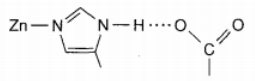
2.15: Strategies for the Investigation of Zinc Enzymes

The Groups to which Zinc(II) is Bound

Zinc(II) is an ion of borderline hardness and displays high affinity for nitrogen and oxygen donor atoms as well as for sulfur. It is therefore found to be bound to histidines, glutamates or aspartates, and cysteines. When zinc has a catalytic role, it is exposed to solvent, and generally one water molecule completes the coordination, in which case the dominating ligands are histidines. It has been noted[20] recently that coordinated histidines are often hydrogen-bonded to carboxylates:



It is possible that the increase in free energy for the situation in which the hydrogen is covalently bound to the carboxylate oxygen and H-bonded to the histidine nitrogen is not large compared to k_BT. Under these circumstances the protein could determine the degree of imidazolate character of the ligand and therefore affect the charge on the metal.

The binding of zinc(II) (like that of other metal ions) is often determined by entropic factors. Water molecules are released when zinc(II) enters its binding position, thus providing a large entropy increase. Most commonly zinc is bound to three or four protein ligands. Large entropy increases are not observed, however, when zinc(II) binds to small polypeptides like the recently discovered zinc fingers, for here the binding site is not preformed (see Section III.B), and zinc(II) must be present for the protein to fold properly into the biologically active conformation.

The Reactivity of Zinc(II) in Cavities

In the preceding section we discussed the properties of zinc(II) as an ion. These properties are, of course, important in understanding its role in biological catalysis, but it would be too simplistic to believe that reactivity can be understood solely on this basis. Catalysis occurs in cavities whose surfaces are constituted by protein residues. Catalytic zinc is bound to a water molecule, which often is H-bonded to other residues in the cavity and/or to other water molecules. The structure of the water molecules in the cavity cannot be the same as the structure of bulk water. Furthermore, the substrate interacts with the cavity residues through either hydrophilic (H-bonds or electric charges) or hydrophobic (London dispersive forces) interactions. As a result, the overall thermodynamics of the reaction pathway is quite different from that expected in bulk solutions. Examples of the importance of the above interactions will be given in this chapter.

The Investigation of Zinc Enzymes

Direct spectroscopic investigation of zinc enzymes is difficult, because zinc(II) is colorless and diamagnetic; so it cannot be studied by means of electronic or EPR spectroscopy. Its NMR-active isotope, ^{67}Zn, the natural abundance of which is 4.11%, has a small magnetic moment, and cannot (with present techniques) be examined by means of NMR spectroscopy at concentrations as low as 10^{-3} M. The enzymes could be reconstituted with ^{67}Zn. However, ^{67}Zn has a nuclear quadrupolar moment, which provides efficient relaxation times, especially in slow-rotating proteins and low-symmetry chromophores, making the line very broad.[210] Of course, ^{1}H NMR can be useful for the investigation of the native enzymes. However, often the molecular weight is such that the proteins are too large for full signal assignment given the current state of the art. At the moment the major source of information comes from x-ray data. Once the structure is resolved, it is possible to obtain reliable structural information on various derivatives by the so-called Fourier difference map. The new structure is obtained by comparing the Fourier maps of the native and of the derivative under investigation. Many x-ray data are now available on carboxypeptidase (Section V.A) and alcohol dehydrogenase (Section V.C).

The zinc ion can be replaced by other ions, and sometimes the enzymatic activity is retained fully or partially (Table 2.4). These new systems have attracted the interest of researchers who want to learn about the role of the metal and of the residues in the cavity, and to characterize the new systems per se. Spectroscopic techniques can be appropriate for the new metal ions; so it is possible to quickly monitor properties of the new derivative that may be relevant for the investigation of the zinc enzyme.

Table 2.4 - Representative metal-substituted zinc enzymes. Percent activities with respect to the native zinc enzyme in parentheses.[a]

Enzyme	Substituted Metals
Alcohol dehydrogenase	Co(II)(70), Cu(II)(1), Cu(I)(8), Cd(II)(30), Ni(II)(12)

Enzyme	Substituted Metals
Superoxide dismutase	Co(II)(90), Hg(II)(90), Cd(II)(70), Cu(II)(100)
Aspartate transcarbamylase	Cd(II)(100), Mn(II)(100), Ni(II)(100)
Transcarboxylase	Co(II)(100), Cu(II)(0)
RNA polymerase	Co(II)(100)
Carboxypeptidase A	Mn(II)(30), Fe(II)(30), Co(II)(200), Ni(II)(50), Cu(II)(0),[b] Cd(II)(5), Hg(II)(0), Co(III)(0), Rh(II)(0), Pb(II)(0)
Thermolysin	Co(Il)(200), Mn(II)(10), Fe(II)(60), Mg(II)(2), Cr(II)(2), Ni(II)(2), Cu(II)(2), Mo(II)(2), Pb(II)(2), Cd(II)(2), Nd(III)(2), Pr(III)(2)
Alkaline phosphatase	Co(II)(30), Cd(II)(1), Mn(II)(1), Ni(II)(0), Cu(II)(0), Hg(II)(0)
β-Lactamase II	Mn(II)(3), Co(II)(11), Ni(II)(0), Cu(II)(0), Cd(II)(11), Hg(II)(4)
Carbonic anhydrase[c]	Cd(II)(2), Hg(II)(0), Cu(II)(0), Ni(II)(2), Co(II)(50), Co(III)(0), Mn(II)(18), $V(IV)O^{2+}$(0)
Aldolase	Mn(II)(15), Fe(II)(67), Co(II)(85), Ni(II)(11), Cu(II)(0), Cd(II)(0), Hg(II)(0)
Pyruvate carboxylase	Co(II)(100)
Glyoxalase	Mg(II)(50), Mn(II)(50), Co(II)(50)

a) Taken from Reference 21. b) Recent data indicate nonnegligible catalytic activity.[22] c) BCA II, except the value for Cd(II) obtained with HCA II