

## Research Article

## An Overview of Inhibition of Enzymatic Activity by Heavy Metal Ions

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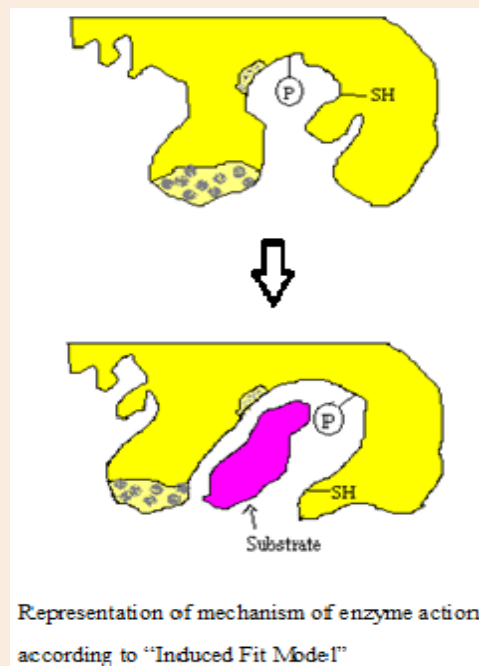
**Abstract**

Enzymes are bio-catalysts. Catalytic activity of enzymes is highly specific and accurate. The basic structure of enzymes is made up of proteins, however one third of the enzymes require metal ions for their activation. The 3-dimensional configuration of enzymes is of immense importance in their proper functioning. The activity of enzymes can be inhibited by various species, which are called as enzyme inhibitors. Enzyme inhibitors can be mainly classified as competitive and non-competitive inhibitors. Inhibition may also be caused by metal ions. Heavy metal ions like those of cadmium, mercury, lead etc are known to cause toxicity by forming extremely stable complexes with amino acid residues on enzymes containing sulfur such as cysteine, cystine and methionine, which deforms and renders the enzymes inactive.

**Keywords:** Enzymes, metal ions, enzyme inhibitors

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**Introduction**

Catalysts are substances that speed up chemical reactions. Enzymes are protein catalysts for chemical reactions in biological systems. Most chemical reactions of living cells would occur very slowly if they were not catalyzed by enzymes. By contrast to non-protein catalysts, each enzyme catalyses a small number of reactions, frequently only one. Enzymes are thus reaction-specific catalysts.

Essentially all enzymes are proteins, which mean the basic structure of enzymes is built of proteins. However many enzymes catalyze reactions only in the presence of a specific non-protein molecule called the co-enzyme. In such cases catalysis will occur only when both enzyme and co-enzyme are present. When co-enzymes are required, the complete system is known as holoenzyme, which consists of the protein part or apoenzyme plus a heat stable, dialyzable non-protein co-enzyme. A co-enzyme can be either organic in nature or else it can simply be a metal ion or a complexed metal ion [1].

The large size of enzymes related to their substrates led the biochemists at the turn of the last century to postulate that some restricted region of the enzymes was concerned with the process of catalysis. This region was termed as the active or catalytic site. X-ray data on a variety of enzymes indicate that active site is a groove or pocket formed by the folding pattern of the protein, which contains the catalytic side chains of the amino acids, and other functionalities such as metal ions that are required for catalysis. This three dimensional structure together with the chemical and electrical properties of the amino acids and cofactors permits only a particular substrate to bind to the active site, thus

determining the enzyme specificity. In order to function properly, enzymes must maintain their correct three dimensional conformational structure, for which they strive to the best of their ability and limitations.

### **Metalloenzymes and Metal – Activated Enzymes**

Metal ions in enzymes exhibit a variety of roles of either catalytic or structural importance. The catalytic importance of metal ions in enzymes is readily attested by the fact that approximately one third of the enzymes known in biochemistry require metal ions for activity. The other category of metal ions in biological systems are those which are associated with numerous types of proteins, without direct catalytic action but none the less are essential for biological function – further emphasizes the structural role of metal ions.

In a general sense, therefore metal ions in proteins and enzymes may be divided into two classes – “chemical” metals and “structural” metals. Chemical metals in enzymes are those that enter directly into biological reactions in a chemical manner, for instance in the oxidation – reduction reactions of peroxidases and ferredoxins. Structural metals, on the other hand, stabilize the protein conformation necessary for biological function e.g. Ca(II) in thermolysin or indirectly promote catalysis by inducing required orientation of substrates or catalytic groups of the protein e.g. Mg(II) in phosphoglucomutase. Thus metal ions in compounds of biological interest, especially proteins and enzymes, appear to have the ability to act in two ways: as indispensable part of the enzyme, removable only by extreme chemical attack and with high metal–ion specificity in function as in metalloenzymes or loosely bound to the enzyme or substrate, readily dialyzable and having lower metal–ion specificity for catalytic function as in metal – activated enzymes.

The most important transition metals that are required by enzymes in biological systems are Zn, Cu, Fe, Co, and Mo. All of these metals except Mo are relatively abundant in the earth’s crust, on the other hand, when we consider the metals that are toxic; we find that they are all extremely rare in their crustal abundance [2]: Pb (0.08), Cd (0.0018), and Hg ( $4 \times 10^{-5}$ ). The conclusion is inescapable, life evolved utilizing those elements that were abundant and available to it and became dependent upon them. Those elements that are rare were not used by living systems because they were not available; neither did these systems evolve mechanism to cope with them.

### **Role of Metal Ions in Catalysis**

Several metal ions, like protons, are Lewis acids or electrophiles and can therefore, accept a share in an electron pair forming a coordinate bond. In addition and unlike protons, metal ions can serve as three dimensional templates for the orientation and binding of basic groups present on the enzyme or substrate.

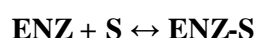
Metal ions can also accept electrons via sigma or pi bonds to activate electrophiles. By donating electrons, via back bonding metals can also activate nucleophiles, or act themselves as nucleophiles. Metal ions also play an essential role in the activation of small lazy molecules such as N<sub>2</sub>, CH<sub>4</sub>, NO, CO and O<sub>2</sub> and make them ready to take part in many biological processes.

The coordination sphere of a metal may bring enzymes and substrate together, which indicates that the metal centers act as collectors of ligands prior to their reaction, thus transforming the uncatalyzed intermolecular reaction into one which is intramolecular. The reaction may thus be facilitated by the entropy advantages of intramolecular vs. intermolecular formation of the transition state. A metal ion may also ‘mask’ a nucleophile and thus prevent an otherwise likely side reaction. Finally, stereochemical control of the course of an enzyme catalyzed reaction may be achieved by the ability of the metal coordination sphere to act as a three dimensional template to hold reactive groups in a specific steric orientation.

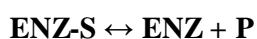
## Mechanism of Enzymatic Action

In most chemical reactions, an energy barrier exists that must be overcome for the reaction to occur. This is the barrier that prevents complex molecules such as proteins and nucleic acids from spontaneously degrading, and make the preservation of life possible. However, when metabolic changes are required in a cell, certain of these complex molecules must be broken down, and this energy barrier must be surmounted, but under the normal conditions of temperature and pressure molecules inside the living cells fail to overcome the energy barrier because most of them possess insufficient kinetic energy to overcome the energy barrier for reaction. Whereas heat could provide the additional needed energy the activation energy, but the rise in temperature would kill the cell. The alternative to this is to lower the activation energy through the use of catalyst. This is the role that enzymes play. Enzymes may be considered to lower energy barriers by providing an alternate reaction path with the same overall change in energy ( $\Delta G^\circ$ ) i.e. by, "tunneling through" the energy barrier.

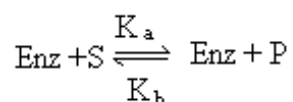
This they do by reacting first with the substrate to form an enzyme- substrate complex, ENZ-S, which is very unstable.



This unstable intermediate quickly breaks down to form a product, P, and free enzyme:



The rate expressions for the forward and backward, reactions may be expressed as under:



$$\text{Rate}_a = K_a[\text{ENZ}][\text{S}]$$

$$\text{Rate}_b = K_b[\text{ENZ}][\text{P}]$$

Where,  $K_a$  and  $K_b$  are the rate constants for the forward and backward reactions. In the expression for the overall equilibrium constant,  $[\text{ENZ}]$  cancels out.

$$K_{eq} = \frac{K_a}{K_b} = \frac{[\text{ENZ}][\text{P}]}{[\text{ENZ}][\text{S}]} = \frac{[\text{P}]}{[\text{S}]}$$

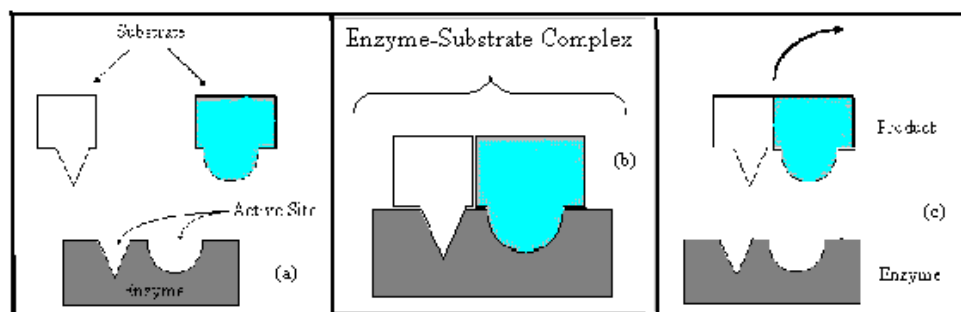
The enzyme concentration thus has no effect on the equilibrium constant. In other words we can say that an enzyme accelerates the approach to equilibrium, without influencing the attainment of equilibrium point. The  $K_{eq}$  of a reaction is the same regardless of whether equilibrium is approached with or without enzymatic catalysis.

Enzymes are more efficient than manmade catalysts operating under the same conditions. One molecule of an enzyme catalase, for example, can produce  $10^{12}$  molecules of oxygen per second. The catalytic groups at the active site of an enzyme act  $10^6$  to  $10^9$  times more effectively than do analogous groups in a non-enzymatic reaction. The reason for the great efficiency of the enzymes is not completely understood, however two models have been proposed to account for the high catalytic efficiency of enzymes, viz i) *Rigid Model of the Catalytic Site* and ii) *Flexible Model of the Catalytic Site*.

### i) Rigid Model of the Catalytic Site

This model, (proposed by Emil Fischer) explained the interaction between the enzyme and substrate in terms of a "Lock and Key" analogy. Just as only particular shaped keys fit into particular shaped locks, similarly only certain

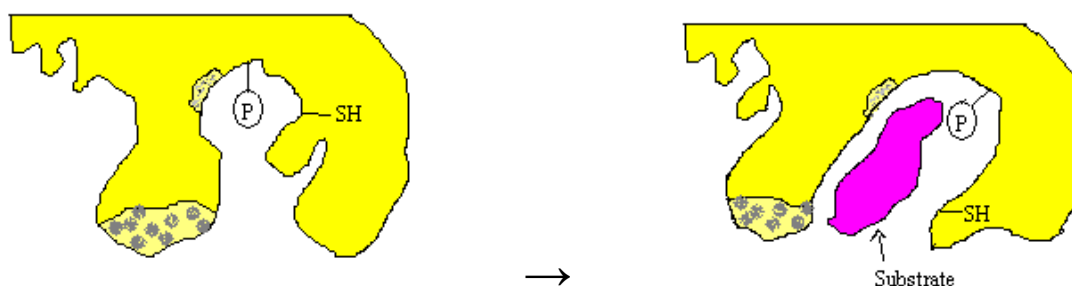
types of substrates will establish a close fit with a given type of enzyme at its active site. According to this concept the active sites of the enzymes are so designed that they accept only those substrate molecules which have matching shape that can fit into the grooves of the active site, as shown in (**figure 1**). The shape of the enzyme must match the shape of the substrate. Enzymes are therefore very specific and they will only function correctly if the shape of the substrate matches the active site. Since the enzyme does not form chemical bonds with the substrate, it remains unchanged and returns to its normal shape after the reaction. As a result, the enzyme molecule can be used repeatedly, making a small amount of an enzyme sufficient for complex reactions.



**Figure 1** Representation of formation of an ENZ-S complex according to “Lock and Key” Model.

## ii) Flexible Model of the Catalytic Site

An unfortunate feature of the Fischer model is the implied rigidity of the catalytic site. A more refined and certainly a more useful model in terms of explaining properties of enzymes, “induced fit model” of Koshland. An essential feature of this model is the flexibility of the region of the catalytic site. In the Fischer model, the catalytic site is presumed to be pre-shaped to fit the substrate. In the induced fit model the substrate induces a conformational change in the enzyme. This aligns amino acid residues or other groups on the enzyme in the correct spatial orientation for substrate binding, catalysis, or both. At the same time, other amino acid residues may become buried in the interior of the molecule.



**Figure 2** Representation of mechanism of enzyme action according to “Induced Fit Model”

In the representative mechanism shown in the (**figure 2**) hydrophobic groups and charged groups (dots) are involved in substrate binding. A phosphoserine ( $-P$ ) and the  $-SH$  of a cysteine residue are involved in catalysis. In the absence of substrate, the catalytic and the substrate – binding groups are several bond distances away from one another. Approach of the substrate induces a conformational change in the enzyme protein, aligning the groups correctly for substrate binding and for catalysis. Experimental evidences for the induced fit model includes demonstration of conformational changes during substrate binding and catalysis with a number of enzymes, for example, creatine kinase, phosphoglucomutase.

## Inhibition of Enzymatic Activity

The chemical species that block or inhibit the activity of an enzyme are called inhibitors. It is customary to distinguish two broad classes of inhibitors as competitive and non-competitive inhibitors.

Competitive inhibitors are those molecules which have a structure very similar to that of substrate due to which they may bind to the active site of the enzyme, forming an enzyme inhibitor complex rather than an enzyme substrate complex. This inhibition of the enzymatic action is of a competitive nature, because the inhibitor molecule actually competes with the substrate for the active site. Penicillin is a competitive inhibitor that blocks the active site of an enzyme that many bacteria use to construct various cell walls.

Non-competitive Inhibitors are those inhibitors that bear no structural resemblance to the substrate. Such inhibitors bind to the enzyme at a location other than the active site. In some cases of non-competitive inhibition, the inhibitor is thought to bind to the enzyme in such a way as to physically block the normal active site. In other instances, the binding of the inhibitor is believed to change the shape of enzyme molecule, thereby deforming its active site and preventing it from reacting with its substrate. This latter type of non-competitive inhibition is called allosteric inhibition; the place where the inhibitor binds to the enzyme is called the allosteric site.

## Inhibition of Enzymatic Activity by Heavy Metal Ions

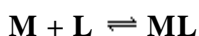
Since the term "Heavy metal" is a loose term and has been taken in many different ways by different researchers, it is not justifiable to give a concise definition to them, nevertheless in a general sense heavy metals may be taken as chemical elements with a specific gravity at least 5 times the specific gravity of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic (5.7), cadmium (8.65), iron (7.9), lead (11.34), and mercury (13.546).

There are 35 metals that concern us because of occupational or residential exposure ; 23 of these are the heavy elements or "heavy metals": antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc. Interestingly, small amounts of these elements are common in our environment and diet and are actually necessary for good health. Living organisms require trace amounts of some heavy metals like iron, cobalt, copper, manganese, molybdenum, vanadium, strontium, and zinc, but their excessive levels can be detrimental to the organism, and may cause acute or chronic toxicity (poisoning). Other heavy metals such as mercury, lead and cadmium, are toxic metals as they have no known vital or beneficial effect on organisms, and their accumulation over time in the bodies of mammals can cause serious illness. Heavy metal ions such as those of lead, mercury, cadmium and arsenic are the examples of non-competitive inhibitors.

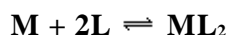
The 3-dimensional structure and many biological properties of enzymes are determined largely by the number and type of the amino acids present, the order in which they are linked together, and the spatial relationship of one amino acid to another. The unique biological properties of enzymes result primarily from specific interactions between the amino acids [3] of which they are composed. To comprehend the chemistry of proteins and enzymes in particular, it is therefore necessary to have some knowledge of chemistry of amino acids. Amino acids i.e.  $RCH(NH_2)COOH$  are species which have both amino and a carboxylic acid function attached to the same ( $\alpha$ ) carbon atom. Amino acids act as negatively charged chelating ligands towards metal ions, and can coordinate through  $-NH_2$  and the  $COO^-$  groups. In addition some of the amino acids have electron pair donor atoms in their side chains that can bond with metal ions as well, for example cysteine (-SH group), aspartic acid (COOH group), threonine (OH group), histidine (5-membered ring of 3 carbon and 2 nitrogen atoms: both nitrogen atoms have lone pairs) etc. Due to the presence of nitrogen, oxygen, and sulfur atoms which act as donor sites, amino acid residues in enzymes are very prone to

complex formation by various metal ions. The poisonous heavy metals like lead, cadmium, mercury etc. exert many of their toxic effects by binding or complexing with important –SH groups [4,5] in enzymes thus blocking or deforming their active sites which results in the inhibition of their activity. In comparison to the enzyme molecule a metal ion is very small entity and thus it cannot coordinate to the whole of the protein chain of an enzyme, but only to few amino acids in accordance with its coordination number, charge and preferred geometry of the ion under consideration. A metal ion say (M) can bind to a particular amino acid residue say (L) on an enzyme in an appropriate stoichiometric proportion and demand of the conformational requirements of the metal – enzyme complexes in the following ways.

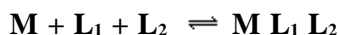
i) The metal ion can coordinate to an amino acid in a 1:1 (metal ion: amino acid) stoichiometric fashion. In such a case the metal ion will bind to that donor atom of the particular amino acid with which it can form most stable bond, the stability being determined by the HSAB [7] Principle.



ii) More common mode of binding of the metal ions will be in the 1.2 stoichiometric fashion.



iii) Another important binding mode (leading to inhibition) will be ternary complex formations where a metal ion M, will bind to two different amino acid moieties say  $L_1$  and  $L_2$ .



(In a 1:1:1, stoichiometric fashion).

### Reasons for preferential affinity of heavy toxic metals for enzymes

The factors that are responsible for formation of metal complexes and favor the formation of certain complexes in preference to others outside the human body should also be important in biological system. A useful description regarding complex formation was first given by Ahrland et al [6] which was then further elaborated by Pearson [7] into the Hard Soft Acid Base Principle (HSAB), to predict the stability of complexes. According to this principle: “Hard acids prefer to bind to Hard bases and Soft acids prefer to bind to Soft bases”. “Hard” acids and bases include species that have small size, high oxidation state, low polarizability and high electronegativity. The affinity of hard acids and hard bases for each other is mainly ionic in nature. On the other hand, “Soft” applies to species which are big, have low charge states and are strongly polarizable. The affinity of soft acids and bases for each other is mainly covalent in nature whereas the intermediate species are known as border line. Some common hard and soft acids and bases are enlisted in (**Table 1**). The essence of this theory is that soft acids react faster and form stronger bonds with soft bases, whereas hard acids react faster and form stronger bonds with hard bases, all other factors being equal. HSAB is widely used in chemistry for explaining stability of compounds, reaction mechanisms and pathways. Now taking HSAB Principle into consideration it can be predicted that ions of soft metals like cadmium, mercury, lead etc will have a special affinity for soft bases for example sulfur and as a result these metal ions will tend to form extremely stable complexes with amino acid residues on enzymes containing sulfur such as cysteine, cystine and methionine, that is soft bioligands.

According to both “Lock and Key” and “Induced Fit Theory” for the enzyme to function properly, it must maintain its correct three dimensional conformational structure, which is held in position by comparatively weak hydrogen bonds and the sulfur-sulfur single bonds found in the component amino acid cystine. The later bond which is formed by the oxidation of the SH bonds of the two cysteine amino acid components is easily ruptured by the action of soft toxic metal ions (giving S- metal bonds instead). This irreversible coordination of the heavy metal ions

to the sulfur-bearing amino acids in the protein chain of an enzyme causes the enzyme to be 'bent out of shape' and thus render it inactive.

**Table 1** Some hard, borderline and soft acids and bases

Hard Acids	Borderline Acids	Soft Acids
H <sup>+</sup> , Na <sup>+</sup> , Li <sup>+</sup> , K <sup>+</sup> , Ca <sup>2+</sup> , Mn <sup>2+</sup> , Al <sup>3+</sup> , N <sup>3+</sup> , Cl <sup>3+</sup> , Gd <sup>3+</sup> , Cr <sup>3+</sup> , Co <sup>3+</sup> , Fe <sup>3+</sup> , Sc <sup>3+</sup> , Ln <sup>3+</sup> , BF <sub>3</sub> , B(OR) <sub>3</sub> , AlCl <sub>3</sub> , SO <sub>3</sub> , CO <sub>2</sub> , RCO <sup>+</sup> , RPO <sub>2</sub> <sup>+</sup> , NC <sup>+</sup>	Fe <sup>2+</sup> , Co <sup>2+</sup> , Ni <sup>2+</sup> , Zn <sup>2+</sup> , Rh <sup>3+</sup> , Ir <sup>3+</sup> , Ru <sup>3+</sup> , Sn <sup>2+</sup> , Ru <sup>2+</sup> , Rh <sup>3+</sup> , Ir <sup>3+</sup> , SO <sub>2</sub> , B(CH <sub>3</sub> ) <sub>3</sub> , R <sub>3</sub> C <sup>+</sup> , C <sub>6</sub> H <sub>5</sub> <sup>+</sup>	M <sup>0</sup> (metal atoms), Pd <sup>2+</sup> , Pt <sup>2+</sup> , Cd <sup>2+</sup> , Ag <sup>+</sup> , Au <sup>+</sup> , Cu <sup>+</sup> , Hg <sup>2+</sup> , Tl <sup>+</sup> , Hg <sup>+</sup> , InCl <sub>3</sub> , BH <sub>3</sub> , RS <sup>+</sup> , Br <sub>2</sub>
Hard Bases	Borderline Bases	Soft Bases
F <sup>-</sup> , OH <sup>-</sup> , H <sub>2</sub> O, NH <sub>3</sub> , CH <sub>3</sub> CO <sub>2</sub> <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> , CO <sub>3</sub> <sup>2-</sup> , NO <sub>3</sub> <sup>-</sup> , PO <sub>4</sub> <sup>3-</sup> , RO <sup>-</sup> , ClO <sub>4</sub> <sup>-</sup> , RNH <sub>2</sub> , ROH, R <sub>2</sub> O	C <sub>6</sub> H <sub>5</sub> NH <sub>2</sub> , C <sub>6</sub> H <sub>5</sub> N, N <sub>2</sub> , N <sub>3</sub> <sup>-</sup> , Br <sup>-</sup> , NO <sub>2</sub> <sup>-</sup> , SO <sub>3</sub> <sup>2-</sup> , NH <sub>2</sub>	R <sub>2</sub> S, RSH, I <sup>-</sup> , SCN <sup>-</sup> , S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> , R <sub>3</sub> P, (RO) <sub>3</sub> P, CN <sup>-</sup> , RNC, CO, C <sub>2</sub> H <sub>4</sub> , C <sub>6</sub> H <sub>6</sub> , H <sup>-</sup> , R <sup>-</sup>

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