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Thioether Coordination in Metalloproteins. A Carbon-13 Nuclear Magnetic Resonance Study of Hg(II)-coordinated Model Complexes

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The coordination of thioether sulfur to intrinsic metals in proteins and enzymes is quite rare. There are two known *naturally* occurring systems where methionine has been demonstrated to be a metal ligand. These are cytochrome *c*, an iron protein [1], and plastocyanin, a copper protein [2], both being involved in electron transport functions. In addition, carboxymethylation of a cysteinyl zinc-ligand in liver alcohol dehydrogenase (LADH) converts the sulfur from a thiolate to a thioether, apparently *without* disruption of the coordination [3]. This observation is somewhat surprising, since the introduced carboxylate is potentially a better ligand than the thioether sulfur in neutral solution, even in the favorable case of coordination to Hg(II) [4].

The observation of methionyl and carboxyl carbon resonances in proteins by ^{13}C NMR has been made particularly simple by the availability of techniques for isotopic enrichment. For example Jones, and coworkers have developed elegant routes to enriching methionyl methyl carbons of proteins [5, 6] that have been utilized in studies on cytochrome *c* [7] and other systems. Similarly, 90%-enriched bromoacetate has been used to carboxymethylate methionine-80, the iron ligand, in cytochrome *c* [8], and to probe the metal environment of carbonic anhydrase [9, 10], a zinc metalloenzyme. Knowledge of the diamagnetic and paramagnetic shifts of such carbons greatly assists in the understanding of their proximity to the functional metals. We wish to report here relevant chemical shifts of Hg(II)-coordinated thioether complexes that serve as potential models for ^{13}C NMR shifts expected in metalloproteins.

Experimental

Materials

S-Carboxymethyl-L-cysteine was obtained from Sigma. S-Methyl-L-cysteine, a Cyclo Chemical Corp. product, was a gift from Professor R. B. Martin. Horse alcohol dehydrogenase was purchased from Boehringer Mannheim and was carboxymethylated with 90%-enriched [1-^{13}C] bromoacetate (Koch Isotopes) according to procedures to be described elsewhere [11]. Reagent grade $Hg(NO_3)_2 \cdot H_2O$ was used.

NMR Measurements

^{13}C NMR spectra were obtained on a JEOL PFT-100/EC 100 Fourier transform spectrometer operating at a carbon resonance frequency of 25.15 MHz. Spectra were obtained on 0.5 *M* samples at 25 °C under full proton noise-decoupling. About 10–15% D_2O was added for locking purposes and 1–10 μl of dioxane (chemical shift = 67.40) were added for referencing.

NMR Titration of Thioethers with Hg(II)

Freshly dissolved ligands (0.5 *M*) in 2 *M* HNO_3 were titrated with 25 μl aliquots (0.1 equivalents) of freshly prepared $Hg(NO_3)_2$ made up in 2 *M* HNO_3. With S-CmCys, Hg ratios exceeding 0.5 led to precipitation. Carbon resonances were assigned from spectra with proton couplings.

Results and Discussion

The selective S-carboxymethylation of Cys-46 of LADH, a zinc ligand, leads to a new resonance at 180.6 ppm (Figure 1) when 90% enriched [1-^{13}C]-bromoacetate is used. The signal comes from the *single* carboxylate carbon covalently introduced into the active site of each sub-unit of this 80,000 dalton dimeric enzyme. The chemical shift, however, is 2.0–2.1 ppm *downfield* from its position in the denatured enzyme (Figure 1) or in model compounds such as S-carboxymethylglutathione [11]. The question thus arises as to whether this deshielding results from zinc coordination of the sulfur of Cys-46 after its con-

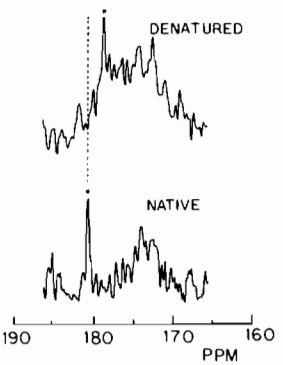

Figure 1. 25.15 MHz ^{13}C NMR spectra (carbonyl region) of liver alcohol dehydrogenase carboxymethylated at cysteine-46 with 90% enriched [1-^{13}C] bromoacetate. Native spectrum at pH 8.7 and denaturation was induced by raising the pH to 9.8 at 25 °C. The enriched carboxylate carbon is indicated by the asterisks.

version to a thioether, a structure deduced from crystallographic studies [3].

Model compounds precisely mimicking this proposed coordination in the carboxymethylated LADH appear to be lacking. This is most probably due to the presence of the carboxymethyl carboxylate which makes *exclusive* coordination at the thioether sulfur unlikely with metals such as Zn(II) [12–16] and Cd(II) [15]. Complexes with Co(III) may be potentially available [17–19], although ligand replacement of S by O still presents problems [17, 19]. We have consequently examined Hg(II) complexes with thioethers that are reported to coordinate exclusively to S under acidic conditions [20, 21]. Studies with the similar thiolate complexes show that the ^{13}C shifts induced by Hg are qualitatively and quantitatively similar to those induced by Zn and Cd [22].

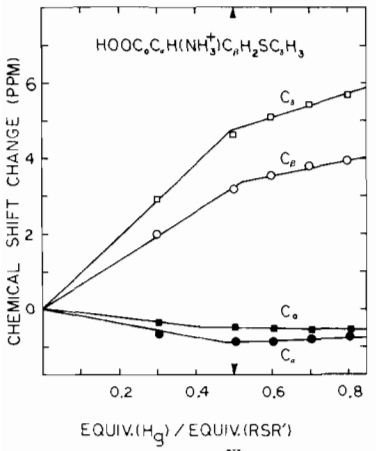

Figure 2. 25.15 MHz ^{13}C NMR shifts of the carbons of S-methyl-L-cysteine induced by complexation with the indicated equivalents of Hg(II) in 2 *M* HNO_3. Positive differences indicate increased deshielding relative to uncomplexed ligand in 2 *M* HNO_3.

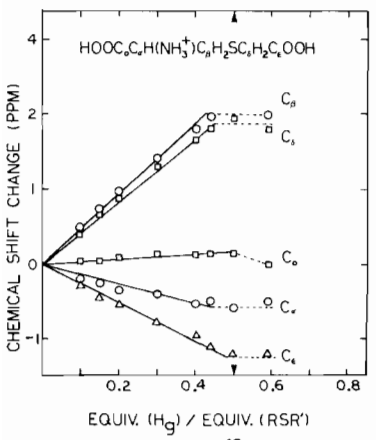

Figure 3. 25.15 MHz ^{13}C NMR shifts of the carbons of S-carboxymethyl-L-cysteine induced by complexation with the indicated equivalents of Hg(II) in 2 *M* HNO_3. Precipitation occurred above 0.5 equivalents, so that the last points (dashed region) are obtained with excess precipitated mercury. Positive differences indicate deshielding relative to uncomplexed ligand in 2 *M* HNO_3.

Figures 2 and 3 present ^{13}C NMR results on the complexation of S-methyl-L-cysteine (S-MeCys) and S-carboxymethyl-L-cysteine (S-CmCys) with $Hg(NO_3)_2$. The data clearly confirm the formation of Hg:L_2 complexes at a mercury ratio of 0.5, as expected [21]. The S-MeCys complex with mercury, and those of cysteine and methionine, have been previously studied by ^1H NMR, where all metal-induced shifts were deshielding [21]. In our study, the carbons adjacent to the sulfur (C_β and C_δ) show a parallel downfield shift, but the next-neighbor carbons (C_α and C_ϵ) undergo *upfield* shifts of somewhat smaller magnitude (0.5–1.1 ppm). Note that C_ϵ is structurally analogous to the enriched carboxyl of the modified LADH. The observed pattern of shifts is in accord with reported observations on complexation of methionine [20] although assignments were not firm in that study.

The *differences* between the Hg-induced shifts of our two model compounds deserve some comment. The amino acid carboxyl carbon (C_0) undergoes an upfield shift in S-MeCys but none in S-CmCys, where instead C_ϵ undergoes an upfield shift. These differences can be rationalized if we attribute the shielding effects to electrical fields arising from the positively charged metal [10, 23, 24]. The acidic medium (2 *M* HNO_3) and the observed stoichiometries rule out strong coordination of the (protonated) carboxyls with the metal but do not exclude weak interactions at the level of rotamer populations around the C_α–C_β bond. The small shielding of C_0 in the S-MeCys complex may reflect a favoring of the so-called [21, 25] *g* rotamer (carboxyl staggered with S) over the *t* rotamer (carboxyl *trans* to S). The latter rotamer does not allow a C_0OOH interaction with the metal. In the S-CmCys complex, a five-membered weak chelate interaction with the side-chain carboxyl ($C_\epsilon OOH$) substitutes instead, thus leading to an upfield shift for C_ϵ and none for C_0. The metal-induced ^{13}C NMR shifts may thus be very useful for conformational analysis.

In conclusion, the downfield chemical shift observed (Figure 1) in native carboxymethylated LADH cannot be attributed to the proposed [3] coordination of the thioether sulfur with the Zn, nor can it provide support for such a proposal. A more searching study of the enzyme is thus required, especially with regard to other interactions possible in the active site.

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