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METAL IONS IN BIOLOGICAL SYSTEMS

JENNY P. GLUSKER, AMY K. KATZ AND CHARLES W. BOCK

1. Introduction

Metal ions play essential roles in about one third of enzymes [1]. These ions can modify electron flow in a substrate or enzyme, thus effectively controlling an enzyme-catalyzed reaction. They can serve to bind and orient substrate with respect to functional groups in the active site, and they can provide a site for redox activity if the metal has several valence states. Without the appropriate metal ion, a biochemical reaction catalyzed by a particular metalloenzyme would proceed very slowly, if at all.

The enzyme provides an arrangement of side-chain functional groups having an appropriate sized hole with the preferred groups on enzyme side chains needed to bind the required metal ion. The optimal number of such binding groups is chosen for the particular metal ion, together with the appropriate hydrophobic or hydrophilic environment in the binding site. Metal ions may be bound by main-chain amino and carbonyl groups, but specific binding is achieved by the amino acid side chains, particularly the carboxylate groups of aspartic and glutamic acid, and the ring nitrogen atom of histidine. Other side chains that bind metals ions include tryptophan (ring nitrogen), cysteine (thiol), methionine (thioether), serine, threonine, tyrosine (hydroxyl groups), and asparagine and glutamine (carbonyl groups, less often amino groups).

No set of general rules exists that describes how a given metal ion will behave in an enzyme [2] . Now that many crystal structures of proteins are being studied by X-ray diffraction, information on the binding of metal ions in the active sites of enzymes is available and should provide clues to the mechanism of action of the enzyme. The examples of catechol O-methyl-transferase [3] and mandelate racemase [4] will be discussed later in this article.

The work described here includes results from examinations of the crystal structures in the Cambridge Structural Database [5] and the Protein Databank [6] . A study of binding, however, also involves an analysis of the energetic consequences of changing the way the binding occurs, so that the most stable binding pattern for a given group of ligands can be deduced. We have approached this using *ab initio* molecular orbital and density functional calculations [7, 8]. In this way we obtain both the binding geometry of ligands and the energetic consequences of changing this binding mode.

Metal ions are generally positively charged and act as electrophiles, seeking the possibility of sharing electron pairs with other atoms so that a bond or charge-charge interaction can be formed. They behave rather like hydrogen ions (the poor man's metal). Metal ions, however, often have positive charges greater than one, and have a larger ionic volume so that they can accommodate many ligands around them at the same time. In addition, metal ion concentrations can be high at neutral pH values, while hydrogen ion concentrations are, by the definition of pH, low at these values.

Ligands are the atoms or groups of atoms that are bonded to the metal ion, generally in an electrostatic manner. They are usually neutral or negatively charged and they donate electron density to the metal ion. The coordination number of a metal ion, that is, the number of ligand atoms bound to it, is viewed in terms of concentric spheres; the inner sphere containing those atoms in contact with the metal ion, the second sphere containing those in contact with the inner sphere ligand atoms. The number of atoms in these spheres will depend on the size of the metal ion and the sizes of the ligand atoms. For example, sodium is smaller than potassium, and sulfur is larger than oxygen. Measurements of metal ion-ligand distances in crystal structures led to the idea of atomic and ionic radii [9-11]; anion radii can also be derived from the minimum anion-anion distances in crystal structures. The radius ratio, a concept introduced by Goldschmidt [11], is the ratio of the radius of the cation to that of the anion and is generally less than 1.0.

Tetrahedral structures have a radius ratio between 0.225 and 0.414, while octahedral structures have a ratio between 0.414 and 0.645. For example, the radius of Mg^{2+} is 0.65 Å, while that of O^{2-} is 1.40 Å and their radius ratio is 0.464; the packing is octahedral.

The charge distribution in the active site of an enzyme is designed to stabilize the transition state of the catalyzed reaction relative to that of the substrate. In enzyme-catalyzed reactions it is essential that the reactants be brought together with the correct spatial orientation, otherwise the chance of the reaction taking place is diminished and the reaction rate will be too low. The electrostatic environment in the active site is a major factor that serves to guide the substrate to the binding site in the correct orientation. Metal ions can assist in this process, often binding groups in a stereochemically rigid manner, thereby helping to control the action of the enzyme. Thus, an enzyme will bind its substrate in such a manner that immobilization and alignment, ready formation of the transition state of the reaction to be catalyzed, and then easy release of the product will result; metal ions often help in accomplishing this process.

Each metal ion has its own chemistry. An example of the differing reactivities of metal cations is provided by their ability to bind or lose water molecules. The exchange of coordinated water with bulk solvent by various cations has been categorized [1] into four groups: those for which the exchange rate is greater than 10^8 per second including alkali and alkaline earth metal ions (except beryllium and magnesium), together with Cr^{3+}, Cu^{2+}, Cd^{2+}, and Hg^{2+}. Intermediate rate constants (from 10^4 to 10^8 per second) are found for Mg^{2+} and some of the divalent first-row transition metal ions. Those with slow rate constants (from 1 to 10^4 per second) include Be^{2+} and certain trivalent first-row transition metal ions. The inert group with rates from 10^{-6} to 10^{-2} per second contains Cr^{3+}, Co^{3+}, Rh^{3+}, Ir^{3+}, and Pt^{2+}. One of the factors involved in rates of exchange is the charge-to-radius ratio; if this ratio is high the exchange rate is low.

An important reaction catalyzed by metal ions in enzymes is the ionization of water to give a hydrated hydrogen ion and a hydroxyl anion. Initial studies of this process will be discussed here as they are relevant to the action of a metal ion in providing a hydroxyl group and a hydrogen ion for use in an enzymatic reaction.

2. Database Analyses and Computational Studies

The structural databases accessed in this study of metal ions were the Cambridge Structural Database (CSD) containing three-dimensional data on crystal structures of small molecules (with carbon atoms), [5] and the Protein Data Bank (PDB) containing information on biological macromolecules [6] . These databases were searched for the three-dimensional coordinates of all reported crystal structures containing the metal ion of interest. Care was necessary in this analysis because metal-ligand interactions have been treated in the CSD as bonds in some structures, and differently in other structures. Therefore each entry was examined individually either by determining all neighbors within a given radius of the metal ion or by displaying the structure on a graphics screen [12] and determining the coordination number by visual inspection. The PDB is readily accessed by way of the World-Wide Web so that atomic coordinates can be extracted. The results of analyses of coordination geometry and ligand type are shown in Table 1.

Ab initio molecular orbital and density functional calculations were carried out using the GAUSSIAN suite of programs [7, 8]; computational details are listed in the individual articles. Initially water molecules were added sequentially to the inner coordination sphere of a particular metal ion. Then, for a selected coordination number of that metal ion, water molecules were moved from the innermost coordination sphere to the second coordination sphere, keeping the total number of water molecules fixed. The energy required to do this provides a measure of the rigidity of the inner coordination shell.

In order to estimate the extent of polarization of the water molecules by a hexahydrated metal ion, the energy of the proton transfer reaction

$$M[H_2O]_6^{2+} + H_2O \rightarrow M[H_2O]_5^{2+}OH^- + H_3O^+$$

was estimated by *ab initio* molecular orbital and/or density functional calculations, giving the energy required to ionize a metal ion-bound water molecule. These calculations were repeated with one water molecule replaced by a formate ion (a model of an aspartate or glutamate side chain in a protein), providing information on the effect of other bound side chains on the ability of the metal ion to ionize water.

3. Coordination Numbers and Ligand Types in Crystal Structures

The first question we asked is what kind of site on a protein will a given metal ion select for binding. Analyses of the environment of metal ions in a wide variety of crystal structures provide data on binding preferences, that is, the most common innermost coordination numbers and types of ligands (those

presenting oxygen, nitrogen or sulfur to the metal ion). We are still in the midst of this study, but provide our current results on the metal ions Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, and Pb^{2+}. These ions include examples of alkaline earth and transition metals. We are currently proceeding with studies of Ni^{2+} and Cu^{2+} (not described here).

Magnesium generally binds oxygen atoms in ligands rather than nitrogen or sulfur, as shown in Table 1. It also has a strong tendency to bind six ligands in a regular octahedral arrangement [13]. Among possible ligands it readily binds water; the hexa-aquated magnesium ion, $Mg[H_20]_6^{2+}$ is common in crystal structures, even when there is a crown ether or strong anion available as a counter-ion which might have been thought to be more suitable as a ligand. The affinity of the magnesium ion for water may be partly connected with the size of the cation in that the six oxygen atoms in the inner coordination sphere are in contact with each other (OAAAO = 2.9 D),'as well as, with the magnesium ion (OAAAMg^{2+} = 2.07 D); some other metal ions of similar size do not show this affinity for water. Thus, the normal liganded state of magnesium is rigidly octahedral with six oxygen atoms (often from water molecules) surrounding the cation.

Calcium ions are larger than magnesium ions and they show a strong tendency to bind oxygen ligands, see Table 1. Their preferred coordination numbers range from 6 to 8 [14, 15]. The geometry of this coordination varies from octahedral (c.n.=6) to square antiprism (c.n.=8) to the latter with additional capping ligand atoms (c.n.=9 or 10). In protein crystal structures calcium ions mainly have six or seven metal ion-bound oxygen ligands. Among these the average calcium ion binds 1.5 water molecules, a lower value than that of 2.2 found for magnesium ions. This is possible because magnesium is used to bind substrates (and generally requires two coordination positions to do so), while many proteins are designed simply to sequester calcium and hold it (in a location that is not very accessible to solvent) ready for action, rather than to use it in an enzymatic reaction. For the latter, nature has devised a specific calcium-binding motif, the EF hand [16] which generally consists of two aspartate residues (or one aspartate and one asparagine) bound in a monodentate manner, one glutamate residue bound in a bidentate manner, one main-chain carbonyl group and one water molecule. The seventh ligand is variable and may be water, serine, asparagine or aspartate.

Manganese has a slightly greater affinity for nitrogen than does magnesium, but otherwise has very similar characteristics [17]. This is not surprising because divalent manganese has long been used as a replacement for magnesium in spectroscopic studies of enzyme activity, using the paramagnetic properties that manganese (but not magnesium) possesses [18]. Like magnesium, manganese forms mainly octahedral complexes, but does not show the great propensity that magnesium does for forming a hexahydrate; manganese tends to bind fewer than six water molecules and to include anions in its inner coordination sphere.

Zinc is very different from magnesium, manganese and calcium. It binds nitrogen and sulfur much more readily and also shows lower coordination numbers in spite of its size (which is intermediate between that of magnesium and divalent manganese [19]). It was found that the predominant ligand to zinc depends on the coordination number of the metal ion, whereas the other metal ions just listed each prefer oxygen at all coordination numbers. Zinc tends to form 4-, 5-, and 6-coordinate complexes with about equal ease. When the coordination number is four, sulfur is as likely a ligand as oxygen (see Table 1), when it is 5 nitrogen is the most common ligand, and for coordination numbers 6 and 7 oxygen predominates as a ligand. Thus, zinc can possibly replace magnesium or divalent manganese (since they both bind oxygen when their coordination number is 6), but it has other options for coordination, in keeping with its reactivity in the active sites of enzymes (often involving a change in coordination number).

Divalent lead provides a more complicated story because of the "inert-pair effect" which involves the inertness of the two other 6s electrons on Pb^{2+} towards removal or participation in covalent bond formation or hydrogen bonding [20]. This is ascribed to a relativistic effect causing the 6s orbital to contract, so that the energy required to remove or interact with this lone pair of electrons is increased. The presence of this lone pair of electrons can cause a nonspherical charge distribution around the Pb^{2+} and there may be an identifiable gap in the ligand arrangement around the metal ion. In this regard the divalent lead ion is very different from all the others mentioned above. In Table 1 we list the surroundings of Pb^{2+} in small crystal structures, and differentiate between those in which the distribution of ligands is spherically symmetrical ("holodirected") and those in which all the ligands are directed in only part of an encompassing globe ("hemidirected"), so that there is a void in the distribution of ligand bonds. The holodirected arrangement is only found for higher coordination numbers and may be influenced by ligand overcrowding.

Table 1. Coordination profiles of selected metal ions (CSD data).

Metal ionic	Ions Radius	CN	% Total[a]	% Composition of Ligands		
Mg2+	0.65 A	4	9%	39% O	38% N	3% S
		5	7%	43% O	56% N	0% S
		6	79%	82% O	9% N	0% S
		7	4%	75% O	18% N	0% S
Ca2+	0.99 A	6	22%	80% O	16% N	0% S
		7	25%	94% O	5% N	0% S
		8	45%	95% O	6% N	0% S
		9	5%	98% O	2% N	0% S
		10	3%	100% O	0% N	0% S
Mn2+	0.74 A	4	7%	22% O	19% N	24% S
		5	7%	33% O	44% N	9% S
		6	75%	61% O	30% N	1% S
		7	7%	54% O	22% N	0.5% S
Zn2+	0.71 A	4	42%	17% O	30% N	17% S
		5	19%	26% O	60% N	8% S
		6	35%	60% O	37% N	2% S
		7	4%	64% O	29% N	6% S
Pb2+	1.12 A	6	18%	14% O	8% N	10% S
		7	4%	11% O	6% N	12% S
		8	9%	57% O	21% N	5% S
		9	2%	56% O	44% N	0% S
		10	5%	85% O	13% N	4% S
		2	2%	25% O	50% N	0% S
		3[b]	9%	28% O	30% N	26% S
		4[b]	24%	30% O	26% N	38% S
		5[b]	9%	37% O	23% N	23% S
		6[b]	8%	15% O	8% N	4% S
		7[b]	8%	46% O	17% N	3% S
		8[b]	1%	6% O	2% N	3% S

[a] Percent of bonds of each coordination number
[b] Hemidirected structures (69% of entries) in which there is a structural gap in the distribution of ligands

4. Energetic Consequences of Changes in Coordination Numbers

Ab initio molecular orbital calculations indicate that the energy penalty for changing the inner coordination number of Mg^{2+} from six is fairly high; $Mg[H_2O]_6^{2+}$ is 12.4 and 6.4 kcal/mol lower in energy than $Mg[H_2O]_4^{2+}$ $A2H_2O$ and $Mg[H_2O]_5^{2+}$ AH_2O respectively. Thus the inner coordination sphere of Mg^{2+} is fairly rigid. Divalent calcium and zinc ions, on the other hand, can have more variable coordination numbers and the calculated energy cost for changing the coordination number is lower, as shown in Tables 1 and 2. Thus the energies of a zinc ion with six water molecules around it are similar no matter whether four, five, or all of these six water molecules are in the inner hydration sphere. This implies that any

Table 2. Relative energies (kcal/mol) of hydrated metal-ion complexes.

Metal ion	Energy	[m.n]*	Energy	[m.n]*	Energy	[m.n]*
Mg2+	0.0	[6.0]	+6.4[a]	[5.1]	+12.4[a]	[4.2]
Mn2+	0.0	[6.0]	+3.5[a]	[5.1]	+7.7[a]	[4.2]
Ca2+	0.0	[6.0]	+8.2[b]	[5.1]	+15.0[b]	[4.2]
	0.0	[7.0]	-1.4[b]	[6.1]	+4.0[b]	[5.2]
	0.0	[8.0]	+1.0[b]	[7.1]	-0.5[b]	[6.2]
Zn2+	0.0	[6.0]	+1.0[a]	[5.1]	+1.4[a]	[4.2]
	0.0	[5.0]	0.6[c]	[4.1]		
	0.0	[4.0]	14.6[c]	[3.1]		

* [m.n] denotes a metal ion surrounded by m water molecules in the inner coordination sphere and n water molecules in the second coordination sphere.
[a] MP2(FULL)/6-311+ +G**//HF/6-31G* level for magnesium complexes and MP2(FULL)/6-311+ +G**//HF/HUZ* level for manganese and zinc complexes [17].
[b] MP2(FULL)/HUZSP*(p,d)+//HF/HUZSP*(p) level [15].
[c] MP2(FC)/HUZSP*//HF/HUZSP* level [19].

chemical reaction around the Zn^{2+}, which involves a change in coordination number, does not incur a serious energy penalty for changing the coordination number. This is in line with the experimental observation that zinc can be used to carry out a catalytic reaction, as in carboxypeptidase, while magnesium is more generally used to hold the substrate firmly in place, as suggested for mandelate racemase and 3',5'-exonuclease.

Molecular orbital studies, combined with database analyses, have provided some basis for understanding the differences between hemidirected geometries (with a void in the ligands) and holodirected geometries (with no such void) of Pb^{2+} complexes. In hemidirected structures the coordination number is low, the ligands may interact with each other, the metalligand bonds are generally ionic and the lone pair has *p* character (indicative of directionality). In holodirected complexes involving higher coordination, numbers, the metal-ligand bonds have more covalent character and the lone pair has little, if any, *p* character. It is also found that in holodirected complexes more electron density is transferred from the ligands to Pb*2+*.

5. Polarizing Potential of Various Ions

Atoms or groups of atoms are considered polarizable if, when they are placed in an electric field, a charge separation occurs and a dipole is acquired. This deformability or polarizability is measured by the ratio of the induced dipole to the applied field. Those atoms that hold on less firmly to their electrons are termed more polarizable. It is found that if two ions have the same inert gas structure (potassium and chloride, for example), the negatively charged anion is more polarizable than the positively charged cation, which holds on to its electrons more tightly.

The word "hard" has been introduced to indicate a low polarizability so that the electron cloud is difficult to deform (like a hard sphere). By contrast "soft" means high polarizability so that the electron cloud is readily deformed [18]. A hard acid or metal cation holds tightly to its electrons and therefore its electron cloud is not readily distorted; its unshared valence electrons are not easily excited. Soft (polarizable) metal cations contain electrons that are not so tightly held and therefore are easily distorted or removed. A hard acid prefers to combine with a hard base, while a soft acid prefers to bind with a soft base by partially forming covalent bonds. The type of binding is related to the highest occupied molecular orbital (HOMO) of the electron-pair donor (a Lewis base, the ligand) and the lowest unoccupied molecular orbital (LUMO) of the electron-pair acceptor (a Lewis acid, the metal ion). If these have similar energies, then electron transfer will give a covalent (soft-soft) interaction, whereas the energy difference is large, electron transfer does not readily take place and the interaction is mainly electrostatic (hard-hard). Hard cations include the alkali and alkaline earth metal ions while soft metal ions include Cu^{2+}, Hg_2^{2+}, Hg^{2+}, Pd^{2+}. In

biological systems, hard ligands generally contain oxygen while soft ligands contain sulfur. Hard acids tend to bind hard bases by ionic forces, while soft acids bind soft bases by partially forming covalent bonds. These hard-soft categorizations are a help in understanding the relative binding preferences of various cations. Most metal ions of biological significance are hard or intermediate between hard and soft. Most soft metal ions and soft ligands are poisonous and they interact with other soft species in the body. For Pb^{2+} the harder ligands are found in hemidirected structures and the softer ligands in holodirected complexes.

Nature has devised many enzyme systems in which a metal ion interacts with the oxygen of a water molecule. If a water molecule can be dissociated into a hydrogen ion and a hydroxyl group, the latter can serve as a nucleophile in chemical and biochemical reactions. Nature has chosen activation of a water molecule as a means to obtain such a nucleophile in situ so that a chemical reaction can occur in a stereochemically controlled manner in the active site of the enzyme. The questions we ask are as follows: 1) how does nature ensure that the specific water molecule will be activated; 2) how does nature compensate for the lower water activation power of some cations over others (since a wide variety of metal ions may not be available in the particular active site and the enzyme has to do the best it can with what is available); and 3) how does nature ensure that the required reaction occurs.

Ab initio molecular orbital and density functional calculations have been carried out to measure the extent to which a series of metal cations can, on binding with water, cause it to be dissociated into its component hydrogen ions (subsequently hydrated in solution) and hydroxyl ions. Initial data indicate that the charge of the metal ion plays a significant role in modifying the pKa of water. The binding enthalpies of a wide variety of metal ion monohydrates, $M[H_2O]^{2+}$, have been published [21] but their deprotonation enthalpies are still under investigation. Calculations for the proton transfer reactions

$$M\left[H_2O\right]_6^{2+} + H_2O \rightarrow M\left[H_2O\right]_5^{2+} \cdot [OH^-] + H_3O^+$$

find values for the ΔG_{298} of -1.8kcal/mol for M=Mg, +1.7kcal/mol for M=Mn and -11.6 kcal/mol for M=Zn. Similar values (+2.8, +3.6, and -10.4kcal/mol respectively) are obtained when $M[H_2O]_6^{2+}$ is replaced by $M[H_2O]_4^{2+}[NH_3]_2$. These values, which indicate that Mg^{2+}, Mn^{2+}, and, (particularly) Zn^{2+}, facilitate the ionization of water; for comparison the values of ΔG_{298} for the reactions $H_2O+H_2O \div 4OH^-+H3O^+$ and $M[H_2O]_5^{2+}[HCO_2]+H_20 \div M[H_2O]_4^{2+}[OH^-][HCO_2]+H_30^+$ (M = Mg, Mn) are +225.5kcal/mol and 81.8 kcal/mol respectively. Thus the presence of a negatively charged carboxylate group makes it significantly more difficult for the ionization of a metal-bound water molecule to proceed.

6. Geometry of Metal-Ion Binding to Functional Groups

The geometries of metal ion-carboxylate interactions have been studied in order to determine the following: 1) which lone pair of an oxygen atom in a carboxylate group, syn or anti, is preferred for metal cation binding; 2) does the metal ion lie in the plane of the carboxyl group; and 3) under what conditions do metal ions share both oxygen atoms of the carboxylate group equally? We found that cations generally lie in the plane of the carboxylate group [22]. The exceptions to this mainly include the alkali metal cations and some alkaline earth cations; these metals ionize readily and form strong bases so it is not surprising that they have less specific binding modes. When the distance of the metal cation to the carboxylate oxygen atoms is on the order of 2.3-2.6 Å, the metal ion tends to share both oxygen atoms equally. Otherwise one oxygen atom of the carboxylate group is bound to the metal ion and the other is not. Calcium ions often form bidentate interactions, while it is less common for the smaller magnesium ions.

Imidazole groups in histidyl side chains of proteins bind metal ions in a variety of enzymes. One imidazole can, by virtue of its two nitrogen atoms, bind one or two metal ions, depending on its ionization state and the suitabilities of the metal ion. The bases in DNA can also bind metal ions. We have analyzed hydrogen bonding to and from nitrogen atoms in nitrogen-containing heterocycles [23, 24] for crystal structures in the Cambridge Structural Database. It was found that for hydrogen bonding, a slight out-of-plane deviation of the binding atom often occurs. Metal ions bind more rigidly in the plane of the imidazole group. The energetic cost of such deviations were analyzed by *ab initio* molecular orbital calculations. In an investigation of protein crystal structures in the Protein Databank it was found that the binding of metal ions to histidine in proteins is more rigid and the location of the metal ion is more directional. Thus, if an enzyme needs to control the location and orientation of a carboxylate or imidazole group, it can accomplish this better with a metal ion than by hydrogen bonding.

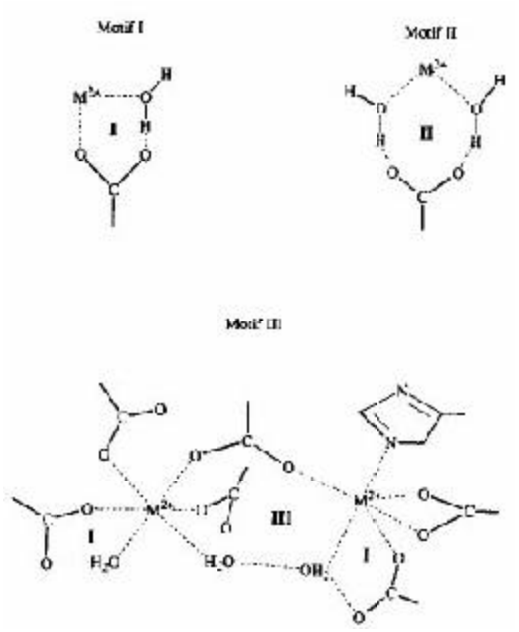

Fig. 1

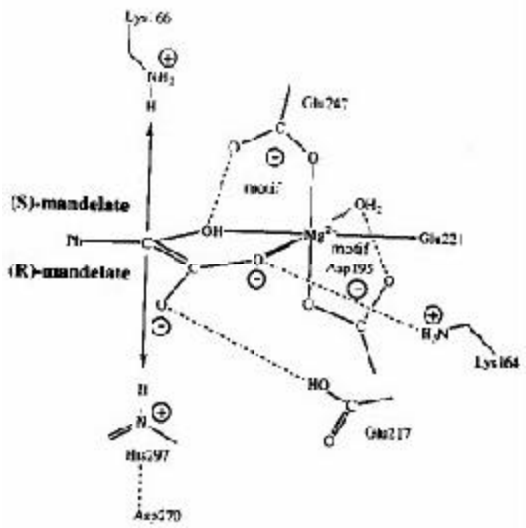

Fig. 2

Metal ions in proteins are often involved in structural motifs as shown in Fig. 1. When a metalloenzyme carries out its catalytic function it uses one of a few possible three-dimensional arrangements of functional groups around the metal ion to ensure the specificity of the required biochemical reaction. Thus, if such catalytic metal-binding motifs can be identified and categorized, then incipient reactivities of enzymes could be inferred from their three-dimensional structures. Such a categorization, however, requires an understanding of the underlying chemistry of any metal ion in the active site. One motif identified in the crystal structure of cobalt(II) formate consists of a carboxyl group in which one oxygen atom is bound to the metal ion and the other is bound to metal-bound water, to give a cyclic structure [13, 17]. This motif has been found in many metalloenzyme crystal structures [25], such as D-xylose isomerase [26].

The roles of these motifs are of interest. The metal ion-hydrated-carboxylate motif (**I**) is planar and commonly found. It does not, however, affect the ability of the metal ion (in studies of Mg^{2+} complexes) to ionize water. On the other hand, for magnesium ions (which generally have a rigid octahedral arrangement of binding groups) it utilizes 2 of the 6 coordination positions and therefore serves to orient the arrangement of ligands, an effect we have labeled "coordination clamping [27]." Motif (**II**) is also found in several crystal structures such as that of the "-subunit of integrin CR3 [28]. It appears to help bind subunits together.

A third motif (**III**) is found in D-xylose isomerase and involves two metal ions with several carboxylate ligands and a histidine ligand [26, 27]. The metal site that binds only oxygen atoms can ' bind substrate in place of the two water molecules and orient the substrate. The second metal ion site (with histidine as one ligand) then positions a metal ion-bound water molecule to attack the substrate.

7. Roles of Metal Ions in Enzyme Action

The crystal structure of mandelate racemase with bound p-iodomandelate provides a useful example of the importance of a metal ion in a reaction [4] . The enzyme binds a magnesium ion by means of three carboxyl groups. The substrate mandelate has displaced water from the magnesium coordination sphere and binds by means of its carboxylate group and an a-hydroxy group. The magnesium ion will lie in the plane of the carboxyl group, as shown by our studies of metal ion-carboxylate interactions [22]. The magnesium holds the substrate firmly in place so that the catalytic abstraction and addition of a hydrogen atom by His 297 or Lys 166 is precisely effected (see Fig. 2). The magnesium probably also aids this activity by affecting the electronic flow in the carboxylate and hydroxyl groups by mild polarization. We have found that metal ion coordination is better than a hydrogen bond in aligning a functional group; there is considerable flexibility in a hydrogen bond as we found for imidazoles [23].

In the reaction catalyzed by the enzyme mandelate racemase the magnesium ion binds substrate as shown in

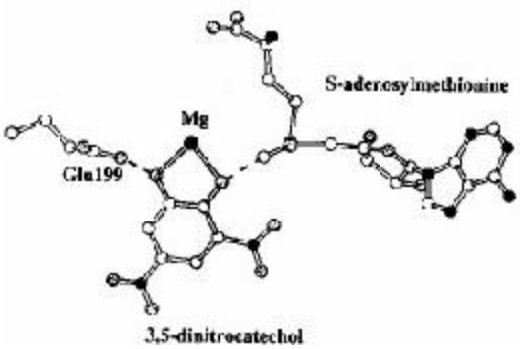

Fig. 3

Fig. 2. A Histidine (His 297) and Lysine (Lys 168) are positioned to abstract a hydrogen ion from the substrate and, if it is added again from the other side, racemization occurs. Hydrogen bonding to a carboxylate group of the substrate helps to stabilize an enolate intermediate in the reaction.

In catechol O-methyltransferase [3], shown in Fig. 3, a methyl group is transferred from the sulfur of S-adenosy[methionine to catechol. The magnesium ion is oriented by a motif of type **I** and it binds substrate in such an orientation that a hydroxyl group is near the S-CH_3 group, and the other hydroxyl group is held in place by a carboxylate group. There are many other examples of two-metal ion active sites, such as hemerythrin, alkaline phosphatase and superoxide dismutases (which have been well documented). These studies of the geometries and energetics of metal-ion ligand binding can therefore aid in our understanding of metalloenzyme function.

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