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$_2$Zn$_2$SOD 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$^I_2$Co$_2$SOD derivative are very similar to those of E$_2$Co$_2$SOD, 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$^I_2$Cd$_2$SOD and E$_2$Cd$_2$SOD 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$_2$Co$_2$SOD and Cu$^{II}_2$Co$_2$ 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$^I_2$Co$_2$SOD derivative.

Cu$^{II}_2$Co$_2$SOD 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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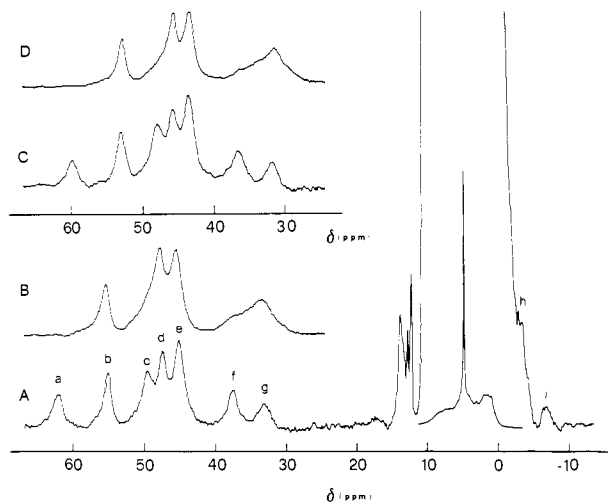(13) Fee, J. A. *J. Biol. Chem.* **1973**, *248*, 4229–4234.

Figure 1. ^{1}H NMR spectra, 300 MHz, of $Cu^I_2Co_2SOD$ in H_2O (spectra A at 30 °C and C at 40 °C) and D_2O (spectra B at 30 °C and D at 40 °C) solutions at pH (or pH*) 5.5.

magnetically coupled to copper(II) could be estimated from the above spectra to be above 95%, since no signal from E_2Co_2SOD species could be detected. The $Cu^I_2Co_2SOD$ derivative was prepared by addition of solid sodium dithionite under helium atmosphere until the copper(II) d–d band disappeared.

The 300-MHz ^{1}H NMR spectrum of $Cu^I_2Co_2SOD$ in water solution at pH 5.5 and 30 °C is reported in Figure 1 (spectrum A); the downfield region of the spectrum at 40 °C is also shown (spectrum C). The spectra show at least nine isotropically shifted signals (labeled a–i) which can be straightforwardly assigned to residues coordinated to the cobalt(II) chromophore. With the likely exception of the upfield signals h and i the resonances are in positions typical of ring protons of coordinated histidines.[14–17] In the native enzyme the zinc ion is coordinated to N1 of His-69, of His-78, and of the bridging histidinato 61 ion. If the latter residue is still coordinated to cobalt(II) but not to copper(I) it should bear an exchangeable proton on the N3 nitrogen analogously to the other two coordinated histidines. Therefore three signals are expected to disappear upon deuteration of the sample.

$Cu^I_2Co_2SOD$ samples that are 95% deuterated give rise to spectra B and D at 30 and 40 °C, respectively. Indeed, it appears that signals a, c, and f are lost almost completely upon deuteration, while the rest of the spectrum (including signals i and h, not shown) is the same, although the signals are somewhat broader. This is to us a definite proof that the imidazolato bridge is broken on the copper(I) side, leaving His-61 regularly coordinated to the cobalt(II) ion. Owing to the close similarity of Cu_2Co_2SOD to Cu_2Zn_2SOD even with respect to the catalytic properties,[4] this conclusion can be confidently extended to the native enzyme, thus settling an important issue in the overall knowledge of the system.

As a final comment, we can assign signals b, d, and e, which are of line widths comparable to or smaller than the NH signals, to the metalike protons of the three histidines coordinated to cobalt(II). Signal g is definitely broader and can possibly be assigned to one of the ortholike protons, whereas the other two may be below the c,d,e group or broad beyond detection. Finally, in analogy with other cobalt(II)-substituted proteins containing histidines coordinated through N1, signals h and i can be assigned to two of the β-methylene protons.

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Early Transition-Metal Carbene Complexes $L_2M=CH_2$: Equilibrium Structures and Rotational Barriers
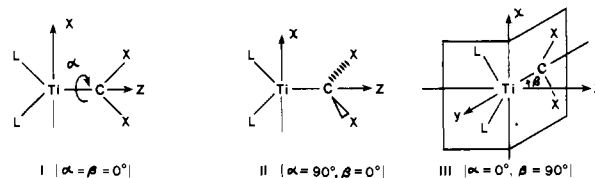
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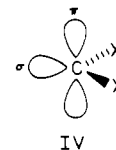*Morgantown, West Virginia 26506-6045*
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In the course of an ongoing experimental and theoretical investigation, which has the synthesis and characterization of hybrid Fischer[1]–Schrock[2] complexes as its ultimate goal, we have obtained theoretical results which predict that some early transition-metal carbene complexes have novel structures. In particular, we have been investigating the structures of a number of d^2 complexes of the type $L_2TiC(X)H$ and L_2TiCX_2, where L = H, Cl, and η^5-cyclopentadienyl (Cp) and X = H, OH, and SH, with a view to understanding the nucleophilicity of an alkylidene carbon coordinated to an electron-poor metal atom[2] and how it might be adjusted for synthetic advantage.[3,4]

Theoretical studies of some of these complexes have been reported,[5] but to date only the familiar "planar", I, and

"perpendicular", II, conformers have been investigated. Limiting geometrical studies to these conformers has the effect of constraining the carbene carbon atom to lie in the plane of the L_2Ti fragment. However, the directional properties of the metal d orbitals are such that a geometry III, with the planes of the metal and carbene fragments vertical and intersecting at 90° along the x axis, affords the opportunity for the formation of σ- and π-bonds that are as strong, in a first approximation, as those formed in the "planar" geometry, I.

In geometry I, the σ-bond is formed from the metal d_{z^2} orbital and the σ frontier orbital of the carbene fragment (IV), which

IV

is composed mainly of the C_{2p_z} orbital. The π-bond is formed from the metal d_{yz} orbital and the π ($2p_y$) frontier orbital of the carbene fragment. The metal $d_{x^2-y^2}$ orbital is left unused as a low-lying nonbonding orbital. In geometry III, the metal $d_{x^2-y^2}$ and d_{z^2}

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