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(10) The solution when frozen under CO is distinctly red in color; removal of CO and addition of acenaphthalenide results in the green color characteristic of $[Fe_4S_4(SPh)_4]^{3-}$. Since the paramagnetic species observed by EPR is only a small fraction of the total iron–sulfur cluster content, it seems likely that the species responsible for the red color are diamagnetic CO adducts, probably containing more than one CO per tetramer.
(11) If, as is suggested by data relating the rate of hydrogen evolution to the solution redox potential, the H_2 evolving species is the tetraanion (L. E. Mortenson and J.-S. Chen, personal communication), the oxidation number of the clusters in our scheme should be decreased by one. The *paramagnetic* CO complexes reported by Erbes et al.[2] should, however, be as indicated.
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Cadmium-113 Nuclear Magnetic Resonance Studies of Metalloproteins. 1. [^{113}Cd]Concanavalin A: A Preliminary Investigation

Sir:

Recent developments in multinuclear Fourier transform (FT) NMR techniques[2-5] have given researchers the capability of using a variety of NMR nuclei as probes to investigate chemical and biological systems. One application of interest is the use of metal nuclides as probes of metal-protein interactions in metalloproteins and enzymes. The native metals found in these proteins have, in general, poor high resolution NMR characteristics but may in many cases be replaced by metals with more favorable properties. One substitute nuclide with excellent NMR properties is ^{113}Cd. Several studies have been published that have investigated the ^{113}Cd NMR of a variety of inorganic and organometallic models systems.[6-10] More recently, Armitage and co-workers[11,13] have investigated the ^{113}Cd NMR of Cd(II) substituted alkaline phosphatases, human and bovine carbonic anhydrase B. Two of these metalloproteins are similar in that each has basically the same symmetry around the metal site (four coordinate or tetrahedral) and, for each protein, three of the ligands binding the metal are nitrogen; bovine carbonic anhydrase may be pentacoordinate. Despite these similarities, the reported ^{113}Cd chemical shifts of these proteins are in a range of over 160 ppm. Sudmeier[12] has also investigated the ^{113}Cd NMR of Cd(II) human carbonic anhydrase B with findings that differ considerably from the former work. The difference in the chemical shifts reported is 80 ppm. The origin of this chemical shift difference is unclear at the present time. In any case, the sensitivity of ^{113}Cd NMR as a probe of metal environment has been clearly demonstrated.

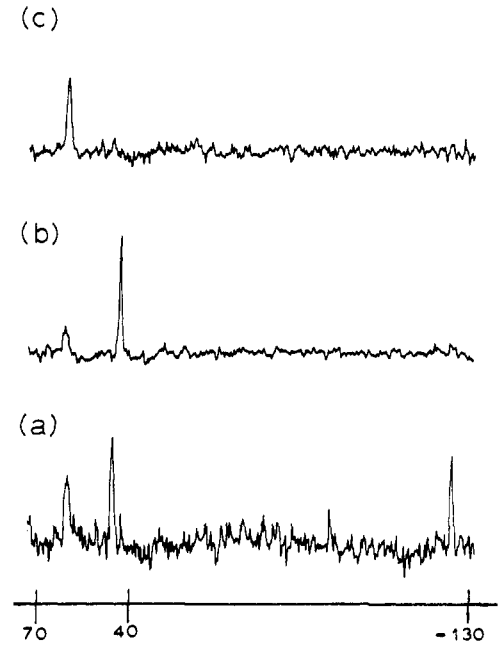

Figure 1. (a) ^{113}Cd NMR spectrum of concanavalin A to which 2 equiv of $^{113}CdCl_2$ per monomer have been added to the previously apoprotein. The same is 2.1 mM in Con A protomer. The buffer used in these experiments is 0.2 M NaCl, 50 mM NaAc, pH* 5.2, with D_2O added to provide an internal lock. The experimental conditions used to obtain all spectra shown follow: flip angle, 45°; recycle time, 0.6 s; spectral window, 10 000 Hz; number of data points collected, 8192. These spectra typically require 70 000 transients. The variations in signal-to-noise ratio within this figure denote differences in total accumulation times and the concentrations of the various samples. A 5-kHz enlargement of the spectra is displayed with 8 Hz of line broadening for sensitivity enhancement. Resonances occur at 68, 43, and −125 ppm. (b) ^{113}Cd NMR spectrum of Con A containing 2 equiv of ^{113}Cd(II) and an excess of Ca(II). The sample is 2.3 mM in Con A protomer. The resonance occurs at 41 ppm. (c) ^{113}Cd NMR spectrum of Con A containing 2 equiv of ^{113}Cd(II) and an excess of Zn(II). The sample is 1.6 mM in Con A protomer. The resonance is at 68 ppm.

We wish to report here preliminary results of a ^{113}Cd FT NMR study of the protein concanavalin A (Con A). This lectin has been of great interest owing to its ability to agglutinate transformed cells selectively in regard to the normal parent cells.[14] In addition, it specifically binds saccharide moieties[15] and induces blastogenesis in lymphocytes.[16] Several reviews have appeared concerning the structure and function of Con A.[17] Con A requires two metals per monomer for saccharide binding activity. In the native protein this consists of Mn(II) occupying a site denoted S1 and Ca(II) occupying a site denoted S2.[18,19] Both of these metals may be removed and replaced with Cd(II) with saccharide binding activity retained.[20] In addition, there are other metal binding site(s) reported for Con A, although none of these site(s) have been shown to bind Cd(II).[21] ^{113}Cd NMR should be able to resolve the number and type of Cd(II) binding sites. Also, any changes in the metal environment upon binding of saccharide to Cd(II) Con A should be reflected in the ^{113}Cd NMR.

Figure 1a presents a ^{113}Cd NMR spectrum of [^{113}Cd]Con

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Figure 2. ^{113}Cd NMR spectrum of Con A containing 2 equiv of $^{113}Cd(II)$ and 1 equiv of methyl α-D-mannopyranoside. The sample is 1.6 mM in Con A protomer. The resonances occur at 43 and −131 ppm.

A. In this experiment enough ^{113}Cd has been added to the apoprotein to occupy in excess of 90% of both the S1 and S2 sites. It is clear that cadmium is found in *three* different environments, with resonances occurring 68, 43, and −125 ppm from an external sample of 0.1 M $Cd(ClO_4)_2$.[22] Since in the native protein only two Cd(II) binding sites (S1 and S2) have been reported, further experimentation was done to assign these resonances.

Preliminary investigation of this system makes two points clear. First, the spectra obtained are independent of the amount of "nicked" subunit present in the Con A preparations.[23] Secondly, none of the resonances are due to "free" Cd(II); that is, Cd(II) not bound in some way to the protein. Figure 1b depicts the ^{113}Cd NMR spectrum of Cd(II) Con A in which the S2 sites are blocked by an excess of Ca(II), while Figure 1c is a similar spectrum where only the S1 sites are blocked, in this case by an excess of Zn(II). The resonance at 68 ppm is therefore assigned to the S2 binding site, while the resonance at 43 ppm corresponds to Cd(II) in the S1 binding site. The absence of the resonance at −125 ppm in both of the above experiments would indicate that the site associated with this resonance may either bind Cd(II), Ca(II), and Zn(II), or that this third binding site is not available in mixed metal proteins. Further experiments to be described elsewhere indicate that the latter explanation is correct.[25]

Further characterization of the resonance at −125 ppm was carried by performing a Pb(II) competition experiment. From x-ray data[21a] Pb(II) is known to bind Con A at sites distinct from the S1 and S2 sites. A preparation of Con A containing 2 equiv of Cd(II) and 1 equiv of Pb(II) per protometer yields a ^{113}Cd spectrum showing the loss of the resonance at −125 ppm indicating that either Pb(II) binds at this site or that the site is unavailable in the presence of Pb(II). Further experiments using ^{207}Pb NMR are currently in progress to confirm this point.

Finally, the ^{113}Cd NMR spectrum of Cd(II) Con A in the presence of methyl α-D-mannopyranoside was obtained. This saccharide specifically binds to Con A.[24] Two changes are noted in this spectrum, shown in Figure 2, when compared with Figure 1a: the resonance at 68 ppm has disappeared, while the resonance at −125 ppm has shifted to higher shielding by 8 ppm. To confirm that the resonance at 43 ppm remains that of the S1 site, the experiment was repeated using a Ca(II)-2Cd(II) Con A preparation. These experiments confirmed the identity of the resonance at 43 ppm is due to Cd(II) in the S1 site. It is obvious that the Cd(II) in the S2 site has been labilized by the presence of saccharide. These observations suggest that similar processes would occur in the native protein. Saccharide binding thus appears to involve a conformation change in the S2 binding site with a resulting destabilization of the metal binding in the S2 site. Recent x-ray studies by Edelman and co-workers[27] confirms that such conformational changes occur within the metal binding region of Con A when a monosaccharide binds. A more detailed analysis of this data and other experiments on Con A will be presented in a later publication.[25]

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