chemical shift contribution that would oppose the normal Curie law term. Moreover, the realization of Curie law behavior for the coordinated O-*t*-Bu proton signals of (tpp)Fe(O-*t*-Bu) points to a role for the acetate carbonyl group in dictating anomalous temperature and solvent effects for the acetate NMR signal.

From the foregoing discussion, one might infer that the carboxylate group in (salen)Fe(OAc) is chelated, given the large value of the acetate methyl shift and the alternating signs of the crotonate proton shifts. Examples of chelated acetate complexes are limited in number (examples include $UO_2(OAc)_4$, $Zn(OAc)_2 \cdot 4H_2O$,[32] and (acetato)ruthenium(II) phosphine complexes[33]), because the small bite angle of the carboxyl group generally disfavors a chelated structure. However, the nitrate ligand in (tpp)Fe(NO_3)[34] and in (salen)Fe(NO_3)[22] has been shown crystallographically and by IR spectroscopy to be unsymmetrically chelated in the solid state, lending credence to the potential for such anions to chelate to iron(III) under appropriate circumstances. Several (salen)FeX complexes exhibit dimeric structures in the solid state, indicating a tendency for these iron centers to be six-coordinate. Upon dissolution, $[(salen)Fe(OAc)]_2$ presumably dissociates into monomeric units, and the tendency toward six-coordination may be satisfied by chelation of the carboxylate ligand.

Conclusions

Regardless of the mechanisms possible for Curie law deviations and solvent dependence, at the empirical level it is clear that protons at the α-positions with respect to an iron(III) bound carboxylate residue can exhibit chemical shift values ranging from at least the normal aromatic region to some 140 ppm downfield. Even the β-proton signals can be shifted as far as 16 ppm downfield. The hypothesis relating coordination mode with chemical shift value would place signals for α-position protons in the 100–140 ppm region for a bidentate carboxylate ion, whereas monodentate coordination would give rise to significantly smaller shifts. Thus, identification of amino acid carboxylate binding in metalloproteins by NMR chemical shift values alone is highly problematic,[35] and other approaches should be used to corroborate suspected carboxylate coordination. On the other hand, once carboxylate coordination and signal assignment are established, NMR spectroscopy would be of value for detection of subtle structural, hydrogen-bonding, and solvation effects at the metal–carboxylate site.

Acknowledgment. This work was supported by NIH Grant GM-28831 (H.M.G.) and by NSF Grant DMB-8314935 (L.Q.). Technical assistance by Gregory Godziela is also acknowledged.

Registry No. (tpp)Fe(OAc), 33393-26-9; (tpp)Fe(O_2CCH_2Br), 109365-20-0; (tpp)Fe(OPr), 109365-21-1; (tpp)Fe($O_2CCCl_2CH_3$), 109365-22-2; (etio)Fe(OAc), 109365-23-3; (salen)Fe(OAc), 41742-84-1; (salen)Fe(O_2CCH_2Cl), 109365-24-4; (salen)Fe(OCr), 109365-25-5; (saloph)Fe(O_2CCH_2Cl), 24844-47-1; (tpp)Mn(OAc), 58356-65-3; $[(HB(pz)_3Fe)_2O(OAc)_2]$, 86177-70-0; $[(HB(pz)_3Fe)_2OH(OAc)_2]^+$, 90886-30-9; $[(hxta)Fe_2(OAc)_2]^-$, 103322-76-5; $[(hxta)Fe_2(OPr)_2]^-$, 109365-18-6; $[(hxta)Fe_2(OCr)_2]^-$, 109365-19-7; $CHCl_3$, 67-66-3; CH_2Cl_2, 75-09-2; C_6H_6, 71-43-2.

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Nuclear Magnetic Resonance Studies of the Solution Chemistry of Metal Complexes. 24. Arylmercury(II) Complexes of Sulfhydryl-Containing Ligands

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Received August 28, 1986

Equilibrium constants were determined by ^1H NMR for the complexes of *p*-mercuribenzenesulfonate (PMBS) with hydroxide and chloride ions and with the thiols glutathione, cysteine, penicillamine, mercaptosuccinic acid, and mercaptoethanol in aqueous solution. The formation constants of the PMBS–thiol complexes are very similar to those of the analogous methylmercury(II) complexes, suggesting that the stability of organomercury(II)–thiol complexes in aqueous solution is not strongly dependent on the nature of the organic moiety. The formation constants do, however, depend strongly on the Brønsted basicity of the sulfhydryl donor group, with the formation constant increasing as the sulfhydryl pK_a increases.

Introduction

The mercury of alkyl- and arylmercury(II) cations shows a strong preference for coordination to only one additional donor atom, and both bind most strongly to deprotonated sulfhydryl groups.[1,2] The kinetics and equilibria for the binding of methylmercury(II) by a variety of sulfhydryl-containing biological molecules has been characterized in detail;[1,3-7] however, similar information is lacking for sulfhydryl binding of arylmercury(II) compounds. The finding by X-ray analysis that the C–Hg–S system of methylmercury(II) and phenylmercury(II) complexes of cysteine is linear and that the Hg–S bond lengths in the two complexes are not significantly different[2] might suggest that the stabilities of alkyl- and arylmercury(II)–thiol complexes are similar; however, the formation constants that would allow a quantitative comparison have not been reported for any aryl-

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mercury(II)–thiol complexes. Because of the importance of sulfhydryl complexation in the toxicology of arylmercury(II) compounds[2] and their use as selective probes of sulfhydryl groups in biochemistry,[8-11] it is of interest to quantitatively characterize the binding of arylmercury(II) by sulfhydryl groups.

In this paper, we present the results of ^{1}H NMR studies to determine the formation constants for complexation of *p*-mercuribenzenesulfonate (PMBS) by glutathione, cysteine, penicillamine, mercaptosuccinic acid, and mercaptoethanol. A sulfonic acid form of arylmercury was studied for water solubility. The sulfhydryl ligands were chosen because the formation constants of their CH_3Hg^{II} complexes have been determined previously, making possible a comparison between arylmercury(II) and alkylmercury(II) binding. Equilibrium constants were also determined for the complexes formed by reaction of PMBS with hydroxide and chloride ions.

Experimental Section

Chemicals. 2-Mercaptoethanol (Fisher), L-cysteine hydrochloride, D-penicillamine (Sigma Chemical Co.), glutathione (Aldrich Chemical Co.), and 2-mercaptosuccinic acid (Kodak Chemical Co.) were used as received.

Methylmercury chloride (Alfa Division, Ventron Corp.) was converted to a stock solution of CH_3HgOH by reaction with Ag_2O as described previously.[3] The solution was standardized by titration with sodium thiosulfate, with endpoint determination by ^{1}H NMR performed by monitoring the chemical shift of the CH_3Hg^{II} protons.[11,12] Stock solutions of *p*-(hydroxymercuri)benzenesulfonate ($^-O_3SPhHgOH$) were prepared from sodium *p*-(chloromercury)benzenesulfonate (Sigma Chemical Co.) by reaction with Ag_2O, under the same conditions as for the preparation of CH_3HgOH. The concentrations of the PMBS stock solutions were determined by titration with a standard solution of mercaptoethanol, with endpoint determination by monitoring the chemical shift of the high frequency doublet for the phenyl protons. The titration was performed at pH ~12.

Solutions of thiols were prepared in deionized and degassed water immediately prior to use. Thiol concentrations were determined by titration of a CH_3HgOH stock solution with a thiol solution at pH ~12. Endpoints were determined by monitoring the chemical shift of the CH_3Hg^{II} protons; the chemical shift changes linearly prior to the end point and remains constant thereafter.

The titrations to determine concentrations were performed directly in an NMR tube.[11,12] The procedure involved carefully weighing an aliquot of sample solution in an NMR tube, adding *tert*-butyl alcohol for a chemical shift reference, D_2O to make the solution 1% in D_2O, and 0.1 M KOH to give pH ~12, and then adding titrant in 10- or 20-μL increments to the NMR tube. The tube was weighed after each addition, and the ^{1}H NMR spectrum was measured. Concentrations were obtained from the weight of solution titrated, the weight of titrant added at the end point, and their densities.

Solutions used for the equilibrium constant measurements were prepared from the stock solutions and typically were 10–20 mM in the compound being observed by ^{1}H NMR. D_2O (1%) was added for a spectrometer lock signal and 0.3 M KNO_3 to give a constant ionic strength comparable to that used in the measurement of acid dissociation constants of the various ligands.[3]

pH Measurements were made at 25 ± 1 °C with a Fisher Accumet-520 pH meter, equipped with a microcombination electrode. Fisher certified buffers of pH 4.00, 7.00, and 10.00 were used for three point calibration of the pH meter.

NMR Measurements. ^{1}H NMR spectra were measured at 25 ± 2 °C by the standard single pulse method with a Varian XL-400 spectrometer equipped with a 15 bit A/D converter. A 5° flip angle was used to avoid overloading the A/D converter by the large signal from the water photons. Spectra with sufficient signal-to-noise for precise measurements of chemical shifts could be obtained by coaddition of 100 transients. Chemical shifts were measured relative to the methyl resonance of internal *tert*-butyl alcohol but are reported relative to the methyl resonance of sodium 4,4-dimethyl-4-sila-1-pentanesulfonic acid (DSS).

Results and Discussion

In aqueous solution, organomercury(II) cations react with hydroxide to form hydroxide complexes.[1,12-15] The formation constant of the PMBS–hydroxide complex, defined by eq 1 and 2, was determined from the pH dependence of the chemical shift

$$^-O_3SPhHg^+ + OH^- \rightleftarrows {}^-O_3SPhHgOH \quad (1)$$

$$K_1 = \frac{[^-O_3SPhHgOH]}{[^-O_3SPhHg^+][OH^-]} \quad (2)$$

of the high-frequency doublet for the phenyl protons of PMBS in solutions containing 0.0176 M PMBS and 0.3 M KNO_3. Exchange of PMBS between its aquated and hydroxide-complexed forms is fast on the NMR time scale, and the fractional concentrations of the two forms were determined from exchange-averaged chemical shifts as a function of pH by procedures described previously.[3,12] An average value of log $K_1 = 9.12 \pm 0.06$ was obtained from two pH titration experiments.

The formation constant of the PMBS–chloride complex (eq 3) was determined from the pH dependence of the chemical shift of the phenyl protons when *p*-(chloromercuri)benzenesulfonate

$$^-O_3SPhHgCl + OH^- \rightleftarrows {}^-O_3SPhHgOH + Cl^- \quad (3)$$

was titrated with base. Because Cl^- competes with OH^- for the PMBS, the change in chemical shift accompanying formation of the hydroxide complex is shifted to higher pH. A value of log $K_f = 4.62 \pm 0.15$ was obtained from the exchange-averaged chemical shifts.

For equimolar concentrations of PMBS and sulfhydryl ligand, the chemical shifts of the phenyl protons are constant and independent of pH from pH <1 to >12. Thus, although displacement of chloride by hydroxide ion could be used to shift the complexation equilibrium for the PMBS–chloride complex to a range where it could be measured by ^{1}H NMR, hydroxide ion does not cause a measurable shift in the PMBS–thiol complexation equilibrium, even at pH 12. The formation constant of the PMBS–mercaptoethanol complex, defined by eq 4 and 5, was determined

$$^-O_3SPhHg^+ + {}^-SCH_2CH_2OH \rightleftarrows {}^-O_3SPhHgSCH_2CH_2OH \quad (4)$$

$$K_f = \frac{[^-O_3SPhHgSCH_2CH_2OH]}{[^-O_3SPhHg^+][^-SCH_2CH_2OH]} \quad (5)$$

from the exchange-averaged chemical shift of the methyl protons of CH_3Hg^{II} as PMBS was titrated into a solution of $CH_3HgSCH_2CH_2OH$. The titration was done at pH 12 to eliminate the possibility of forming binuclear complexes, e.g. $(CH_3Hg)_2SCH_2CH_2OH$.[13] The added PMBS displaces $CH_3Hg(II)$ from its mercaptoethanol complex according to

$$^-O_3SPhHgOH + CH_3HgSCH_2CH_2OH \rightleftarrows {}^-O_3SPhHgSCH_2CH_2OH + CH_3HgOH \quad (6)$$

for which the displacement equilibrium constant is

$$K = \frac{[^-O_3SPhHgSCH_2CH_2OH][CH_3HgOH]}{[^-O_3SPhHgOH][CH_3HgSCH_2CH_2OH]} = \frac{K_{CH_3HgOH}K_f}{K_{CH_3HgSR}K_1} \quad (7)$$

where

$$K_{CH_3HgOH} = \frac{[CH_3HgOH]}{[CH_3Hg^+][OH^-]} \quad (8)$$

$$K_{CH_3HgSR} = \frac{[CH_3HgSCH_2CH_2OH]}{[CH_3Hg^+][^-SCH_2CH_2OH]} \quad (9)$$

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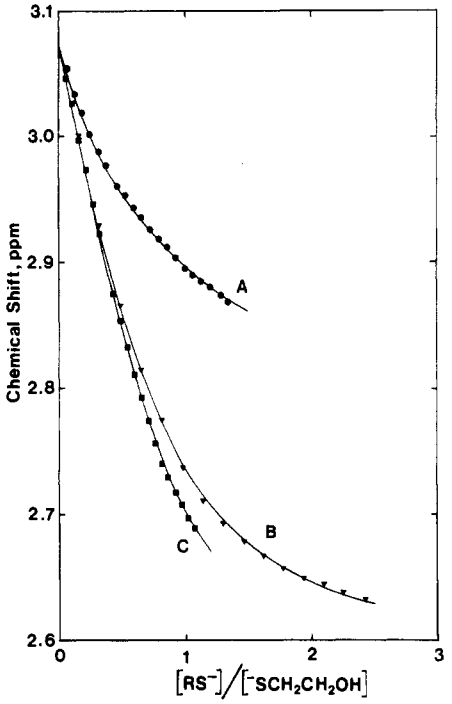

Figure 1. Chemical shift data for the exchange-averaged resonance for the methylene protons adjacent to the sulfur of mercaptoethanol vs. the added thiol (RS^-) to mercaptoethanol ratio as glutathione (A) and cysteine (B) were added to a 0.0282 M PMBS–mercaptoethanol solution and penicillamine (C) was added to a 0.0189 M PMBS–mercaptoethanol solution.

and K_1 is defined by eq 1. The displacement equilibrium constant was calculated directly from the chemical shift of the exchange-averaged resonance by procedures similar to those used previously for determining formation constants for CH_3Hg^{II}–thiol complexes.[3] An average value of $K = 2.55 \pm 0.28$ was obtained from 32 individual measurements. With this value and log $K_{CH_3HgOH} = 9.29$,[15] log $K_{CH_3HgSR} = 16.12$,[13] and log $K_1 = 9.12$, log K_f for the PMBS–mercaptoethanol complex is calculated to be 16.35 ± 0.10.

The formation constants of the PMBS complexes of glutathione, cysteine, penicillamine, and mercaptosuccinic acid were determined at pH 12 relative to that of the PMBS–mercaptoethanol complex by measuring the equilibrium constant for the displacement reaction

$$^-O_3SPhHgSCH_2CH_2OH + RS^- \rightleftarrows {}^-O_3SPhHgSR + {}^-SCH_2CH_2OH \quad (10)$$

where RS^- represents fully deprotonated glutathione, cysteine, penicillamine, and mercaptosuccinic acid. The fractions of free and complexed mercaptoethanol were determined from the chemical shift of the exchange-averaged resonance for the CH_2 protons next to the sulfur of mercaptoethanol, and then the concentrations of the various species in eq 10 were calculated from these fractions, the total concentrations, and mass balances.

The exchange-averaged chemical shift is shown as a function of the added thiol (RS–) to mercaptoethanol ratio in Figure 1 for glutathione, cysteine, and penicillamine. The data for mercaptosuccinic acid were essentially identical with those for penicillamine. As the ratio increases, the chemical shift of the exchange-averaged resonance moves toward that of free mercaptoethanol (2.570 ppm), indicating displacement of mercaptoethanol by thiol. Displacement equilibrium constants, which equal the ratio of the formation constants of the two thiol complexes in eq 10, of 0.368 ± 0.033, 4.18 ± 0.19, 8.21 ± 0.70, and 8.36 ± 0.62 were calculated for glutathione, cysteine, penicillamine, and mercaptosuccinic acid, respectively, from these data. The solid curves through the points in Figure 1 are theoretical curves calculated with these displacement constants. The formation constants given in Table I for the PMBS complexes of the fully deprotonated ligand were calculated from the displacement constants and the formation constant of the PMBS–mercaptoethanol complex.

Table I. Formation Constants for Organomercury(II)–Thiol Complexes[a]

ligand	log K_f $^-O_3SPhHg^{II}$ complex	log K_f CH_3Hg^{II} complex	pK_a of ligand SH
$HOCH_2CH_2S^-$	16.35 ± 0.10	16.12[b]	9.46[c]
$^-O_2CCHCH_2CH_2CONHCHCOHNCH_2CO_2^-$ (NH₂; CH₂–S⁻)	15.91 ± 0.12	16.00[d]	9.08[e]
$H_2NCHCO_2^-$ (CH₂–S⁻)	16.97 ± 0.11	16.67[f]	9.74[f]
$H_3N^+CHCO_2^-$ (CH₂–S⁻)	15.50 ± 0.13	15.38[f]	8.38[f]
$H_2NCHCO_2^-$ ($H_3C-C(S^-)-CH_3$)	17.26 ± 0.09	16.94[f]	9.70[f]
$H_3N^+CHCO_2^-$ ($H_3C-C(S^-)-CH_3$)	15.07 ± 0.12	14.79[f]	8.05[f]
$^-O_2CCHCH_2CO_2^-$ (S⁻)	17.28 ± 0.12	17.31[f]	10.26[f]

[a] 25 °C and 0.3 M KNO_3. [b] Reference 13. [c] This work. [d] Reference 6. [e] Rabenstein, D. L. *J. Am. Chem. Soc.* **1973**, *95*, 2797. [f] Reference 3.

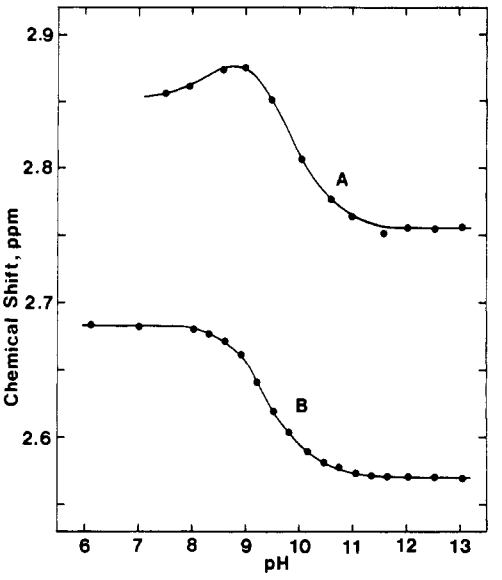

Figure 2. pH dependence of the chemical shift of the methylene protons adjacent to the sulfur of mercaptoethanol in solutions containing (A) 0.0204 M mercaptoethanol, cysteine, and PMBS and (B) 0.020 M mercaptoethanol. The solid curves through the points are theoretical curves calculated by using the appropriate formation and acid dissociation constants in Table I.

Formation constants for the amino-protonated PMBS–cysteine and PMBS–penicillamine complexes were determined from the pH dependence of the exchange-averaged mercaptoethanol resonances for solutions containing PMBS, mercaptoethanol, and cysteine or penicillamine in a 1:1:1 ratio. Representative data are shown in Figure 2 for the PMBS–mercaptoethanol–cysteine system, together with chemical shift data for mercaptoethanol. Over the entire pH range in Figure 2, the mercaptoethanol res-

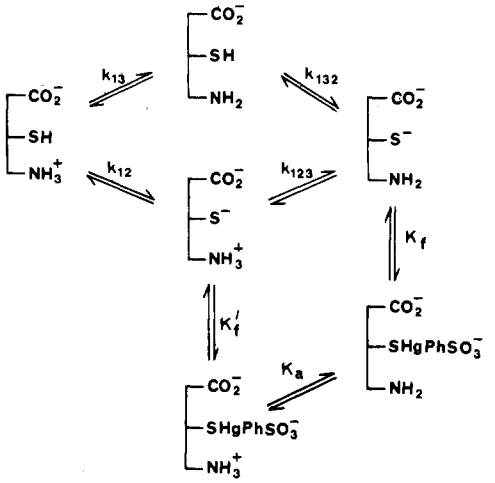

Figure 3. Microscopic protonation and complexation equilibria occurring in aqueous solutions of PMBS and cysteine or penicillamine.

onance for the PMBS–mercaptoethanol–cysteine system is between those of free (curve B in Figure 2) and complexed mercaptoethanol (3.072 ppm over this pH range), indicating some displacement of mercaptoethanol by cysteine. However, the extent of displacement is pH dependent due to competition of PMBS and hydrogen ions for the sulfhydryl donor groups. Because the pK_a's of the sulfhydryl groups of mercaptoethanol and cysteine are different (Table I), competitive protonation of the sulfhydryl groups causes the position of the displacement equilibrium to be pH dependent. Chemical shift vs. pH data for the PMBS–mercaptoethanol–cysteine and penicillamine systems were fitted to the microscopic protonation–complexation model shown in Figure 3 by methods used previously for determining formation constants of the analogous CH_3Hg^{II} complexes.[3] Literature values were used for the microscopic acid dissociation constants.[3,16,17] The results are listed in Table I. The solid curve through data set A in Figure 2 is the theoretical curve calculated with the formation constants listed in Table I for the PMBS–mercaptoethanol and PMBS–cysteine complexes and the acid dissociation constants for mercaptoethanol and cysteine.

Comparison of the formation constants for the PMBS–thiol complexes with those for the analogous CH_3Hg^{II} complexes (Table I) reveal that the formation constants of the arylmercury(II) complexes are remarkably similar to those of the alkylmercury(II) complexes, suggesting that the nature of the organic moiety of the organomercurial has little influence on the stability of its complexes with simple thiol molecules in aqueous solution. The only related information in the literature is the report that the Hg–S bond lengths in the methylmercury(II) and phenylmercury(II) complexes of cysteine are not significantly different.[2] Whether this lack of dependence of the formation constants on the nature of the organic moiety is general for other organometal(II) complexes is not known since the formation constants in Table I are the only ones that have been reported for the alkyl- and arylmetal(II) complexes of the same series of ligands in aqueous solution. The formation constants of both the PMBS–thiol and the CH_3Hg^{II}–thiol complexes increase as the Brønsted basicity of the coordinating site increases (Table I); this is a general characteristic of the complexation chemistry of CH_3Hg^{II}.[1]

The rate of exchange of mercaptoethanol and a second thiol ligand between their free and PMBS-complexed forms in solutions containing PMBS, mercaptoethanol, and a second thiol ligand is pH dependent. Above pH ~7, exchange is fast on the ^1H NMR time scale and sharp, exchange-averaged resonances are observed. Below pH ~7, however, resonances for both mercaptoethanol and the second thiol broaden, indicating a decrease in the rates of exchange between their free and complexed forms. It has been shown previously in studies of the ligand-exchange kinetics of CH_3Hg^{II}–thiol complexes that the deprotonated sulfhydryl group is the reactive species in the displacement of complexed ligand by free ligand.[18] The decreased rate of exchange in the PMBS–thiol systems at pH <7 is due to protonation of the sulfhydryl groups of the free ligands, with the fraction protonated increasing as the pH decreases.

Acknowledgment. This research was supported in part by the University of California, Riverside. Partial support of the NMR instrumentation by Grant BRSG 2 S07 RR07010-20 awarded by Biomedical Research Resources, National Institutes of Health, and the Standard Oil Co. (Ohio) is gratefully acknowledged.

Registry No. PMBS, 87101-05-1; OH^-, 14280-30-9; Cl^-, 16887-00-6; glutathione, 70-18-8; cysteine, 52-90-4; penicillamine, 52-67-5; mercaptosuccinic acid, 70-49-5; mercaptoethanol, 60-24-2.

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