Registry No. 5, 95250-95-6; **6**, 95250-96-7; **7**, 3005-61-6; camphor, 76-22-2.

Supplementary Material Available: Listing of ^1H and ^{13}C NMR data of **1**, INADEQUATE and full $^1H,^{13}C$ correlation spectra of **1**, and summary of Eu(fod)$_3$ experiments (5 pages). Ordering information is given on any current masthead page.

Cadmium-113 Chemical Shift Tensor in Cadmium Diethyl Phosphate: A Step toward Understanding Divalent Cation–Phospholipid Interactions

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Received October 3, 1984

Solids NMR methods have made major contributions to the characterization of biological membranes. The partial averaging of ^{13}C and ^{31}P chemical shift tensors, in particular, have been useful in studies of the motional and structural properties of phospholipids.[1-5] Such studies could be extended to the ionic portions of divalent ion–anionic lipid complexes important in membrane function,[6] if a suitable probe nucleus and sufficient model compound studies existed for the cations involved. We present here an illustration that ^{113}Cd can be used as such a probe and present model compound data for this nucleus.

Model compound studies are normally conducted on a single crystal, which can be rotated in an applied magnetic field to uniquely determine tensor elements in a molecular frame. In the case of ^{113}Cd some crystalline complexes have been studied,[7-11] but these do not include the phosphate complexes that would be most suitable as models for membrane work.[4,5]

We present here a partial determination of the ^{113}Cd chemical shift tensor of Cd in a diethyl phosphate complex in an effor to provide suitable model compound data. Our inability to obtain crystals of sufficient size for single-crystal studies has prevented a complete determination. However, the needlelike form of the crystals and the coincidence of one tensor element with the long axis of the crystals has allowed unique orientation of one element in the molecular frame and accurate determination of the magnitude of all three tensor elements.[12] To illustrate the feasibility of extension to membrane preparations, data on a dispersion of
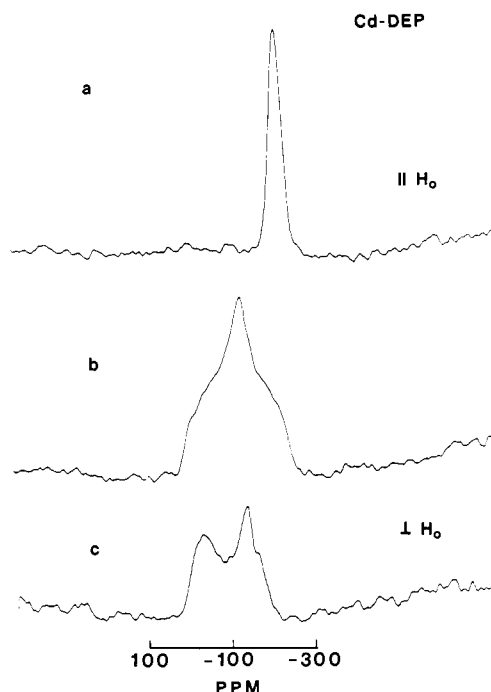

Figure 1. ^{113}Cd spectra of Cd–diethyl phosphate crystals (a) aligned parallel to the field, (b) as a powder, and (c) aligned perpendicular to the field.

Table I. Orientational Data for the Most Shielded Element of the Cadmium Diethyl Phosphate Shift Tensor

bond	bond dist, Å	angle from tensor element, deg
Cd–O12	2.228	56.6
Cd–O22	2.228	123.6
Cd–O21′	2.451	71.9
Cd–O11′	2.448	108.2
Cd–O21	2.290	34.1
Cd–O11	2.289	145.9

the Cd complex with the anionic lipid dimyristoylphosphatidic acid (DMPA) are also presented.

Cd((EtO)$_2$PO$_2$)$_2$ was prepared as described elsewhere.[13] The needlelike crystals of approximate dimensions 0.5 mm by 0.5 mm by 5.0 mm were packed into a 5-mm NMR tube with long axes parallel to the tube. Dimyristoylphosphatidic acid was synthesized from dimyristoylphosphatidylcholine (Sigma, St. Louis, MO) as described previously.[14,15] The Cd–DMPA complex formed by the addition of equimolar CdCl$_2$ to an aqueous dispersion of phosphatidic acid[14] was collected and transferred to a 10-mm-diameter NMR tube for study.

NMR spectra were obtained on a Bruker CXP-200 spectrometer operating at 44 MHz for ^{113}Cd. In all cases, spectra were obtained using cross-polarization from protons for sensitivity enhancement. A 4-μs proton pulse was followed by a 5-ms contact time and acquisition with a ^1H decoupling field present. In most cases, the spectra are the result of overnight accumulation with a 2–4-s recycle time.

Shift tensor elements were extracted from powder spectra using computer simulation of line shapes. The simulation is based on that described by Seelig[1] and assumes a constant Lorentzian line width, independent of orientation. Tensor elements are referenced to 1 M Cd(ClO$_4$)$_2$ with negative values indicating upfield shifts.

Figure 1 presents powder and oriented ^{113}Cd spectra of Cd–diethyl phosphate. The powder spectrum (b) is totally asymmetric

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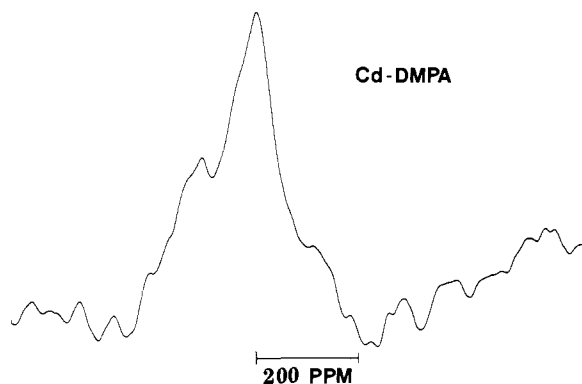(15) Liao, M.-J.; Prestgard, J. H. *Biochim. Biophys. Acta* **1979**, *550*, 157–173.

Cd-DMPA

200 PPM

Figure 2. ^{113}Cd spectrum of dimyristoylphosphatidic acid–Cd complex as a powder at approximately 30 °C.

and can be fit to extract tensor elements of –7, –111, and –217 ppm. When microcrystals are aligned parallel to the field (a), a single narrow line coincident with the most shielded element, –217, is observed. The width of the line would suggest that the most shielded element of the tensor departs by at most 15° from the long axis of the microcrystals. When crystals are aligned perpendicular to the field (c), a spectrum consistent with a nearly random orientation of the –7 and –111 ppm tensor elements relative to the field direction is observed.

We have recently determined the molecular structure of cadmium diethyl phosphate using X-ray diffraction methods.[13] The Cd ions exist in the form of a long chain running parallel to the crystal "a" axis with each Cd ion bridged to adjacent Cd ions by an eight-membered ring composed of two O–P–O groups and two Cd ions and by a four-membered ring composed of two phosphate oxygens and two Cd ions. Each Cd is in a distorted octahedral environment with six nonesterified phosphate oxygens at distances of 2.225, 2.446, 2.448, 2.287, 2.289, and 2.227 Å. The distortion from octahedral symmetry is moderate, yet the dispersion of shift tensor elements is large (~200 ppm). The most shielded element is coincident with the *a* crystal axis and thus the chain of Cd ions.

Honkonen and Ellis have recently introduced an analysis that leads to an empirical correlation of shift tensor elements with structure, suggesting the most shielded element to lie perpendicular to the longest Cd–ligand bonds.[11] As summarized in Table I, we find our data to be in reasonable agreement with this suggestion. In addition to being along the chain of Cd ions, the most shielded element in cadmium diethyl phosphate is just 18° off being perpendicular to the two longest Cd–O bonds.

Given this data and structural interpretation, it is useful to attempt application to an ion–lipid complex of unknown structure. Figure 2 presents a ^{113}Cd spectrum of a Cd–dimyristoylphosphatidic acid complex. Assuming a single site to exist, it appears that the CSA powder pattern arises from an asymmetric shift tensor and that the complex is not undergoing the rapid axial rotation often found in membrane systems which would give an axially symmetric tensor. This is consistent with the well-ordered gel-phase structure and intricate ion bridging network proposed for the phosphatidic acid–Ca system.[14–17] The width of the spectrum is more than 200 ppm, indicating a dispersion of shift elements at least as large as that found in cadmium diethyl phosphate. One could expect an octahedral coordination shell with distortions somewhat larger than that observed in the model. While studies of oriented samples were not attempted, it is obvious that identification of the direction of greatest shielding relative to the membrane normal would provide information useful in proposing specific structural models.

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Evidence of the Breaking of the Copper–Imidazolate Bridge in Copper/Cobalt-Substituted Superoxide Dismutase upon Reduction of the Copper(II) Centers

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Copper/zinc superoxide dismutase (E.C.1.15.1.1, Cu_2Zn_2SOD hereafter) exhibits catalytic activity for the dismutation of superoxide ions.[1] The reaction is believed to take place in two steps, where the native copper(II) ion is reduced by a first O_2^- ion to give molecular oxygen and the reoxidized by a second O_2^- ion to give hydrogen peroxide.[2–4] In the resting state copper(II) is bridged to zinc(II) by an imidazolato group from His-61. Two more histidines and an aspartate residue complete the coordination sphere of the zinc ion.[5]

Several experiments have suggested that the imidazolato bridge may be broken upon reduction of copper. For example, (i) the redox properties of copper are indicative of a proton being taken up by a protein residue upon reduction,[6] (ii) the electronic spectra of the cobalt(II) chromophore in the $Cu^I_2Co_2SOD$ derivative are very similar to those of E_2Co_2SOD, where E stands for empty copper site,[7] (iii) ^{113}Cd NMR spectra show again very similar coordination environments for Cd^{2+} in the $Cu^I_2Cd_2SOD$ and E_2Cd_2SOD derivatives,[8] and (iv) from a recent EXAFS study copper(I) has been proposed to be three-coordinated.[9] All of these data are consistent with the breaking of the copper–imidazolate bond with subsequent protonation of the latter group, which would remain coordinated to zinc(II); however, none of them constitutes a direct proof for such a picture.

We have recently shown in a ^{1}H NMR study that well-resolved isotropically shifted signals from the ring protons of the coordinated histidines can be obtained on both E_2Co_2SOD and $Cu^{II}_2Co_2$ derivatives.[10,11] The former species shows spectra typical of high-spin cobalt(II)-containing proteins; in the latter derivative magnetic coupling between the two paramagnetic centers allowed us to observe the proton signals from the histidines coordinated to both the cobalt(II) and copper(II) chromophores. In particular, the NH protons from the coordinated imidazoles could be easily assigned through deuteration of the samples. Therefore, it seemed to us possible to obtain unambiguous information through ^{1}H NMR on the number of histidine residues coordinated to cobalt(II) in the reduced $Cu^I_2Co_2SOD$ derivative.

$Cu^{II}_2Co_2SOD$ solutions at pH 5.5 were prepared according to established procedures[12,13] from native enzyme of commercial source (Diagnostic Data Inc., Mountain View, CA). The electronic and CD spectra of the above derivative were the same as those previously reported.[10,13] The ^{1}H NMR spectra, obtained on a Bruker CXP 300 instrument, were also found identical with those previously obtained by us.[10] The fraction of cobalt(II) ions

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