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Supplementary Material Available. The concentrations, measured heats, and calculated thermodynamic parameters will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20 × reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N. W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-6935.

Nuclear Magnetic Resonance Studies of the Solution Chemistry of Metal Complexes. IX. The Binding of Cadmium, Zinc, Lead, and Mercury by Glutathione[1]

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Abstract: The binding of cadmium, zinc, lead, and mercury ions by the tripeptide glutathione has been investigated by carbon-13 magnetic resonance spectroscopy. Binding to the potential coordination sites was monitored as a function of solution conditions by observing the chemical shifts of the carbon atoms of glutathione. The results indicate that each of these metal ions binds to the potential coordination sites of glutathione with a high degree of specificity, with the actual sites involved in metal binding being dependent on the metal ion and the solution pD, with the exception of mercury which binds only to the sulfhydryl group at a mercury to glutathione ratio up to 0.5. At a metal to glutathione ratio of 0.5, Cd^{2+} and Zn^{2+} bind to both the sulfhydryl group and the amino group, the extent of binding to the two different sites being a function of pD, while Pb^{2+} binds only to the sulfhydryl group. Some binding of the glutamyl and glycyl carboxylic acid groups to cadmium, zinc, and lead was detected in certain pH regions. The chemical shift data for the carbonyl carbons of the two peptide linkages suggest zinc-promoted ionization of the peptide protons with subsequent binding of zinc to the ionized peptide nitrogen at pD greater than 10.5, while no evidence for this metal-promoted reaction was observed in the cadmium, lead, and mercury complexes. The results are discussed in terms of the possible structures of the complexes.

The binding of metal ions by peptides and proteins is of fundamental interest in view of the importance of metal ions in biological systems. Peptides and proteins are comprised of a number of functional groups, many of which are potential coordination sites as shown by studies on metal binding by simple amino acids and other model compounds.

Proton magnetic resonance (pmr) spectroscopy has proven useful for elucidating, at the molecular level, the binding of selected metal ions by simple peptides, particularly polyglycine peptides. The functional groups involved in coordination to diamagnetic cadmium, zinc, lead,[2] and nickel[3,4] have been established from changes in the chemical shifts of carbon-bonded protons close to the binding site, while those involved in coordination to paramagnetic copper and nickel[4,5] have been identified from the dependence of the pmr line widths on their proximity to the binding sites. The application of pmr is limited, however, to relatively simple peptides because of the need for distinct, well-resolved resonances for monitoring interactions at the potential binding sites. The pmr spectra of peptides comprised of more than three or four amino acid residues are characterized by overlapping resonances which are of little use in metal binding studies, particularly if proton-proton coupling is present as, for example, in the pmr spectra of cysteinyl, glutamyl, and lysyl residues.

Carbon-13 magnetic resonance (cmr) spectroscopy should be more useful than pmr for elucidating the binding of metal ions by peptides and proteins; single lines are obtained for nonequivalent carbons in proton-decoupled cmr spectra and the range of chemical shifts is at least an order of magnitude greater. Thus, by cmr it may be possible to elucidate metal binding by larger peptides and by peptides which contain amino acid residues difficult to study by pmr due to proton–proton coupling. In additon, carbon atoms are bonded directly to the potential binding sites making cmr potentially more sensitive as a probe for studying binding at the molecular level.

In the present paper, the results of a cmr study of the binding of cadmium, zinc, lead, and mercury by the tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine) are reported. This peptide, which is widely distributed in nature, was chosen to evaluate the potential of cmr for the elucidation of metal binding by peptides because there is a lack of agreement as to which of the six potential coordination sites are involved in binding to

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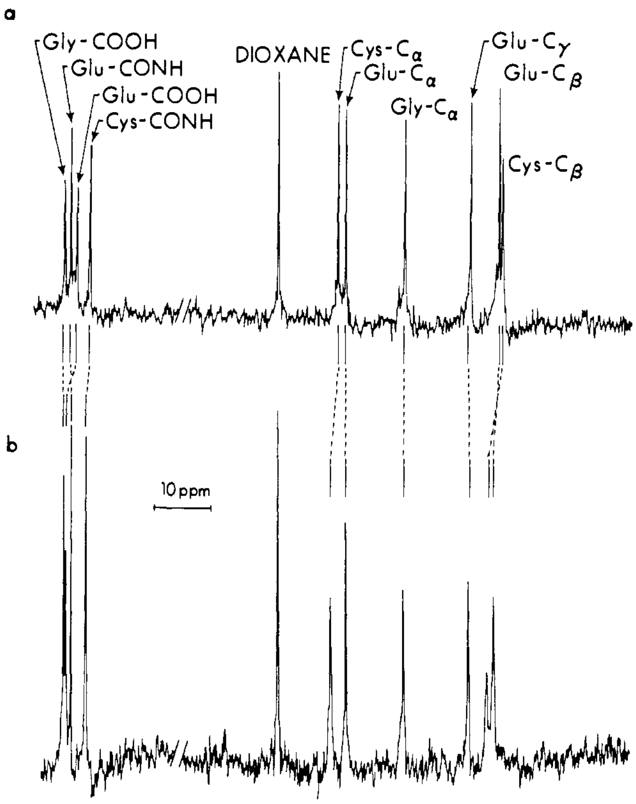

Figure 1. Carbon-13 magnetic resonance spectra of glutathione: (a) 0.30 *M* glutathione in D_2O at pD 7.37; (b) 0.30 *M* glutathione and 0.15 *M* $Zn(NO_3)_2$ in D_2O at pD 7.84.

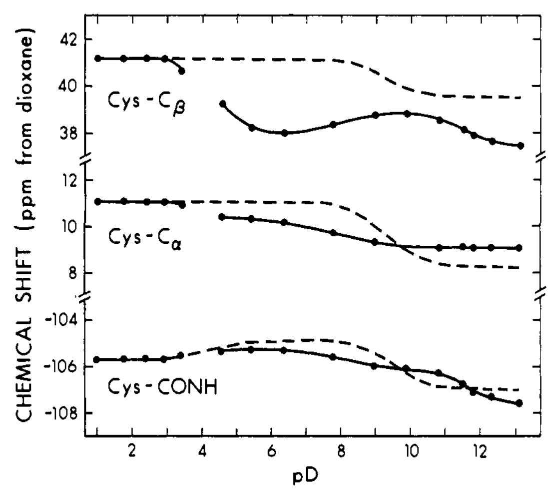

Figure 2. pD dependence of the chemical shifts of the cysteinyl carbons of glutathione in a D_2O solution containing 0.30 *M* glutathione and 0.15 *M* $Zn(NO_3)_2$. The dashed curves represent the chemical shift behavior of the same carbon atoms in a D_2O solution containing no complexing metal ion. See ref 16 for nomenclature used to identify the carbon atoms.

cadmium, zinc, and lead.[6-9] Previous studies have employed the pH titration method, which does not provide definitive information at the molecular level since the measurement is of a macroscopic property. Binding to Hg^{2+} has been investigated previously by the polarographic method,[10] and binding to CH_3Hg^+ has been investigated by pmr.[11] In addition, the acid–base chemistry of the four acidic groups of glutathione, which is necessary for quantitative studies of metal binding, has been characterized at the molecular level.[11,12]

Experimental Section

Reduced glutathione (Nutritional Biochemicals Corp. and Terochem Laboratories) was washed with a water-ethanol mixture and dried at 110° before use. Reagent grade metal nitrate salts were used as received. Solutions, usually 0.30 *M* in glutathione and 0.15 *M* in metal salt, were prepared in D_2O under an atmosphere of nitrogen to minimize oxidation of the sulfhydryl group. No attempt was made to control the ionic strength because of the high concentration used in the cmr experiment. The pD was adjusted with a 40% KOD solution and concentrated nitric acid. All pD measurements were made at 25° with an Orion Model 801 pH meter equipped with a standard glass electrode and a fiber-junction, saturated calomel reference electrode. Saturated potassium acid tartrate and 0.01 *M* sodium tetraborate solutions were used to standardize the pH meter. The meter readings were converted to pD values using the expression of Glascoe and Long.[13]

The ^{13}C spectra were obtained using a Bruker HFX-90 spectrometer operating at a frequency of 22.63 MHz and equipped with a Nicolet 1085 computer. The Fourier transform mode was used with proton decoupling. When D_2O was the solvent, the deuterium resonance from the D_2O was used for the heteronuclear lock signal. When H_2O was the solvent, the ^{19}F resonance from C_6F_6 in a coaxial capillary was used for the lock. 8K data points were collected in the computer for each free induction decay signal, and 4K accumulations were carried out to achieve an adequate signal-to-noise ratio. The frequency range of the transformed spectra was 5000 Hz. Chemical shift measurements were made with respect to dioxane which was added as an internal reference at a concentration of about 0.1 *M*. Chemical shifts are reported in ppm relative to dioxane, positive chemical shifts corresponding to greater shielding than in dioxane. For all measurements the temperature was 32 ± 2°.

Results

Spectrum a in Figure 1 is the cmr spectrum of glutathione (I) at pD 7.37. The assignments were made by comparison with the chemical shifts of the constituent

$$^{-}O_2CCHCH_2CH_2CONHCHCONHCH_2CO_2^{-}$$
$$NH_3^+ \qquad CH_2SH$$

I

amino acids and from the dependence of the chemical shift of each of the resonances on pH. The assignments are in agreement with those reported by Jung, *et al.*,[14,15] who have presented chemical shift *vs.* pH curves for each of the carbon atoms of glutathione.

Zinc–Glutathione. Addition of zinc to a solution of glutathione causes the chemical shifts of selected resonances to change, as illustrated by spectrum b in Figure 1, indicating the carbon-13 chemical shifts to be sensitive to metal binding. The chemical shifts of the three cysteinyl carbon resonances are shown as a function of pD in Figure 2 for a zinc to glutathione ratio of 1:2, while the chemical shifts of selected carbon

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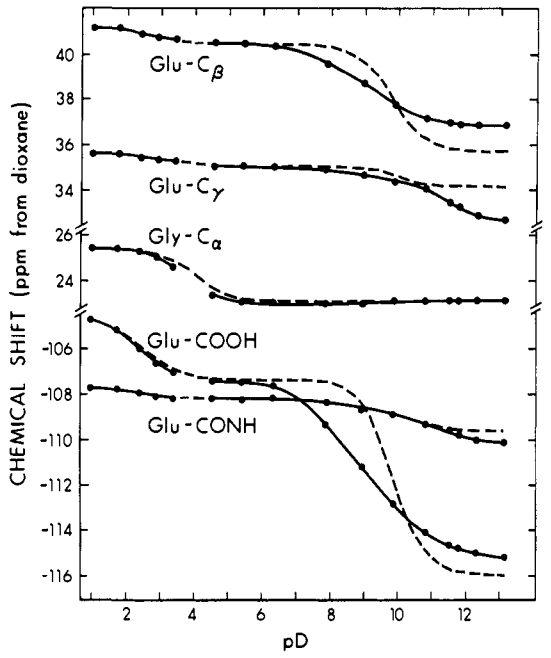

Figure 3. pD dependence of the chemical shifts of selected carbons of the glutamyl and glycyl residues in a D_2O solution containing 0.30 *M* glutathione and 0.15 *M* $Zn(NO_3)_2$. The dashed curves represent the chemical shift behavior of the same carbon atoms in a D_2O solution containing no complexing metal ion.

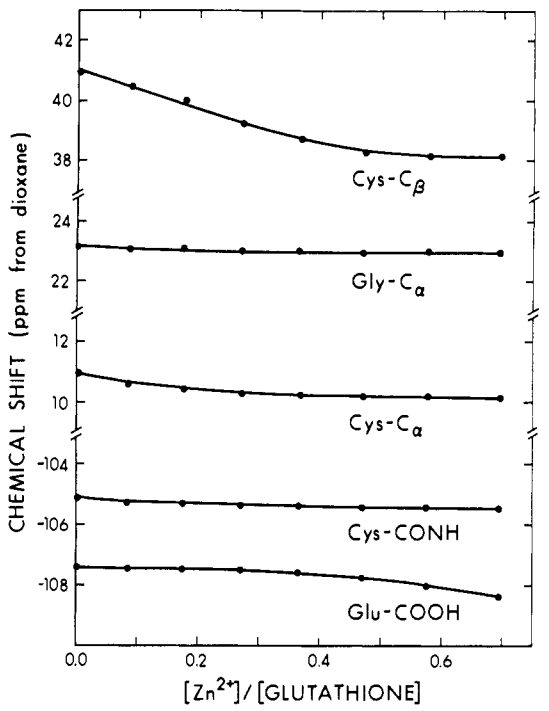

Figure 4. The chemical shifts of selected carbons as a function of the Zn^{2+} to glutathione ratio, pH 5.51. Precipitation occurred at ratios >0.7.

atoms of the glutamyl and glycyl residues are shown for the same conditions in Figure 3.[16] The dashed curves in Figures 2 and 3 show the chemical shift *vs.* pD behavior of these same carbon atoms in the absence of complexing metal ion.

The lack of any large dependence of the chemical shifts plotted in Figures 2 and 3 in the presence of zinc at pD <3.0 indicates no significant coordination in this pD range. A small amount of binding to the carboxylic acid groups, which previous studies[11] have shown to be partially ionized at pH >0, is indicated by the small differences observed for the Gly-C_α, Gly-COOH, Glu-C_α, and Glu-COOH chemical shifts. Some binding to the carboxylic acid groups is consistent with the known binding of zinc by the carboxylic acid groups of acetylglycine and the C-terminal end of polyglycine peptides.[2,17]

Between pD 3.0 and 13.2 the chemical shifts of the cysteinyl carbon resonances are different in the presence of zinc, indicating that zinc is bound to some extent to the sulfhydryl group and possibly to the peptide linkage between the cysteinyl and glycyl residues over this pD range. The chemical shift data for the Glu-CONH carbon indicate no binding to the peptide linkage between the glutamyl and cysteinyl residues at pD <10.5. The Glu-COOH resonance indicates a small amount of binding to the glutamyl residue up to pD 6, presumably involving only the carboxylic acid group, and an increase in the binding at pD >6. At pD >6, zinc is binding simultaneously to both the amino and carboxyl groups, by analogy with the coordination of zinc by glycine. The small downfield shift in the Gly-C_α and Gly-COOH resonances over the pD range 3 to 9 is consistent with a small amount of binding to the glycyl carboxylic acid group.

The change in the chemical shifts of the Cys-CONH, Cys-C_β, Glu-CONH, and Glu-C_γ carbons at pD >10.5 indicates a change in the nature of the binding to the peptide linkages in this pD range. If binding does occur to the peptide linkages at pD <10.5, it presumably is to the carbonyl oxygen of the neutral peptide linkages.[4,18-21] The chemical shift data suggest that, in the presence of zinc, ionization of the peptide protons can occur at pD >10.5 with subsequent binding of the zinc to the ionized nitrogen atom. Resonances for the free amino acids of which glutathione is composed were not observed, indicating hydrolysis had not occurred.

The data in Figures 2 and 3 indicate that in the pD range 3 to 6 binding is almost exclusively to the cysteinyl group. The specificity of the binding was investigated further by monitoring the chemical shifts as a function of the zinc to glutathione ratio at pH 5.51; the results are presented in Figure 4. The chemical shift of the Cys-C_β carbon, in particular, varies continuously (total shift of 2.7 ppm) as the ratio goes from 0 to 0.5 and then levels out at a constant value of 38.2 ppm, suggesting that a complex consisting of one Zn^{2+} ion and two glutathione molecules is the main species at pH 5.51, up to a ratio of 0.5. The very small chemical shift variation for the Glu-COOH carbon (total shift of 0.2 ppm up to a ratio of 0.5, compared with a shift of 8.4 ppm upon ionization of the glutamyl ammonium proton) suggests only a very small amount of binding to the glutamyl end. Above a ratio of 0.5, however, when presumably all the sulfur is complexed, the glutamyl

(16) The carbon atoms are identified by Glu, Cys, or Gly, to identify the amino acid residue in which the carbon is located, and C_α, C_β, C_γ, CONH, or COOH, to indicate the particular carbon of that residue.

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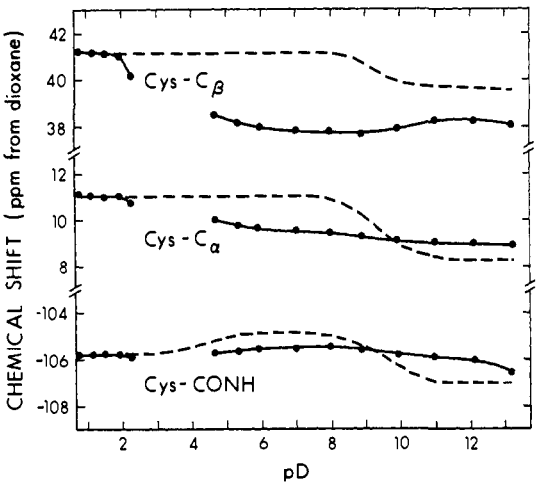


Figure 5. pD dependence of the chemical shifts of the cysteinyl carbons in a D_2O solution containing 0.30 *M* glutathione and 0.15 *M* $Cd(NO_3)_2$. The dashed curves represent the chemical shift behavior of the same carbon atoms in a D_2O solution containing no complexing metal ion.

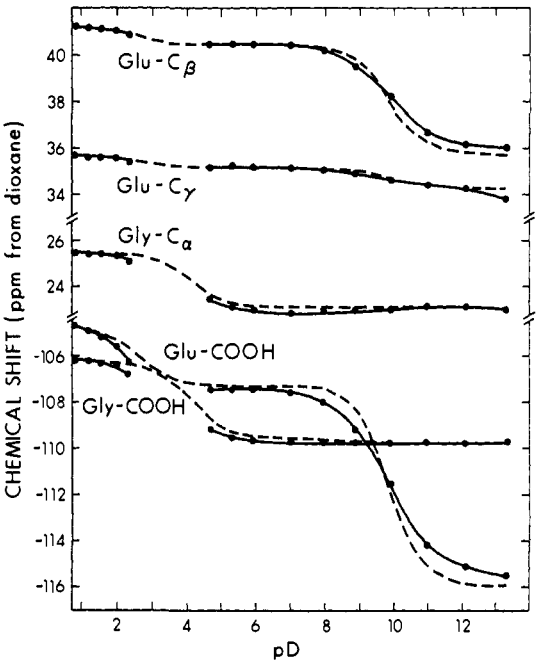


Figure 6. pD dependence of the chemical shifts of selected carbons of the glutamyl and glycyl residues in a D_2O solution containing 0.30 *M* glutathione and 0.15 *M* $Cd(NO_3)_2$. The dashed curves represent the chemical shift behavior of the same carbon atoms in a D_2O solution containing no complexing metal ion.

end becomes more important as a coordination site. The constant chemical shift of the Gly-C_α carbon indicates no observable glycyl carboxyl binding at this pH. Precipitation occurred at ratios greater than 0.7.

Cadmium–Glutathione. The chemical shifts of the three cysteinyl carbons are shown as a function of pD in Figure 5 for a cadmium to glutathione ratio of 1:2, while the chemical shifts of selected carbon atoms of the glycyl and glutamyl residues are shown for the same conditions in Figure 6. At pD <2, there is some binding to the two carboxylic acid groups but no detectable binding to the amino group, the sulfhydryl group, or the peptide linkages. Between pD 2 and 13.2, cadmium is bound to some extent to the sulfhydryl group and possibly to the peptide linkage between the cysteinyl and glycyl residues, as evidenced by the large differences between the solid and dashed curves for the cysteinyl carbon resonances in Figure 5. The chemical shift of the Glu-CONH carbon resonance is not changed by the presence of cadmium over the entire pD range studied in the present work, indicating no detectable binding to the peptide linkage joining the glutamyl and cysteinyl residues.

As in the zinc system, the chemical shift curves for the Glu-COOH carbon suggest a small amount of binding to the glutamyl end up to pD 6.5, presumably involving only the carboxylic acid group. At pD >6.5, the Glu-COOH, Glu-C_α, -C_β, and -C_γ resonances indicate an increase in binding to the glutamyl end, presumably involving both the amino and carboxyl groups. The small downfield shift observed for the Gly-C_α and Gly-COOH carbon resonances at pD <10 indicates weak binding to the glycyl carboxylic acid group.

In Figure 7, the chemical shifts of selected carbons are plotted *vs.* cadmium to glutathione ratio at a pH of 6.59. The sulfhydryl group is the principal coordination site up to a ratio of 0.5, as evidenced by the chemical shift of the Cys-C_β carbon. Coordination to the glutamyl end becomes important only when the ratio is greater than 0.5.

Lead–Glutathione. The chemical shifts of the three cysteinyl, the Gly-C_α, and the Glu-COOH carbons are

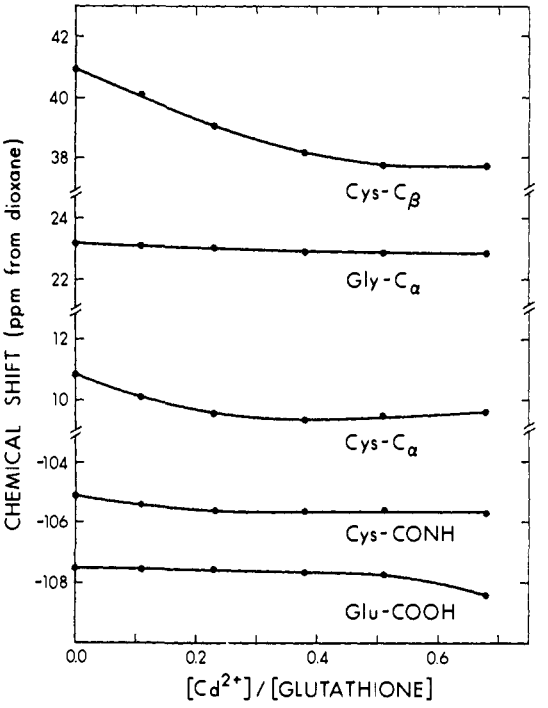


Figure 7. The chemical shifts of selected carbons as a function of the Cd^{2+} to glutathione ratio, pH 6.59. Precipitation occurred at ratios >0.7.

shown as a function of pD in Figure 8 for a lead to glutathione ratio of 1:2. Precipitation over the pD range 2.3 to 5.4 and at pD >12 limited the pD range over which binding could be studied. The chemical shift curves for the cysteinyl carbons indicate binding to the sulfhydryl group from pH 5.4 to 12.0. The only other binding site of any significance over this pD range is the glycyl carboxylic acid group, as evidenced

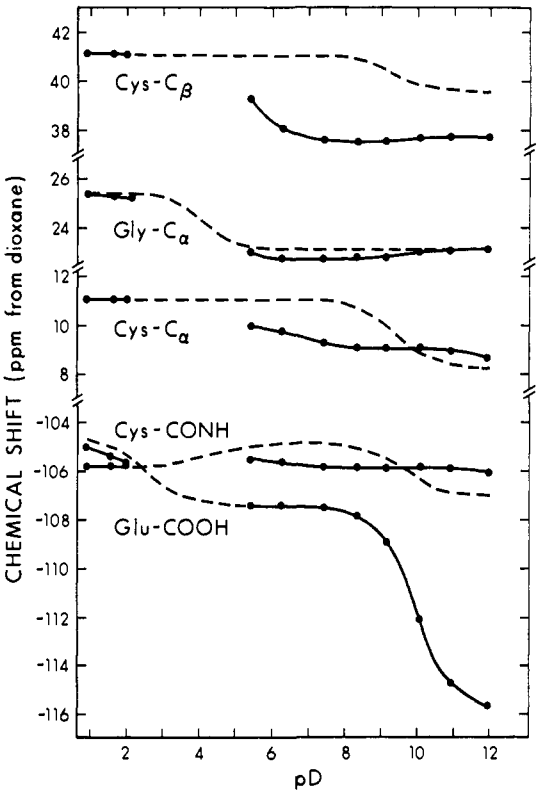


Figure 8. pD dependence of the chemical shifts of selected carbon atoms in a D_2O solution containing 0.30 M glutathione and 0.15 M $Pb(NO_3)_2$. The dashed curves represent the chemical shift behavior of the same carbon atoms in a D_2O solution containing no complexing metal ion.

by the Gly-C_α chemical shift. The extent of binding to the glycyl end decreases at pD >9, presumably due to displacement by hydroxide ions with formation of lead–hydroxy–glutathione mixed complexes. The chemical shift data for the Glu-COOH and Glu-C_α carbons indicate no detectable binding to the amino group. At pD <2, weak coordination occurs to the two carboxylic acid groups, as evidenced by the chemical shifts of the Gly-C_α and Glu-COOH carbons.

Mercury–Glutathione. The chemical shifts of the three cysteinyl carbons are shown as a function of pD in Figure 9 for a mercury to glutathione ratio of 1:2. The chemical shifts for all the other carbon atoms of glutathione were identical with those obtained for solutions containing no complexing metal ion. The nearly constant chemical shift values for the three cysteinyl carbons over the entire accessible pD range in Figure 9 and the results of experiments in which the mercury to glutathione ratio was varied from 0 to 0.5 at pH 5.01 indicate binding is exclusively to the sulfhydryl group at mercury to glutathione ratios up to 0.5. Precipitation prevented study at higher ratios.

Discussion

At a metal to glutathione ratio of 1:2 and a pD >3, all of the metal ions studied in the present work interact strongly with the sulfhydryl group. All the metal ions except Hg^{2+} coordinate rather weakly to the glycyl and glutamyl carboxyl groups, while only Zn^{2+} and Cd^{2+} bind to the amino group. Binding of Zn^{2+} to the carboxylic acid groups of glutathione could not be detected in pH titration experiments,[6-9] presumably be-

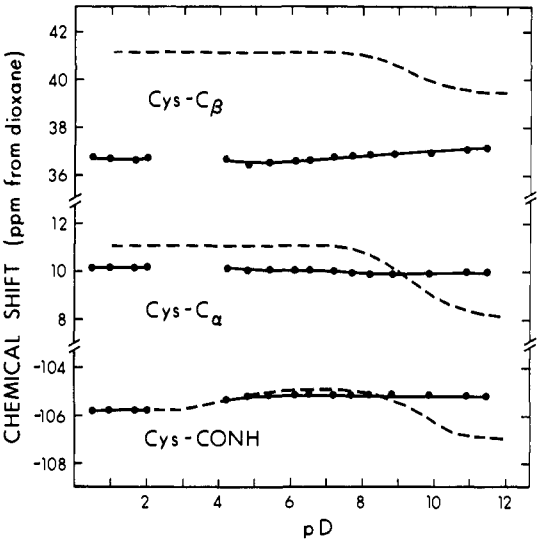
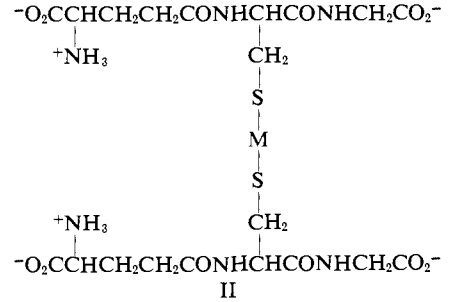


Figure 9. pD dependence of the chemical shifts of the cysteinyl carbon atoms in a D_2O solution containing 0.30 M glutathione and 0.15 M $Hg(NO_3)_2$. The dashed curves represent the chemical shift behavior of the same carbon atoms in a D_2O solution containing no complexing metal ion.

cause the formation constants are so small.[2,17] The extent of binding to the various sites is dependent on solution pD, except in the case of Hg^{2+} which binds strongly to the sulfhydryl group over the entire accessible pD range.

Because of the similarity in the binding of Cd^{2+} and Zn^{2+} by glutathione, the results for these systems will be discussed together. The strongest binding of these two metal ions occurs to the sulfhydryl group, as evidenced by the fact that the metal ions bind to this group at a much lower pD than to the glutamyl amino group, even though the pK_A of the sulfhydryl group is only 0.2 units less than the pK_A of the amino group.[11] The chemical shift of the Cys-C_β carbon indicates that, for a glutathione to metal ratio of two, all the sulfhydryl groups are complexed at pD 6 in the zinc system and at pD 7 in the cadmium system. The results of the experiments in which the metal to glutathione ratio was varied at pH 5.51 for the zinc system and at pH 6.59 for the cadmium system also indicate that binding is almost exclusively to the sulfhydryl group at these pH values up to a ratio of 0.5. Thus, a 1:2 complex is the major species in solution for these conditions, with the sulfhydryl group as the coordination site (structure II).



II

This is analogous to the complex $M(HL)_2^{2-}$, where M is Cd^{2+} or Zn^{2+} and HL^{2-} represents monoprotonated glutathione, proposed by Perrin and Watt.[9] These workers were unable to assign the binding sites due to uncertainty in the pK_A values of the amino and thiol

Table I. Fraction of Glutamyl Groups of Glutathione Complexed by Zn^{2+} and Cd^{2+} as a Function of pD[a]

pD	Zn^{2+}	Cd^{2+}
6.0	0.03	0.03
7.0	0.14	0.03
8.0	0.33	0.11
8.5	0.44	0.14
9.0	0.56	0.29
10.0	0.60	0.34
10.5	0.52	0.27

[a] Metal to glutathione ratio of 1:2.

groups. The amino group is still protonated, as evidenced by the chemical shift curves for the Glu-C_α, -C_β, and Glu-COOH carbons. Li and coworkers[6,7] interpreted pH titration data for the binding of Cd^{2+}, Zn^{2+}, and Pb^{2+} by glutathione in terms of complexes in which the two carboxyl groups, the amino group, and the sulfhydryl group are all ionized; the chemical shift data show this not to be the case. Rather, several different complexes form and, in some, those functional groups which are not coordinated are protonated.

The Cys-CONH carbon indicates that, in the above Cd^{2+} and Zn^{2+} complexes, some binding might also occur to the glycyl peptide linkage. Of the two sites in the peptide linkage, protonated nitrogen and carbonyl oxygen, the oxygen is the more basic[18-21] and hence a more probable binding site. Simultaneous coordination to the peptide oxygen would result in a six-membered chelate ring.

The chemical shifts of the Glu-COOH and Glu-C_β carbons indicate little binding to the glutamyl amino group below pD 6 for zinc and pD 7 for cadmium. At higher pD, the glutamyl end is bound, the extent of binding being pD dependent, as illustrated by the results in Table I. The fractions in Table I were calculated by methods described previously,[2,17] using as the chemical shifts for the Glu-COOH and Glu-C_β carbons when the glutamyl end is complexed, the chemical shifts at the point where the solid and dashed curves cross in Figures 4 and 6.[17]

It is unlikely that the sulfhydryl and glutamyl groups of a glutathione molecule are simultaneously coordinated to the same metal ion; this would involve an unstable ten-membered ring[22] since the glutamyl peptide linkage is not bonded to Cd^{2+} over the pD range accessible in this study nor to Zn^{2+} at pD <10.5. Thus the chemical shift data are not consistent with the structure proposed by Perrin and Watt[9] for the ML_2^{4-} complexes, where M is Cd^{2+} and Zn^{2+} and L^{3-} represents fully ionized glutathione. These authors proposed that in ML_2^{4-} one glutathione ligand is coordinated simultaneously at the glutamyl amino and carboxyl groups, the glutamyl peptide linkage, and the sulfhydryl group while the other glutathione is coordinated only at the glutamyl amino and carboxyl groups. More likely structures are ones in which the cysteinyl or glutamyl group of one glutathione molecule and the cysteinyl or glutamyl group of another are bound to the same metal ion, as considered by Martin and Edsall[8] in the analysis of pH titration data, or complexes in which the cysteinyl and glutamyl ends of a glutathione molecule are bound to different metal ions resulting in polynuclear complexes.[2,4] Molecular models indicate that polynuclear complexes in which a metal is bonded to the sulfhydryl groups of two glutathione ligands and the glutamyl amino and carboxyl dentates of two other sulfhydryl-complexed glutathione molecules are possible. To elucidate the structures of these complexes, experiments are in progress on the formation of mixed complexes from II and bidentate ligands, for example, glycine.

It is known that, in certain metal ion peptide complexes, the metal ion promotes the ionization of peptide protons with subsequent binding to the negatively charged nitrogen atom.[23] The chemical shift data at pD >10.5 suggest that Zn^{2+} might similiarly promote ionization of the peptide protons of glutathione. Chemical shift data for the other three metal ions studied provided no evidence for peptide proton ionization. Perrin and Watt[9] suggested that ionization of the peptide protons can occur on complex formation with both Cd^{2+} and Zn^{2+} and that the metal–peptide bond interaction is slightly greater in the zinc system. However, the pK_A of 9.86, which they interpreted as corresponding to ionization of a peptide proton from a coordinated peptide group in the ZnL_2^{4-} complex, is approximately two orders of magnitude too low to be consistent with the chemical shift data for the Glu-CONH carbon.

The binding of Hg^{2+} and Pb^{2+} is somewhat different from the binding of Zn^{2+} and Cd^{2+}; neither Hg^{2+} nor Pb^{2+} binds to the glutamyl end at pD >3 for metal to glutathione molar ratios of 0.5. Hg^{2+} binds to sulfhydryl groups of the two glutathione molecules over the whole pD range accessible in this investigation, resulting in complexes of structure similar to II except that the state of protonation of the carboxyl and amino groups may be different than in II, depending on the solution pH. This is in agreement with the results of Stricks and Kolthoff,[10] who found that Hg^{2+} is firmly bound to glutathione as a mercaptide and is similar to the binding of CH_3Hg^+ to the sulfhydryl group of glutathione.[11,24] Because of the high degree of specificity in the binding of Hg^{2+} to the sulfhydryl group and the large shift in the Cys-C_α and Cys-C_β resonances that results from such binding, it may be possible to identify the Cys-C_α and Cys-C_β resonances in the cmr spectra of proteins by observing changes in the spectrum as the protein is titrated with Hg^{2+} or CH_3Hg^+ at constant pH. The binding of Pb^{2+} to the sulfhydryl group is not as strong, as evidenced by the decrease in the extent of binding at pD <7.0.

The differences between the chemical shifts of the three cysteinyl carbon resonances in the sulfur-coordinated form and the uncomplexed form in which the sulfhydryl group is protonated are listed in Table II. The order of decreasing differences for the Cys-C_β carbon is $Hg^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+}$, the same as the order of affinity of the sulfhydryl group of bovine serum albumin for these metal ions.[25] The data in Table II indicate that the differences in the chemical shifts of the three cysteinyl carbons between the sulfur-protonated and sulfur-complexed forms decrease as the number of bonds separating the carbon and the sulfhydryl group increases.

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Table II. ^{13}C Chemical Shift Changes for Selected Carbon Resonances of Glutathione upon Complexation[a]

Carbon	Δ, ppm Zn^{2+}	Cd^{2+}	Pb^{2+}	Hg^{2+}
Cys-C_β	3.1	3.3	3.5	4.4
Cys-C_α	1.0	1.5	1.6	1.0
Cys-CONH	0.5	0.5	0.8	0.2
Glu-COOH	6.0	3.9	*b*	*b*
Glu-C_β	2.6	1.8	*b*	*b*
Gly-C_α	0.1	0.2	0.3	*c*

[a] The chemical shifts of the carbons in noncomplexed glutathione at pD 7.0 were used in the above calculations. The differences listed for the Gly-C_α carbon are the differences between the solid and dashed curves for this carbon in Figures 3, 6, and 8. [b] No coordination of Hg^{2+} or Pb^{2+} to the glutamyl end was detected. [c] No coordination of Hg^{2+} to the glycyl carboxylic acid group was detected.

The differences between the chemical shifts of the Glu-COOH and Glu-C_β carbon resonances of the form in which the glutamyl amino and carboxyl groups are simultaneously coordinated by Cd^{2+} and by Zn^{2+}, and the form in which the glutamyl carboxyl group is ionized and the amino group is protonated, are also listed in Table II. The difference is larger for Zn^{2+} than for Cd^{2+}, the same as the relative stabilities of the glutamic acid complexes of these metal ions.[26] Complexation affects the chemical shift of the Glu-COOH carbon more than the chemical shift of the Glu-C_β carbon, which in turn experiences a larger effect than the Glu-C_α carbon. The larger effect at the Glu-C_β carbon compared with the effect at the Glu-C_α carbon is similar to what is observed on protonation of the amino group of amino acids and peptides.[27]

Also listed in Table II are the differences between the solid and dashed curves for the Gly-C_α carbon at pD 7.0 in Figures 3, 6, and 8. The order of decreasing difference is $Pb^{2+} > Cd^{2+} > Zn^{2+}$, the same order as the relative affinities of these metal ions for acetylglycine and the C-terminal end of polyglycine peptides.[2,17]

Conclusions

Carbon-13 chemical shift data indicate that the metal ions Zn^{2+}, Cd^{2+}, Pb^{2+}, and Hg^{2+} bind to the potential coordination sites of glutathione with a high degree of specificity. All four metal ions bind to the sulfhydryl group while only Zn^{2+} and Cd^{2+} bind to the glutamyl amino group under the conditions used in this work. These results demonstrate the potential of carbon-13 magnetic resonance as a technique for elucidating the mode of binding of metal ions by biological molecules.

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Proton Nuclear Magnetic Resonance Line Widths and Spin Relaxation in Paramagnetic Metalloporphyrins of Chromium(III), Manganese(III), and Iron(III)

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Abstract: The proton nmr line widths of a series of metalloporphyrins of general structure PMX (P = tetra-*p*-tolylporphyrin, M = trivalent transition ion, X = halide or azide) have been investigated as a function of metal ion and apical ligand, X. The line widths depend strongly on M, decreasing in the order Cr > Mn > Fe. For fixed M, the sensitivity of the line width to X depends on the metal ion. In the case of Cr(III), the insensitivity of the line width to X is interpreted in terms of a dipolar relaxation mechanism where the correlation time is the tumbling time of the complex. The iron system exhibits dramatic variations in line width as a function of X which parallel the reverse trend in the zero-field splitting, ZFS, parameter *D*. This is consistent with a dipolar relaxation mechanism where the correlation time is the electron spin relaxation time, T_{1e}, which is in turn determined by the modulation of the ZFS by the motions of the complex. A semiquantitative relationship holds between the relative line width as a function of X and the relative value for D^{-2} for the related deuterohemins. The proton relaxation mechanism for the Mn(III) complexes is intermediate between that of the Cr(III) and Fe(III) systems, with the tumbling time in solution and T_{1e} making comparable contributions to the correlation time for dipolar relaxation. The relationship between narrow nmr lines and large ZFS is also demonstrated for the iron(III) complexes in the unusual intermediate $S = {}^3/_2$ spin state, the bis(diethyldithiocarbamato)iron halides. This investigation reveals that in cases where T_{1e} makes significant contributions to the correlation time for paramagnetic relaxation of nuclei, the nmr spectral resolution can be controlled, permitting high resolution studies in high-spin iron systems. In favorable cases, the nmr line width data may be used to determine the sign of the ZFS parameter, *D*.

The utility of nuclear magnetic resonance as a tool for investigating electronic structure and dynamic properties of paramagnetic molecules[2] has developed

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