and render the NMR signals difficult to detect.[5] This problem notwithstanding, ^{195}Pt NMR spectroscopy offers important advantages for the study of complex natural products. Since the ^{195}Pt chemical shifts span a range greater than that of any other nucleus, they are highly sensitive to the nature of the ligands and, as this and other studies showed, even to the subtle changes in the molecular environment. Labeling of different groups on a biomolecule, and even of similar groups differently located, should give rise to well-separated ^{195}Pt NMR signals. We are working to develop the $PtCl_3^-$ complex and its derivatives into NMR labels for the studies of structural and dynamic properties of proteins.

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Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada

Nuclear Magnetic Resonance Studies of the Solution Chemistry of Metal Complexes. 23. Complexation of Methylmercury by Selenohydryl-Containing Amino Acids and Related Molecules

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The acid–base chemistry and the methylmercury(II) (CH_3Hg^{II}) complexation chemistry of selenoacetic, selenopropionic, and selenobutyric acids, selenocysteine, selenocysteamine, selenopenicillamine, and 2-hydroxyethaneselenol have been studied in D_2O solution. Aqueous solutions of the selenohydryl ligands were prepared by reduction of diselenides at a mercury-pool cathode. Acid dissociation constants for the ligands were determined by pH titration, and those for the CH_3Hg^{II}–selenol complexes were determined from ^1H NMR chemical shift data. Equilibrium constants for displacement of mercaptoacetic acid from its CH_3Hg^{II} complex by the selenol ligands were obtained from ^1H NMR chemical shift data for the mercaptoacetic acid. Formation constants for the CH_3Hg^{II}–selenol complexes were calculated from the displacement constants and the formation constant of the CH_3Hg^{II}–mercaptoacetic acid complex. Formation constants for the CH_3Hg^{II}–selenol complexes are 0.1–1.2 log K units larger than those for the corresponding thiol complexes. Despite the large formation constants, exchange of CH_3Hg^{II} between selenol ligands, and between selenol and thiol ligands, is fast on the ^1H NMR time scale. The possibility of in vivo complexation of CH_3Hg^{II} by selenocysteinyl sites of glutathione peroxidase is discussed.

Introduction

Since the outbreak of Minimata disease in Japan in the 1950s, there has been widespread interest in the biological chemistry of methylmercury(II) (CH_3Hg^{II}). CH_3Hg^{II} has such a high affinity for sulfur that it is assumed to be all complexed by thiol groups of amino acids, peptides, and proteins in vivo.[1-3] Direct experimental evidence for thiol complexation has been obtained from ^1H NMR studies of CH_3Hg^{II}-containing intact human erythrocytes.[4] These ^1H NMR studies also show that, despite the large formation constants of the CH_3Hg^{II}–thiol complexes,[3,5-7] CH_3Hg^{II} exchanges rapidly among thiol ligands in intact cells.[4,7-9]

An intriguing aspect of CH_3Hg^{II} toxicology is that various selenium-containing compounds have significant protective effects in animal studies of CH_3Hg^{II} poisoning.[10,11] The molecular basis for this effect has not yet been established; however, it is possible that it involves complexation of CH_3Hg^{II} by selenium donor groups. The complexation of CH_3Hg^{II} by selenium is also of interest since selenium is present, as a selenolate anion, at the active site of glutathione peroxidase.[12] If CH_3Hg^{II}–selenolate complexes are more stable than CH_3Hg^{II}–thiolate complexes, and if exchange of CH_3Hg^{II} between thiolate and selenolate groups is sufficiently fast, a significant fraction of the enzyme would be CH_3Hg^{II}-complexed, which presumably would affect its ability to protect cells from peroxidative damage.[13]

Since selenium is softer in a Lewis base sense than sulfur, it would be expected to form more stable complexes with class B metal ions such as CH_3Hg^{II}.[14] In support of this, the formation constant of $CH_3HgSeCN$ is larger than that of CH_3HgSCN[15] and the ^{199}Hg–^1H coupling constant, which decreases as the formation constant increases,[3] is smaller for the CH_3Hg^{II} complexes of Se^{2-}, $^+NH_3CH_2CH_2Se^-$, CH_3Se^-, and $PhSe^-$ than for the complexes of the sulfur analogues.[15-17] Structural data also suggest that the Hg–Se binding in $CH_3HgSeCH_2CH(NH_3)CO_2 \cdot H_2O$ is stronger than the Hg–S binding in the analogous cysteine complex.[18] However, formation constants with which the relative strengths of CH_3Hg–Se and CH_3Hg–S binding can be quantitatively compared have not been reported.

In this paper, we present the results of ^1H NMR studies of the

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binding of CH_3Hg^{II} by selenocysteine, selenocysteamine, and selenopenicillamine, 2-hydroxyethaneselenol, and the homologues $HSe(CH_2)_nCO_2H$ (n = 1–3). The selenohydryl ligands were prepared in aqueous solution by cathodic reduction of the corresponding diselenides. Formation constants for the CH_3Hg^{II}–selenolate complexes were determined by allowing the various selenols to equilibrate with the CH_3Hg^{II} complex of mercaptoacetic acid (MAA) according to the reaction

$$CH_3HgSCH_2CO_2^- + RSe^- \overset{K_d}{\rightleftharpoons} CH_3HgSeR + {}^-SCH_2CO_2^- \quad (1)$$

Displacement equilibrium constants, K_d, were obtained from chemical shift data for the exchange-averaged ^1H resonance for MAA. Formation constants for the selenolate complexes were calculated from the displacement equilibrium constants and the formation constant of the CH_3Hg^{II}–MAA complex. These studies also provide information about the kinetics of the exchange of CH_3Hg^{II} between thiol and selenol groups.

Experimental Section

Chemicals. Mercaptoacetic acid and 3-mercaptopropionic acid (Aldrich Chemical Co.) were fractionally distilled under reduced N_2 pressure. 4-Mercaptobutyric acid was synthesized from γ-butyrolactone and thiourea[19] and was purified by fractional distillation under reduced N_2 pressure (bp 89–90 °C (0.05 mmHg)). Cysteamine hydrochloride (Aldrich Chemical Co.) was sublimed in vacuo to remove some disulfide. Solutions of thiols in D_2O were prepared immediately prior to use.

Methylmercury(II) iodide (Alfa Division, Ventron Corp.) was converted to a stock solution of methylmercury(II) deuteroxide by reaction with silver oxide in a manner described previously.[5] The solution was standardized with respect to CH_3Hg^{II} by titration with iodate-standardized thiosulfate in 50% MeOH at pH 4, with use of Michler's thioketone as the indicator.[20] The usual precautions were followed in the handling of methylmercury solutions.

The diselenides D,L-selenocystine and selenocystamine dihydrochloride (Sigma Chemical Co.) contained no impurities that could be detected by ^1H NMR and were used as received. 2,2′-Diselenodiacetic acid was prepared by acid hydrolysis of the selenocyanate (prepared from bromoacetic acid and potassium selenocyanate in acetone)[21] and recrystallized from benzene–ethyl acetate. 3,3′-Diselenodipropionic acid was prepared from 3-bromopropionic acid and aqueous sodium diselenide[22] and recrystallized from hot water. 4,4′-Diselenodibutyric acid and bis(2-hydroxyethyl)diselenide were prepared by hydrolysis of the selenosulfates, produced from the corresponding bromides and potassium selenosulfate in water. Bis(2-hydroxyethyl)diselenide was fractionally distilled under reduced pressure as an orange oil, and 4,4′-diselenodibutyric acid was reprecipitated several times from bicarbonate solution with dilute sulfuric acid.

The diselenide of selenopenicillamine was prepared by reaction of isopropylidene-2-methyl-5(4*H*)oxazolone and benzenemethaneselenol in pyridine.[23] The resultant Se-benzylselenopenicillamine was deprotected with Na in liquid ammonia and the ammoniacal residue dissolved in water and oxidized in situ by aeration overnight in the presence of a catalytic amount of $FeCl_3$ at pH 9. Dibenzyl diselenide was removed by extraction with benzene and the aqueous phase acidified to pH 6 to precipitate the pale yellow product, which was contaminated with valine. After two reprecipitations from alkaline solution, selenopenicillamine diselenide dihydrochloride contained no impurities detectable by ^1H NMR.

Reduction of Diselenides. Deaerated solutions of the diselenides in D_2O containing 0.3 M KNO_3 were reduced at a Hg cathode (12.5 cm^2) held at a potential of −1.0 V vs. the saturated calomel electrode. The argon-purged, sealed reduction cell contained a double salt bridge (0.3 M KNO_3 in D_2O) with ground-glass-sleeve junctions separating the auxiliary electrode compartment (Ag wire in saturated KCl) from the diselenide solution. A Princeton Applied Research Model 174A polarographic analyzer was used as the current source. The reduction potentials of the diselenides used in this work were determined by differential pulse polarography to be in the range −0.5 to −0.7 V over the pH range 3–10, in agreement with published potentials.[24] The half-times of the potentiostatic reductions were 30–40 min for 25 mL of 10 mM diselenide. Yellow diselenide solutions, initially at pH 8–9, were reduced until colorless, after which time the pH had risen to 11. Proton NMR spectra of the selenolate solutions were not detectably exchange broadened, indicating the absence of diselenide.[25]

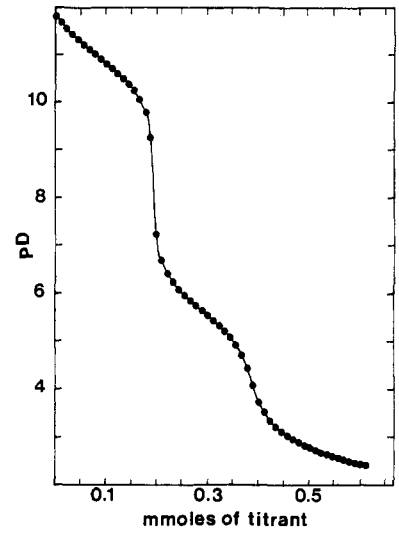

Figure 1. Potentiometric data for the titration of 20 mL of 10 mM selenocysteine with DNO_3 (0.3 M ionic strength and 25 °C). The solid curve through the points is the theoretical titration curve calculated with pK values obtained from the data.

Solution Preparation. D_2O solutions containing ~10 mM CH_3Hg^{II} and selenol at 1:1 ratios were prepared by adding deaerated CH_3HgOD to the selenolate solutions prepared by reduction of the diselenides. Near the expected equivalence point, CH_3HgOD was added in small increments and ^1H NMR spectra were measured after each addition. Exchange of ligand between free and complexed forms is fast, and CH_3HgOD was added until the chemical shift of resonances from protons adjacent to the selenium, which change due to complexation, remained constant. The exact stoichiometry was obtained from a plot of chemical shift vs. volume of CH_3HgOD added, and selenolate solution was then added as necessary to give a 1:1 stoichiometry. In the case of selenocysteamine, it was necessary to use thiouracil to locate the end point in the titration of ligand with CH_3HgOD because the carbon-bonded protons of selenocysteamine give a complex multiplet pattern.

The pD of the resulting CH_3Hg^{II}–selenol solutions was adjusted to 12 with KOD, and samples were withdrawn under an argon atmosphere into NMR tubes at appropriate intervals as the pD was decreased with DNO_3. The pD dependence of the chemical shifts of protons adjacent to ammonium and/or carboxylic acid groups was used to calculate acid dissociation constants for the CH_3Hg^{II}–selenol complexes.

D_2O solutions containing CH_3Hg^{II}, selenol, and mercaptoacetic acid (~10 mM) at exactly known, approximately equimolar amounts were prepared by adding mercaptoacetic acid to CH_3Hg^{II}–selenol solutions prepared as described above. Samples were withdrawn into NMR tubes at appropriate intervals as the pD was increased from pD 2 by titration with KOD.

pH measurements were made at 25 °C with an Orion Model 701A pH meter equipped with a standard glass electrode (Philips GAT 130) and a double-junction saturated calomel reference electrode (Philips R44/2-SD1); the outer junction solution was 0.3 M KNO_3 in D_2O. The pH meter was calibrated with pH 4.008 (0.05 *m* phthalate) and pH 6.865 (0.05 *m* phosphate) standard solutions prepared according to NBS specifications. pH measurements were corrected for deuterium isotope effects with the relation pD = pH meter reading + 0.40.[26]

pH titration data for the determination of acid dissociation constants were collected with the automated equilibrium titrator described previously.[27] Twenty-milliliter aliquots of the selenol solutions were transferred with an argon-flushed pipet into the argon-flushed titration cell and titrated with 2.184 M KOD or 1.114 M DNO_3. After each addition of titrant, the solution was considered to be at equilibrium when the drift

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Table I. Acid Dissociation Constants for Selenol Ligands

ligand	acid group	p$K^{a,b}$	lit. pK
selenoacetic acid	CO_2D^c	3.91 ± 0.03	
	SeD^c	6.91 ± 0.03	7.33[d,e]
selenopropionic acid	CO_2D^c	4.70 ± 0.02	
	SeD^c	7.32 ± 0.01	7.68[d,e]
selenobutyric acid	CO_2D^c	4.96 ± 0.02	
	SeD^c	7.23 ± 0.02	7.78[d,e]
selenocysteine	CO_2D	2.21 ± 0.03	2.01[f]
	SeD	5.43 ± 0.02	5.63,[d,e] 5.24[f]
	ND_3^+	10.68 ± 0.01	9.96[f]
selenopenicillamine	CO_2D	2.24 ± 0.11	
	SeD	5.26 ± 0.07	
	ND_3^+	10.59 ± 0.04	
selenocysteamine	SeD	5.50 ± 0.02	5.0[g]
			4.93[d,h]
			5.01[d,i]
	ND_3^+	11.45 ± 0.02	10.8[h]
2-hydroxyethaneselenol	SeD	6.60 ± 0.02	

[a] In D_2O at 0.3 M ionic strength and 25 °C. [b] Mixed constants in terms of "a_D" as obtained from the pH meter reading and the concentrations of the acid and its conjugate base. [c] pK_1 and pK_2 for the $HSe(CH_2)_nCO_2H$ compounds are actually mixed constants, although the CO_2H group is somewhat more acidic than the SeH group (Kurz, J. L.; Harris, J. C. *J. Org. Chem.* **1970**, *35*, 3086). [d] Corrected for ionic strength differences with the Davies equation (Davies, C. W. *J. Chem. Soc.* **1938**, 2093). [e] Reference 24. [f] Huber, R. E.; Criddle, R. S. *Arch. Biochem. Biophys.* **1967**, *122*, 164. [g] Odom, J. D.; Dawson, W. H.; Ellis, P. D. *J. Am. Chem. Soc.* **1979**, *101*, 5815. [h] Reference 16. [i] Tanaka, H.; Sakurai, H.; Yokoyama, A. *Chem. Pharm. Bull.* **1970**, *18*, 1015.

in pH was ≤0.005 pH unit/min, or after 60 s near equivalence points.

NMR Measurements. ^1H NMR spectra were obtained at 360 MHz and 25 °C with a Bruker WM-360 spectrometer operating in the pulse/Fourier transform mode. 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-silapentanesulfonic acid (DSS).

Results

Acid dissociation constants were determined for the selenol ligands from pH titration data. Data for the titration of a pH 11 solution of selenocysteine with DNO_3 are shown in Figure 1. Protonation occurs over three distinct regions, in contrast to the case for the analogous sulfur compound, where the pH regions for protonation of the amino and thiolate groups overlap. A comparison of this titration curve with those for the selenocarboxylic acids indicates that titration proceeds in the order amino group, selenolate group, and then carboxylate group. pK_A values were calculated by fitting pH titration data to the appropriate protonation equilibria with the computer program MINIQUAD,[27,28] and the results are presented in Table I.

Acid dissociation constants were determined for the ammonium and/or carboxylic acid groups of the CH_3Hg^{II}–selenol complexes by ^1H NMR. Exchange of the CH_3Hg^{II}-complexed ligands between their various protonated forms is fast on the NMR time scale. The chemical shifts of the exchange-averaged resonances for the carbon-bonded ligand protons are the weighted averages of the chemical shifts of the various protonated species, and thus the chemical shift changes as the pH is varied as shown by the data in Figure 2 for the CH_3Hg^{II} complex of selenopenicillamine. Acid dissociation constants were calculated from chemical shift titration data by fitting the data to the appropriate protonation equilibria with the computer program KINET[29] as described previously.[5] The results are presented in Table II. ^{199}Hg–^1H coupling constants for the CH_3Hg^{II} group in the selenol complexes were also measured in these titration experiments, and the results are summarized in Table III.

Acid dissociation constants for MAA and for the CH_3Hg^{II}–MAA complex were determined from chemical shift data for use in the formation constant studies. The pK values in D_2O at 0.3 M ionic strength are 3.68 and 10.12 for MAA and 3.91 for the CH_3Hg^{II}–MAA complex. Acid dissociation constants were also determined for 3-mercaptopropionic acid (4.40 and 10.28 for the free ligand and 4.42 for the CH_3Hg^{II} complex), 4-mercaptobutyric acid (4.63 and 10.28 for the ligand and 4.72 for the CH_3Hg^{II} complex), and cysteamine (8.37 and 11.12 for the ligand and 9.95 for the CH_3Hg^{II} complex) for use in the determination of the

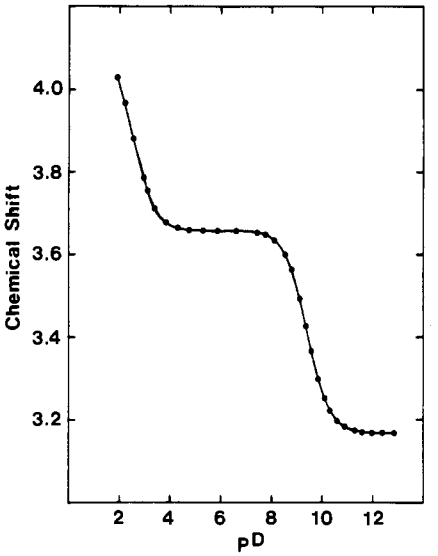

Figure 2. pD dependence of the chemical shift of the resonance for the methine proton of the CH_3Hg^{II}–selenopenicillamine complex (0.3 M ionic strength and 25 °C). The solid curve is the theoretical curve calculated from acid dissociation constants determined in this work for the complexed ligand.

Table II. Acid Dissociation Constants for CH_3Hg^{II}–Selenol Complexes[a,b]

complex	acid group	pK
$CH_3HgSeCH_2CO_2D$	CO_2D	4.45 ± 0.01
$CH_3HgSeCH_2CH_2CO_2D$	CO_2D	4.77 ± 0.01
$CH_3HgSeCH_2CH_2CH_2CO_2D$	CO_2D	5.09 ± 0.01
$D_3N^+CHCO_2D$ CH_2 $SeHgCH_3$	CO_2D	2.69 ± 0.04
	ND_3^+	9.61 ± 0.03
$D_3N^+CH_2CH_2SeHgCH_3$	ND_3^+	10.40 ± 0.03
$D_3N^+CHCO_2D$ $C(CH_3)_2$ $SeHgCH_3$	CO_2D	2.53 ± 0.02
	ND_3^+	9.41 ± 0.01

[a] In D_2O at 0.3 M ionic strength and 25 °C. [b] Mixed constants in terms of "a_D" as obtained from the pH meter reading and the concentrations of the acid and its conjugate base.

Table III. $J_{^{199}Hg-^1H}$ for Selenol-Complexed Methylmercury(II)

ligand	pD	$J_{^{199}Hg-^1H}$, Hz
selenoacetic acid	2.48	169.7
	12.40	165.3
selenopropionic acid	2.45	168.5
	12.43	163.1
selenobutyric acid	2.44	164.6
	13.19	161.6
selenocysteamine	2.39	169.9
	12.70	163.6
selenocysteine	2.90	168.0
	7.91	167.0
	12.53	166.8
selenopenicillamine	2.55	168.0
	7.76	168.0
	12.86	165.0
2-hydroxyethaneselenol	13.22	164.3

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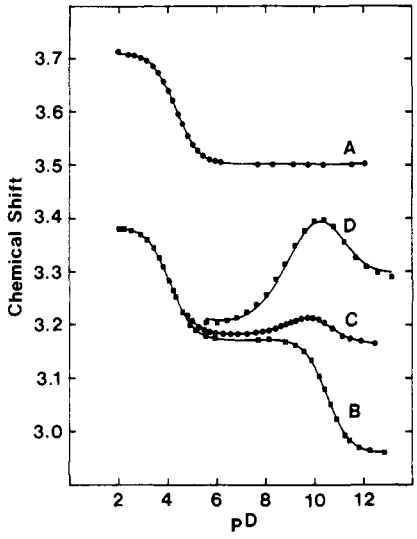

Figure 3. pD dependence of the chemical shift of the methylene resonance of mercaptoacetic acid (MAA) in solutions containing (A) 14.63 mM MAA and 14.66 mM CH_3Hg^{II}, (B) 14.63 mM MAA, (C) 8.88 mM MAA, 9.003 mM CH_3Hg^{II}, and 9.003 mM selenoacetic acid, and (D) 7.14 mM MAA, 10.14 mM CH_3Hg^{II}, and 10.11 mM selenocysteamine. The solid curves are theoretical curves calculated from formation constants determined in this work.

formation constants of the $CH_3Hg(II)$ complexes of these ligands.

Formation constants for complexation of CH_3Hg^{II} by the selenol ligands were determined from the pH dependence of the chemical shift of the methylene resonance of MAA is solutions containing approximately equimolar concentrations of selenol, MAA, and CH_3Hg^{II}. Chemical shift data are presented in Figure 3 for the methylene protons of MAA in solutions containing CH_3Hg^{II}–MAA (A), MAA (B), CH_3Hg^{II}–MAA and selenoacetic acid (C), and CH_3Hg^{II}–MAA and selenocysteamine (D). The pD dependence of the chemical shift in curve A is due to titration of the carboxylic acid group of the CH_3Hg^{II}–MAA complex, while the pD dependence in B is due to titration of the carboxylic acid and thiol groups of free MAA. Curves C and D lie between curves A and B over the pD regions studied, indicating displacement of some of the complexed MAA by selenol, as described by eq 1, and fast exchange of MAA between its free and complexed forms. The positions of curves C and D relative to curves A and B are pD-dependent, indicating that the relative affinities of the thiol and selenol ligands for CH_3Hg^{II} are pD-dependent. Displacement constants (defined by eq 2) were calculated from exchange-averaged chemical shift data of the type shown in Figure 3 with use of procedures described previously[5] for determining the analogous constants for thiol displacement reactions

$$K_d = \frac{[CH_3HgSeR][^-SCH_2CO_2^-]}{[CH_3HgSCH_2CO_2^-][RSe^-]} \quad (2)$$

where RSe^- is the fully deprotonated form of the ligand. The values determined for K_d are presented in Table IV. Formation constants for the selenol complexes (defined by eq 3) were calculated from K_d and the formation constant of the CH_3Hg^{II}–MAA complex[5] by using the relation $K_{f,CH_3HgSeR} = K_d K_{f,CH_3Hg\text{-}MAA}$. The results are presented in Table III. For comparison, values determined previously[5] for the cysteine, mercaptoethanol, and penicillamine complexes and values determined in this work for the 3-mercaptopropionic acid, 4-mercaptobutyric acid, and cysteamine complexes are also presented in Table IV.

$$K_{f,CH_3HgSeR} = \frac{[CH_3HgSeR]}{[CH_3Hg^+][RSe^-]} \quad (3)$$

Discussion

The formation constants listed in Table IV indicate that CH_3Hg^{II} forms stronger complexes with selenol ligands than with the corresponding thiol ligands. Consistent with this, $^2J_{^{199}Hg-^1H}$ is less for the selenolate complexes than for the thiolate complexes.[3,16] For example, $^2J_{^{199}Hg-^1H}$ for the CH_3Hg^{II} complex of mercaptoacetic acid at high pH is 172.0 Hz while that of the cysteine complex is 174.0 Hz.[3]

Table IV. Formation Constants for CH_3Hg^{II} Complexes of Selenol and Thiol Ligands[a]

complex	log $K_{d,X=Se}$	log $K_{f,X=Se}$	log $K_{f,X=S}$
$CH_3HgXCH_2CO_2^-$	0.438 ± 0.004	17.36	16.92[b]
$CH_3HgXCH_2CH_2CO_2^-$	0.955 ± 0.005	17.88	16.72
$CH_3HgXCH_2CH_2CH_2CO_2^-$	0.221 ± 0.005	17.14	16.57
$CH_3HgXCH_2CH_2NH_2$	−0.428 ± 0.014	16.49	16.17
$CH_3HgXCH_2\underset{\displaystyle NH_2}{\overset{\displaystyle |}{C}}HCO_2^-$	0.455 ± 0.014	17.38	16.67[b]
$CH_3HgXC(CH_3)_2\underset{\displaystyle NH_2}{\overset{\displaystyle |}{C}}HCO_2^-$	0.478 ± 0.010	17.40	16.94[b]
$CH_3HgXCH_2CH_2OH$	−0.714 ± 0.023	16.21	16.12[c]

[a] In D_2O at 25 °C and 0.3 M ionic strength unless otherwise noted. [b] In H_2O at 25 °C and 0.3 M ionic strength; from ref 5. [c] From ref 6 and: Schwarzenbach, G.; Schellenberg, M. *Helv. Chim. Acta* **1967**, *48*, 28.

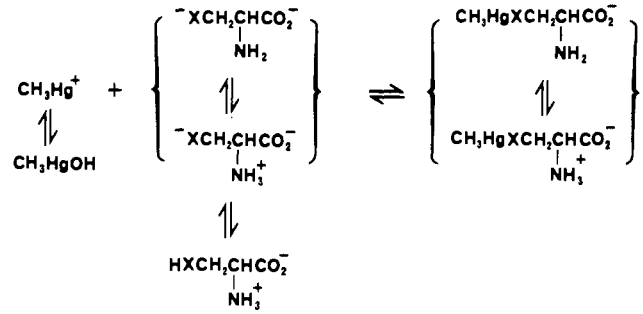

Figure 4. Competing complexation and protonation reactions occurring in aqueous solutions of CH_3Hg^{II} and selenocysteine (X = Se) and CH_{Hg}^{II} and cysteine (X = S).

The formation constants listed in Table IV for the CH_3Hg^{II} complexes of selenocysteamine, selenocysteine, and selenopenicillamine are for the amino-depronated form of the ligand. The formation constants calculated from the results in Tables II and IV are log K_f = 15.44, 16.31, and 16.22 for the amino-protonated complexes of selenocysteamine, selenocysteine, and selenopenicillamine, respectively. Comparison of these formation constants with those in Table IV indicates that the formation constants of the selenolate complexes increases considerably when the ammonium group is deprotonated. The increase in formation constant upon deprotonation of the ammonium group is also reflected by a decrease in $^2J_{^{199}Hg-^1H}$ (Table III).

It is important to note that, because the formation constants in Table IV refer to the reaction of CH_3Hg^+ with the fully deprotonated ligands, their magnitudes can be misleading with respect to the extent of complexation. At all pH values, there are competing reactions, as summarized by the various equilibria involving CH_3Hg^{II} and selenocysteine (and CH_3Hg^{II} and cysteine) in Figure 4. At high pH, hydroxide competes with ligand for CH_3Hg^+, while at lower pH, protonation competes with CH_3Hg^+ for the ligands. Since the acid dissociation constants of selenol and thiol groups are considerably different, the pH dependence of the extent to which complexation occurs will be different for the selenol and thiol complexes. The effect of the various competitive equilibria on the extent of complexation can most easily be accounted for with conditional formation constants, K_{fc}, which are pH-dependent equilibrium constants defined by the reaction

$$(CH_3Hg)_f + L_f \rightleftarrows (CH_3HgL)_t \quad (4)$$

where $(CH_3Hg)_f$ represents the sum of CH_3Hg^+ and CH_3HgOH and L_f and $(CH_3HgL)_t$ denote the sums of the concentrations of the various protonated forms of free and complexed ligand. To illustrate the effect of pH, the conditional formation constants for the cysteine and selenocysteine complexes are plotted as a

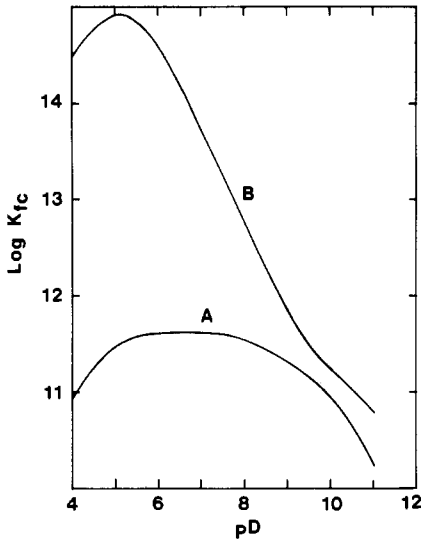

Figure 5. Logarithm of the conditional formation constants vs. pD for the CH_3Hg^{II} complexes of (A) cysteine and (B) selenocysteine.

function of pH in Figure 5. Over the entire pH range, the conditional formation constants are less than those given in Table IV. At high pH (≥12), the difference between the conditional formation constants is the same as the difference in Table IV because both ligands are fully deprotonated and both conditional formation constants are affected equally by the competitive reaction of CH_3Hg^+ with hydroxide. However, as the pH is decreased, the difference increases because competitive protonation of the thiolate group occurs at high pH. The increased stability of the selenol complexes relative to that of the thiol complexes as the pH is decreased is also apparent from the pH dependence of curves C and D in Figure 3; as the pH is decreased, curves C and D shift closer to curve A, indicating a larger fraction of the total MAA has been displaced by selenol.

The conditional formation constants at pH 7.4 are of particular interest with respect to the possible binding of CH_3Hg^{II} by the selenol group of glutathione peroxidase. log K_{fc} for the selenocysteine complex is 13.35, while log K_{fc} of the CH_3Hg^{II} complexes of glutathione and hemoglobin, the two most abundant thiol-containing molecules in human erythrocytes,[2] are 11.55 and 10.7, respectively.[7] With use of the conditional formation constant of the selenocysteine complex as an estimate for the formation constant of the CH_3Hg^{II}–glutathione peroxidase complex and concentrations of 2×10^{-6}, 2×10^{-3}, and 5×10^{-3} M for glutathione peroxidase,[30] glutathione,[2] and hemoglobin in red blood cells, 1.6, 7.7, 14, and 47% of the selenol groups of glutathione peroxidase are predicted to be complexed by CH_3Hg^{II} at total CH_3Hg^{II} concentrations of 1×10^{-6}, 5×10^{-6}, 1×10^{-5}, and 5×10^{-5} M. Even though K_{fc} for the selenocysteine complex is somewhat larger than those of the glutathione and hemoglobin complexes, a somewhat larger fraction of the total amount of CH_3Hg^{II} is complexed by the thiol ligands because of their much higher concentrations. As a reference point, red blood cell concentrations of CH_3Hg^{II} up to $\sim 3 \times 10^{-5}$ M were reported in the 1972 epidemic of CH_3Hg^{II} poisoning in Iraq.[31]

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Registry No. $CH_3HgSeCH_2CO_2D$, 102493-99-2; $CH_3HgSeCH_2CH_2CO_2D$, 102494-00-8; $CH_3HgSeCH_2CH_2CH_2CO_2D$, 102494-01-9; $D_3N^+CH(CH_2SeHgCH_3)CO_2D$, 102494-02-0; $D_3N^+CH_2CH_2SeHgCH_3$, 102494-03-1; $D_3N^+CH(C(CH_3)_2SeHgCH_3)CO_2D$, 102494-04-2; $CH_3HgSeCH_2CO_2^-$, 102494-05-3; $CH_3HgSeCH_2CH_2CO_2^-$, 102494-06-4; $CH_3HgSCH_2CH_2CO_2^-$, 102494-07-5; $CH_3HgSeCH_2CH_2CH_2CO_2^-$, 102494-08-6; $CH_3HgSCH_2CH_2CH_2CO_2^-$, 102494-09-7; $CH_3HgSeCH_2CH(NH_2)CO_2^-$, 102494-10-0; $CH_3HgSeCH_2CH_2NH_2$, 102494-11-1; $CH_3HgSCH_2CH_2NH_2$, 102494-12-2; $CH_3HgSeC(CH_3)_2CH(NH_2)CO_2^-$, 102494-13-3; $CH_3HgSeCH_2CH_2OH$, 102494-14-4; CH_3Hg^{II}, 22967-92-6; selenoacetic acid, 25244-47-7; selenopropionic acid, 60746-32-9; selenobutyric acid, 42905-03-3; selenocysteine, 3614-08-2; selenopenicillamine, 36969-38-7; selenocysteamine, 21681-94-7; 2-hydroxyethaneselenol, 60718-59-4.

(30) This value is calculated for an average selenium blood level of 230 μg/L (Shamberger, R. J. *Biochemistry of Selenium*; Plenum: New York, 1983; p 227) by assuming that all the selenium is in the erythrocytes in the form of glutathione peroxidase and that the blood has a hematocrit of 50%.

(31) Calculated from whole blood mercury concentrations reported in: Bakir, F.; Damluji, S. F.; Amin-Zaki, L.; Murtadha, M.; Khalidi, A.; Al-Rawi, N. Y.; Tikriti, S.; Dhahir, H. K.; Clarkson, T. W.; Smith, J. C.; Doherty, R. A. *Science (Washington, D.C.)* **1973**, *181*, 230. The calculations assume that there was a hematocrit of 50% and that 90% of the whole blood mercury was in the red blood cells.

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Electron Self-Exchange by Hexakis(aryl isocyanide)manganese(I/II): Concentration, Electrolyte, and Temperature Dependences

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The rate of electron self-exchange has been measured for the tetrafluoroborate salts of $Mn(CNR)_6^{+/2+}$ complexes, where R is p-$C_6H_4CH_3$ and p-$C_6H_4OCH_3$, as a function of concentration, temperature, and added tetrabutylammonium tetrafluoroborate in CD_3CN by ^1H NMR line broadening. The measured rate constants (M^{-1} s^{-1}), at 26 °C and 0.1 M added salt, and the activation parameters $\Delta H^{\ddagger}$ (kcal/mol) and $\Delta S^{\ddagger}$ (cal/(mol K)) are 3.0×10^7, 2.4, and −16 for the tolyl isocyanide complex and 4.5×10^7, 3.4, and −12 for the anisyl isocyanide complex, respectively. Through an analysis of the ionic strength dependence of the rate constant for the tolyl isocyanide complex, a rate constant of 6.1×10^7 M^{-1} s^{-1} was estimated for the condition of no electrostatic interaction between the complexes. These rate constant values are the highest measured for complexes of this type and are ca. 100 times the rate constants previously determined for $Mn(CN(C(CH_3)_3)_6^{+/2+}$ and $Mn(CNC_6H_{11})_6^{+/2+}$. It is concluded that the most likely reason for the rate enhancement is improved overlap between the donor and acceptor orbitals.

Introduction

Significant progress is being made in the understanding of electron-transfer reactions through complementary experimental and theoretical studies.[1,2] In this area we have been especially concerned with the analysis of well-defined, outer-sphere electron-transfer systems that can be studied with the use of solvents other than water. As part of this effort we have sought to ex-

(1) Newton, M. D.; Sutin, N. *Annu. Rev. Phys. Chem.* **1984**, *35*, 437.

(2) Cannon, R. D. *Electron Transfer Reactions*; Butterworths: London, 1980.

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