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

JOACHIM SEELIG

Biocenter, University of Basel, Klingelbergstrasse 70, CH-4056 Basle, Switzerland

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].

1 H. Akutsu and J. Seelig, 'Interaction of metal ions with phosphatidylcholine bilayers membranes', *Biochemistry, 20,* 7366 (1981).
2 Ch. Altenbach and J. Seelig, unpublished results.

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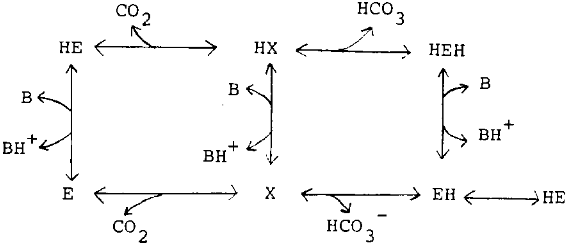
Diamagnetic Bivalent Metal Ion NMR Studies of Metalloproteins; Zn^{2+}–Insulin and Zn^{2+}-Concanavalin Complexes

MASAHIRO HATANO* and TORU SHIMIZU

Chemical Research Institute of Nonaqueous Solutions, Tohoku University, Sendai, 980, Japan

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.