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Binding of Metal Ions to Phospholipid Membranes. Application of Deuterium Magnetic Resonance

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Phosphatidylcholine is one of the predominant phospholipids in membranes and a large fraction of most membrane surfaces is occupied by phosphocholine groups. The interactions of metal ions with the uncharged phosphatidylcholine bilayer can be expected to be relatively weak compared to those with negatively charged lipids such as phosphatidylglycerol or phosphatidylserine. Nevertheless, even small changes in the head group orientation and flexibility could significantly alter the electrical properties of the membrane surface. The problem of metal ion binding to phosphatidylcholine bilayers has attracted much attention and deuterium magnetic resonance is a particularly promising method in this respect. We have therefore studied the interaction of mono-, di-, and trivalent metal ions with bilayers of saturated and unsaturated phosphatidylcholines by means of deuterium magnetic resonance. Using selectively deuterated lipids the measurements of the residual deuterium quadrupole splitting provided a sensitive handle to monitor directly the binding of ions, including the weak binding of Na^+. From a systematic comparison of various ions the following conclusions could be derived. (1) Addition of metal ions led to a structural change at the level of the polar groups. The glycerol backbone or the beginning of the fatty acyl chains were not affected. (2) The strength of interaction increased with the charge of the metal ion in the order $Na^+ < Ca^{2+} < La^{3+}$. However, distinct differences were also noted between ions of the same charge. Furthermore, the strongly hydrophobic tetraphenylammonium ion induced almost the same change as La^{3+}. (3) The variation of the quadrupole splittings with ion concentration exhibited is a plateau value at high concentrations of lanthanum. The titration curves of phosphatidylcholine bilayers with calcium and lanthanum could be described in terms of a Langmuir adsorption isotherm with an interaction potential and apparent binding constants were derived [1, 2].

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Diamagnetic Bivalent Metal Ion NMR Studies of Metalloproteins; Zn^{2+}–Insulin and Zn^{2+}-Concanavalin Complexes

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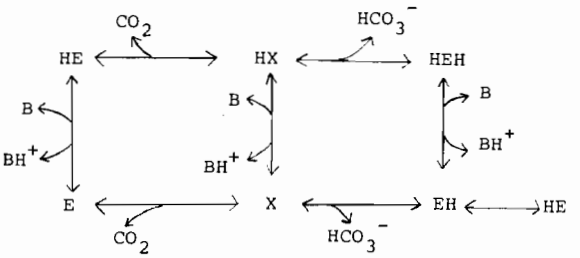
Diamagnetic bivalent metal ions such as Mg^{2+}, Ca^{2+}, and Zn^{2+} are indispensable for the full activity of metalloenzymes, but are non-chromophoric. Thus, paramagnetic and chromophoric metal ions such as Co^{2+}, Mn^{2+}, and Tb^{3+} were substituted for Mg^{2+}, Ca^{2+}, or Zn^{2+} in the metalloenzymes and have been thought to be useful as a mimetic probe to the diamagnetic bivalent metal ions. However, the enzymatic activities and structures of the metal-binding sites in the metal substituted enzymes might be different from the native enzymes, and then it is necesary to analyze directly the structural role of the divalent ions such as Mg^{2+}, Ca^{2+}, and Zn^{2+} in the metalloenzymes. Thus, metal ion NMR has shown in studying the structure of the metalloenzymes, especially the metal-binding site, the ligating candidates, or the motional behavior of the bound metal ions. In succession to our bivalent diamagnetic metal ion NMR studies [1–5], we would like to present ^{67}Zn NMR studies on Zn^{2+}–insulin and Zn^{2+}–concanavalin A complexes in this paper.

NMR characteristics of ^{67}Zn (I = 5/2) are similar to those of ^{25}Mg (I = 5/2) and ^{43}Ca (I = 7/2), since all the nuclei have a quadrupole moment. However, ^{67}Zn NMR spectra of aqueous Zn^{2+} are different from ^{25}Mg and ^{43}Ca NMR spectra of aqueous Mg^{2+} and Ca^{2+}. ^{67}Zn NMR spectra of aqueous Zn^{2+} have a marked concentration dependence in terms of the half-band widths compared with those of ^{25}Mg and ^{43}Ca NMR of aqueous Mg^{2+} and Ca^{2+} [1–3]. For example, $ZnCl_2$ (2 *M*), pH 4.0, exhibited a very broad ^{67}Zn NMR with a half-band width of 170 Hz, and dilution of the $ZnCl_2$ solution to 50 m*M*



Scheme 1. Proposed kinetic mechanism of carbonic anhydrase. H to the right of E represents protonated catalytic group (zinc-bound H_2O). H to the left of E represents protonated His-64.

led to a narrower ^{67}Zn NMR with a half-band width of 12 Hz. By adding 1 m*M* bovine insulin to the 50 m*M* $ZnCl_2$ solution, at pH 2.95, the half-band width of ^{67}Zn NMR increased by three times. Addition of insulin more than 1 m*M* led to a broader ^{67}Zn NMR. The determined T_1/T_2 values of the 2 *M* and 50 m*M* $ZnCl_2$ solutions are almost unity. But, the T_1/T_2 ratio of ^{67}Zn NMR of the Zn^{2+}-insulin complex is not unity (1.36). From these findings, the correlation time, τ_c, and the quadrupole coupling constant are evaluated. The halfband widths of ^{67}Zn NMR are temperature dependent, when Zn^{2+} is bound to polymeric ligands. It is suggested from the temperature dependence that molecular weights of the ligands are closely correlated with the mechanism of the ^{67}Zn NMR of the Zn^{2+} complexes. The relaxation mechanism on the ^{67}Zn NMR will be discussed.

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X-Ray Absorption Spectrometry as a Tool for the Study of Molecular Structure

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X-ray absorption spectroscopy is a relatively new tool for the investigation of electronic structure and the local environment of specific atoms in biological molecules. Until recently, these techniques were not practical for the low concentrations required in biological preparations. With the development of high flux X-ray beams produced by synchrotrons, much interest has been generated in development of its application to biological problems. It is a unique tool in that it probes only the local environment of specific atoms and is not limited by the physical state of the sample (*e.g.*, gases, liquids, solids, solutions, gels, etc.). Unlike X-ray diffraction, the entire structure of a crystal need not be solved to obtain information concerning the structure of the active site of a molecule. In these respects, X-ray absorption spectroscopy is ideally suited for biologically problems. It has grown enormously as a practical technique since the opening of the Stanford Synchrotron Radiation Laboratory in 1974.

In many biological systems the active sites include one or more transition metal atoms. In most cases, however, the metal atoms are very dilute and are responsible for only a small part of the total absorption. Fluorescence detection is usually the preferred measurement technique, and one is often forced to work with spectra of less than ideal signal-to-noise quality. Mathematical techniques have been developed to extract information from this type of experimental data. A further complication is that care must be taken to avoid X-ray damage of the biological samples.

EXAFS (extended X-ray absorption fine structure) experiments have been performed on a large number of macromolecules of interest to biochemistry. These experiments can roughly be divided into two groups. The first involves proteins where the ligands of the metal ion are already known from diffraction, and EXAFS is used to obtain more accurate bond length and geometry information. The second group of experiments deals with proteins where some or all of the ligands are unknown, and the goal is to determine the coordination number, the bond lengths, and ultimately the local structure. In both these groups model compounds play an important role in aiding the analysis and establishing confidence in the results.

Absorption features below the K X-ray absorption edge of 3*d* transition metal complexes can be assigned, in sufficiently ionic sites, to transitions from the 1*s* orbital to vacant 3*d*, 4*s*, and 4*p* orbitals. The spacing between these levels can be perturbed from those obtaining in the analogous free ion states since the vacant orbitals are hybridized with the filled orbitals of ligand atoms, leading to antibonding orbitals of elevated energy. This hybridization is, of course, quite different for the *d*, *s*, and *p* orbitals as they are of quite different sizes and symmetries. A study of a number of Cu(I) and Cu(II) complexes shows that the $1s \rightarrow 4s$ and $1s \rightarrow 4p$ ranges of observed energies of the features of the two charge states partly overlap, indicating that effects of covalency outweigh effects of valency itself.

In addition, there are multiple scattering modes in this region of the spectrum which, if properly interpreted, can give information on the local structure around the metal atom. Series of metal–ligand complexes in which the total charge state is changed while keeping the ligand atoms constant show how these atomic and scattering features depend on local structure.

Several examples of metalloproteins studied by the Bell Laboratories group will be described.
