substrate (green).**

3- Zinc ion **stabilizes the negative charge on oxygen** in the tetrahedral transition state.

Enzyme **active site residues** are indicated by **black coloring,** and the dashed red arrow indicates the bond cleaved**;** Figure 11.39

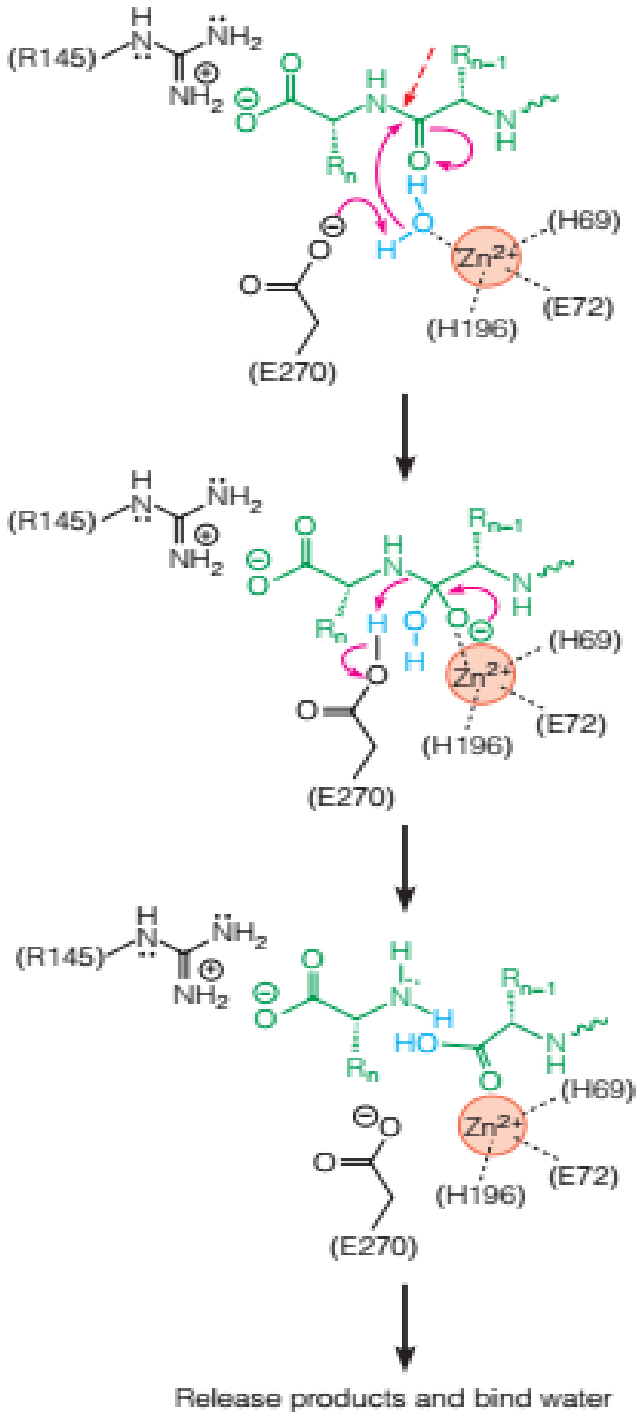


FIGURE 11.39 The mechanism of the **protease carboxypeptidase A**.