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Co–C Bond Cleavage in B_{12} Systems and Organocobalt Compounds: The Role of Structure

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In this talk, we will discuss the relationship between structure and Co–C bond cleavage in organocobalt compounds. At least two organocobalt compounds are required in humans [1]. These are deoxyadenosylcobalamin (coenzyme B_{12}) and methylcobalamin (methyl B_{12}). Both compounds have Co–C bonds in one coordination position. The ligand is simply a methyl group in methyl B_{12} but is 5′-deoxyadenosine in coenzyme B_{12}. The deoxyadenosine is bound to Co via the 5′ carbon of the ribose sugar. It is generally accepted that the cobalt in these compounds is in oxidation state III (low spin d^6). The four coordination positions *cis* to the carbon donor ligand are occupied by four N's of a corrin ligand. The position *cis* to these four N donors and *trans* to the alkyl ligand is occupied by N(3) of a 5,6-dimethylbenzimidazole which is linked to the corrin ring via a complex chain containing a phosphodiester linkage. Thus the macrocyclic corrin and its pendant benzimidazole chain is a pentadentate ligand. There is considerable flexibility in the corrin ring system and the structures of metallocorrin compounds have recently been reviewed in depth [2].

Coenzyme B_{12} is known [1] to be required in about a dozen enzymatic reactions, mostly in bacterial systems. Although there are substantial differences between the enzymes both in the nature of the reactions catalyzed and in the requirement for additional cofactors [1], there is little doubt that, for several catalytic processes, homolytic cleavage of the Co–C bond is an essential step. The initial products of this cleavage are cob(II)alamin (B_{12r}) and the deoxyadenosine radical.

Since the coenzyme is reasonably stable to Co–C bond cleavage under physiological conditions (in the absence of light), most workers in the field believe that interaction between the coenzyme and the enzyme promotes or 'triggers' the cleavage reaction [3, 4]. The most convincing explanation for the enzyme-promoted cleavage involves a conformational change of the coenzyme induced in the holoenzyme. Such a conformational change could weaken the Co–C bond either through steric or electronic effects, or a combination of the two.

The extensive evidence that the strength or stability of the cobalt–carbon bond is sensitive to steric or electronic changes in the ligands, including the alkyl group, attached to the cobalt cannot be reviewed here. The reader is referred to an extensive literature [3–5]. In particular, using elegant kinetic and equilibrium studies of model organocobalt compounds, Halpern has assessed the influence of both the electronic and steric properties of *trans* ligands on Co–C bond strengths [6–8]. These studies are described in another talk in this symposium, which emphasizes the dynamic and energetic aspects of Co–C bond cleavage. In this talk, we will focus on structure.

At the time of Glusker's review of structure [2], only one organocobaltcorrin had been structurally characterized by X-ray crystallography. However, this compound is the very important coenzyme B_{12} itself [9, 10]. The unexpected presence of the Co–C bond was revealed in this study – however, no organocobaltcorrin structures have been reported since. We have crystallized and are structurally characterizing methyl B_{12}, the other recognized coenzyme in human metabolism. Structural details will be available at the time of the meeting.

Acknowledgments. This research was supported by NIH grants GM 29225 (LGM) and CA 10925 (JPG) and by the CNR, Rome (LR), and by NATO (LGM and LR). We are grateful to these organizations for this support.

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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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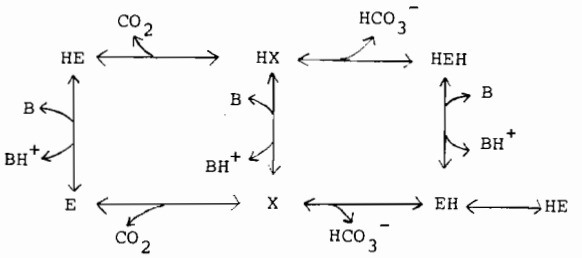
Diamagnetic bivalent metal ions such as Mg^{2+}, Ca^{2+}, and Zn^{2+} are indispensable for the full activity of metalloenzymes, but are non-chromophoric. Thus, paramagnetic and chromophoric metal ions such as Co^{2+}, Mn^{2+}, and Tb^{3+} were substituted for Mg^{2+}, Ca^{2+}, or Zn^{2+} in the metalloenzymes 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.