Methylmercury(II) sulfhydryl interactions. Potentiometric determination of the formation constants for complexation of methylmercury(II) by sulfhydryl containing amino acids and related molecules, including glutathione

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Formation constants for the interaction of methylmercury(II) with 2-mercaptoethanol, mercaptoacetic acid, *O*-methylmercaptoacetate, 2-mercaptosuccinic acid, L-cysteine, D,L-penicillamine, *N*-acetyl-D,L-penicillamine, glutathione, thiocholine, and 4-mercapto-*N*-methylpiperidine have been determined by potentiometric titration. For the equilibrium $MeHg^{+} + RS^{-} \rightleftharpoons [MeHgSR]^{+}$, log β occurs in the range 14.60(1)–17.14(1), with the lowest value obtained for the cationic complex of thiocholine, $MeHgSCH_2CH_2\dot{N}Me_3$. Acid dissociation constants for the ligands are reported. Formation constants for the addition of both one and two equivalents of MeHg(II) to L-cysteine have been determined, giving log *K* ca. 4.1 for the reaction

$$MeHgSCH_2CH(\dot{N}H_3)(CO_2H) + MeHg^{+} \rightleftharpoons (MeHg)_2\dot{S}CH_2CH(\dot{N}H_3)(CO_2H)$$

occurring at low pH, and log *K* 8.8 for the reaction

$$MeHgSCH_2CH(NH_2)CO_2^{-} + MeHg^{+} \rightleftharpoons MeHgSCH_2CH(\dot{N}H_2HgMe)CO_2^{-}$$

occurring at higher pH.

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On a déterminé par titrage potentiométrique les constantes de formation pour l'interaction du méthylmercure(II) avec le mercapto-2 éthanol, l'acide mercaptoacétique, le *O*-méthylmercaptoacétate, l'acide mercapto-2 succinique, la L-cystéine, la D,L-pénicillamine, la *N*-acétyl-D,L-pénicillamine, la glutathione, la thiocholine et la mercapto-4 *N*-méthylpipéridine. Dans le cas de l'équilibre $MeHg^{+} + RS^{-} \rightleftharpoons [MeHgSR]^{+}$, log β est de l'ordre de 14,60(1) à 17,14(1), le complexe cationique de thiocholine, $MeHgSCH_2CH_2\dot{N}Me_3$ donne la plus faible valeur. On rapporte les constantes de dissociation acide des ligands. On a déterminé les constantes de formation dans le cas de l'addition d'un et de deux équivalents de MeHg(II) sur la L-cystéine qui donne log K = 4,1 environ pour la réaction

$$MeHgSCH_2CH(\dot{N}H_3)(CO_2H) + MeHg^{+} \rightleftharpoons (MeHg)_2\dot{S}CH_2CH(\dot{N}H_3)(CO_2H)$$

se produisant à faible pH et log K = 8,8 pour la réaction

$$MeHgSCH_2CH(NH_2)CO_2^{-} + MeHg^{+} \rightleftharpoons MeHgSCH_2CH(\dot{N}H_2HgMe)CO_2^{-}$$

se produisant à un pH plus élevé.

[Traduit par le journal]

Introduction

The interaction of methylmercury(II) with thiols to form complexes MeHgSR is believed to be important in both the toxicology of MeHg(II) and the use of antidotes containing thiol groups (1). Thus, a fundamental requirement in studying the biological behaviour of MeHg(II) is accurate values of β_{110} for equilibria [1], where RSH are thiols that occur *in vivo*.

[1] $MeHg^{+} + RS^{-} \rightleftharpoons MeHgSR$

[2] $\log \beta_{110} = [MeHgSR]/[MeHg^{+}][RS^{-}]$

Early determinations of log β_{110} with mercaptoalbumin (distribution method) (2, 3), cysteine and glutathione (distribution) (3), and 2-mercaptoethanol (potentiometric) (4) gave values in the range 15.7–16.9. Some recent determinations agree well with these values. Thus, Jawaid and Ingman (5) obtained log β_{110} 15.7 ± 0.12 for L-cysteine (potentiometric); and it has been reported that substituted thiophenols form complexes with log β_{110} in the range 10.48–15.60 (potentiometric) (6, 7) to give the relationship log $\beta_{110} = 0.798pK(RSH) + 8.77$, which is also obeyed by the early values (2–4) of log β_{110} within 0.5pK units.

However, Hojo *et al.* (8) in a recent potentiometric study obtained values for several thiols, including L-cysteine and glutathione, which are well below the values reported by other workers (log β_{110} 7.19–9.03). Jawaid and Ingman (5), and Reid and Rabenstein (9), have commented on these results and the latter workers have reported log β_{110} for mercaptoacetic acid, mercaptoethanol, mercaptosuccinic acid, cysteine, penicillamine, homocysteine, and *N*-acetylpenicillamine. Reid and Rabenstein obtained values consistent with the early workers and Jawaid and Ingman, but not Hojo *et al.*

The results of Reid and Rabenstein were obtained using novel ^1H nuclear magnetic resonance competition experiments involving determination of K_d for the equilibrium [3], from which β_{110} for MeHgSR can be evaluated from β_{110} for $MeHgSCH_2CO_2^{-}$ [4] (9).

[3] $MeHgSCH_2CO_2^{-} + RS^{-} \rightleftharpoons MeHgSR + SCH_2CO_2^{-}$

[4] $$K_d = \frac{[MeHgSR][SCH_2CO_2^{-}]}{[MeHgSCH_2CO_2^{-}][RS^{-}]} = \frac{\beta_{110}\text{ for MeHgSR}}{\beta_{110}\text{ for MeHgSCH}_2\text{CO}_2^{-}}$$

Swarzenbach and Schellenberg's value of β_{110} for the 2-mercaptoethanol complex was used to calculate β_{110} for $MeHgSCH_2CO_2^{-}$ after measurement of K_d for mercaptoacetate/2-mercaptoethanol competition.

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However, discrepancies between reported values for MeHg(II) thiolate formation constants, and reliance of the nmr method on the single determination of β_{110} for 2-mercaptoethanol, indicate that further measurement of β_{110} for a range of thiols is desirable. In addition, MeHg(II) binds to glutathione in red blood cells (10), and thus competitive equilibria between MeHg(II), glutathione, and sulfhydryl containing antidotes for MeHg(II) poisoning (e.g. D-penicillamine (11) and *N*-acetyl-D,L-penicillamine (11, 12)) are important, but the formation constant for interaction of MeHg(II) with glutathione has only been determined in one of the early investigations (distribution method) (3). We report here a potentiometric study of the interaction of MeHg(II) with all of the ligands studied by the nmr method (except for homocysteine), glutathione, *O*-methylmercaptoacetic acid, and the cationic thiols thiocholine and *N*-methyl-4-mercaptopiperidine. Our potentiometric results confirm early values for glutathione and 2-mercaptoethanol, are in complete agreement with constants determined using the nmr method and provide values for the formation constants for addition of a second MeHg(II) group to L-cysteine to form $(MeHg)_2SCH_2CH(\dot{N}H_3)(CO_2H)$ and $MeHgSCH_2CH(\dot{N}H_2HgMe)CO_2^-$.

Experimental

Chemicals

The thiols 2-mercaptoethanol (Koch-Light) and mercaptoacetic acid (Aldrich) were fractionally distilled under reduced nitrogen pressure and stored under nitrogen at −20°C. The crystalline thiols L-cysteine, *N*-acetyl-D,L-penicillamine (Koch-Light), D,L-penicillamine (Ega-Chemie), 2-mercaptosuccinic acid (Aldrich), and glutathione (Sigma) were used as received and stored under nitrogen over P_2O_5. Thiocholine perchlorate was a gift from P. Guerney (University of New South Wales). The thiol *O*-methylmercaptoacetic acid was prepared by acid catalysed *O*-alkylation of mercaptoacetic acid in refluxing methanol (bp 46−47.5°C (4 mm), lit. (13) 42−43°C (10 mm)), and 4-mercapto-*N*-methylpiperidine was prepared as described (13) (bp 31−32°C (1 mm), lit. (14) 62°C (0.8 mm)).

A solution of methylmercury(II) hydroxide was obtained by stirring MeHgI (20 mmol, purified as described elsewhere (15, 16)) with freshly prepared silver oxide (ca. 20 mmol) for 12 h under nitrogen in 50 mL of freshly boiled water. The resultant suspension was filtered (Whatman No. 54), in a nitrogen-flushed glovebox, directly into a 1 L volumetric flask containing a slight excess of nitric acid (20 mmol) to give a $MeHg^+/H^+/NO_3^-$ solution with $[MeHg^+]$ and $[H^+]$ nominally 0.02 *M* and 0.005 *M*, respectively. The solution was stored under nitrogen. Values of $[H^+]$ were determined by alkalimetric titration, monitored potentiometrically, after addition of excess KI to remove $MeHg^+$ as insoluble MeHgI. Values of $[MeHg^+]$ were determined as described elsewhere (15, 16), involving titration with thiosulfate in 50% aqueous methanol with Michlers thioketone as an indicator. The composite concentration, $[H^+] + [MeHg^+]$, was also determined by alkalimetric titration in the absence of iodide, and was in agreement with values for $[H^+]$ and $[MeHg^+]$. Concentration of H^+ and $MeHg^+$ remained constant for 15 months.

The 0.1 *M* potassium hydroxide titrant (Merck Titrisol®) was stored in leached borosilicate glass and was protected by Carbasorb®. The alkali was frequently standardised by potentiometric titration against primary standard grade potassium hydrogen phthalate. The equivalent points of these titrations were determined by the program TITRAT, and produced identical titrant concentrations to alternate titrations against borax standardised 0.1 *M* nitric acid in 0.1 *M* potassium nitrate when equivalent points were found by Gran type 1 or type 2 methods (17). These latter titrations also provided estimates of pK_w' (see below) and indicated the presence of less than 0.1% weakly acidic impurities, e.g. carbonate, silicate.

Water used for preparation of all titration solutions was deionised by mixed bed ion exchange to a specific resistance greater than 4×10^6 ohm cm, then boiled and cooled under nitrogen prior to use.

The ionic strength of potentiometric titration solutions was adjusted to 0.1 *M* with twice recrystallised potassium nitrate or potassium iodide. The latter also serves as a competitive ligand for MeHg(II) in the formation constant titrations (see below).

Potentiometric measurements

All pH measurements were performed in a thermostatted double walled cell based on that of Perrin and Sayce (18), in which the entire assembly was maintained at 25.00 ± 0.02°C to minimise thermal gradients within the electrodes. An Orion Model 7010 digital pH meter was equipped with a standard glass electrode (Philips GA 110) or low resistance glass electrode (Philips GAT 130), together with a glass sleeve double junction saturated calomel reference electrode (Philips R44/2-SD/1) in which the outer bridge solution was 1 *M* potassium nitrate. The inner (reference) half cell of this electrode was vented outside of the titration vessel to preclude contamination by volatile thiols. The electrode pair was standardised before each titration with thermostatted NBS standard solutions (19) (pH 4.008, 6.865, and 10.012).

Solutions for thiol pK determination

Immediately following standardisation of the electrode assembly, 50.0 mL of thiol solution were transferred to the dried purged titration vessel. For thiols containing carboxylic acid groups 0.1 *M* nitric acid was then added to given an initial pH in the range 2.5−3. Titrant (0.1 *M* potassium hydroxide) was delivered by a motorised 5 mL piston burette (Metrohm D535) through a finely drawn glass capillary placed just beneath the solution surface. The solution was purged with high purity nitrogen which has been passed through a column of Carbasorb® (to remove CO_2) and a thermostatted solution of 0.1 *M* potassium nitrate. Magnetic stirring was stopped and nitrogen redirected over the solution surface during pH measurement. The pH readings were recorded when drift was less than 0.001 units per min. Equilibrium was obtained within 1−5 min in at least moderately buffered regions.

Using this procedure 30−60 titration points were collected over periods of 1−3 h. The electrode standardisation was then verified with the pH 6.865 NBS buffer. On the rare occasions that the electrode standardisation had drifted by more than 0.002 pH, the titration was repeated. Early attempts to use combination electrodes, particularly with solutions of some vicinal dithiols, resulted in restandardisation discrepancies of up to 1 pH unit after such titrations, forcing the use of separate glass and reference electrodes.

Solutions for formation constant determination

An aliquot of $MeHg^+/H^+/NO_3^-$ stock solution was added to 50.0 mL of vigorously stirred thiol solution (containing 0.1 *M* potassium iodide) in order to achieve 1:1 or 2:1 $MeHg^+$:thiol stoichiometry. If precipitation of MeHgI was evident during MeHg(II) addition potassium hydroxide titrant was simultaneously added to achieve the minimum pH consistent with a clear solution. The titration procedure was then identical to that for p*K* determinations. It was often convenient to combine the two procedures by titrating the thiol ligand in 0.1 *M* potassium iodide, then adding $MeHg^+/H^+/NO_3^-$ solution and retitrating after readjustment of the ionic strength with potassium nitrate.

Calculation of equilibrium constants

Calibration of the glass electrode in terms of $[H]^+$

The equilibrium constants recorded in this work are concentration constants, requiring that the electrode assembly be calibrated in terms of log $[H^+]$ rather than log $\{H^+\}$. The approach to this problem advocated by Irving *et al.* (20), and expanded by McBryde (21), and Hedwig and Powell (22) was used in this work, where $[H^+]$ is derived from the measured pH by the relationship

$$-\log [H^+] = pH + pH_{cal}$$

where pH_{cal} includes activity coefficient corrections and accounts for

systematic errors inherent in transferring electrodes from buffers to titration solutions.

Initially pH_{cal} was measured in a series of titrations of strong acid (0.01 *M* HNO_3 in 0.1 *M* KNO_3) with 0.1 *M* potassium hydroxide. However, it is more convenient to treat pH_{cal} as an unknown parameter in the multiparametric curve fitting procedures used to calculate thiol acid dissociation constants or MeHg(II) complex formation constants. In this way pH_{cal} can be estimated from data below pH 4 for the actual titration under study, rather than from separate preliminary titrations. Values of pH_{cal} calculated in this way over many titrations were always in the range −0.08 to −0.05, in agreement with similar values found elsewhere (22, 23) or in this work by separate strong acid – strong base titrations. It should be emphasised that extraction of low p*K*'s (less than 3) from titration data by any calculation or method requires accurate values of $[H^+]$ in this low pH region (20).

For solutions of moderately strong acids in the multimolar level (p*K* less than 3) small errors in $[H^+]$ result in large *systematic* errors in the estimated p*K* value which are not reflected by the usual error estimates produced by least squares procedures.

A related problem is that of accurate values of K_w^c, which equals $[H^+][OH^-]$ in the ionic medium of interest. Estimates of p*K* values greater than 10–11 are highly correlated to errors in K_w^c and to electrode calibration above pH ~ 11, e.g. it can be shown that the uncertainty in pK_w^c produces 10 times this error in the acid dissociation constant of a weak monoprotic acid with pK_w^c approximately 11.5 (20). Values of pK_w^c were obtained from the difference in $E°$ of the electrode assembly in acid and alkaline regions of strong acid/strong base titrations (24), giving $pK_w^c = 13.74 \pm 0.02$ at 25°C in 0.1 *M* potassium nitrate, which agrees favourably with $pK_w^c = 13.784 \pm 0.006$ calculated from the molal value of 0.1 molal KCl at this temperature (25). However, it was found that the thiol p*K* titration data could not be adequately fitted in the region above pH 10 using pK_w^c 13.74. Multiparametric refinement by TITRAT or MINIQUAD 81 (see below) over many titrations produced values of pK_w^c in the range of 13.86 ± 0.04. This value probably includes a small term due to electrode non-linearity above pH 10. It should be noted that there seem to be many reported instances of equilibrium constants calculated at high pH, e.g. thiol p*K*, in which the value of pK_w^c is either not recorded or has been taken from sources using different experimental conditions. Difficulties in this area are reflected by the general lack of agreement between such constants.

Acid dissociation constants of the thiol ligands were obtained together with values of the proton purity of the ligands with the computer program TITRAT. This program is a generalised version of the interactive rigorously weighted non-linear least-squares programs used by Schwartz and Gelb to evaluate two simultaneous acid dissociation constants (26). TITRAT can refine any combination of the parameters applicable to the titration of a polyprotic acid or base mixture by a stock monoprotic acid or base.[2] The running time of the original program was decreased by an order of magnitude by the use of analytical expressions for condition function derivatives, and the convergence properties improved by quadratic optimisation of the parameter shifts during the refinement.

In this work the acid dissociation constants in the concentration form were refined simultaneously with the number of millimoles of ligand and electrode calibration parameters pH_{cal} and pK_w^c.

Protons do not compete effectively in aqueous solutions with MeHg(II) for the thiolate ligands studied here. One means of overcoming this limitation to the use of pH titrations is to reduce the effective MeHg(II) thiolate constant by competition with another ligand. Iodide is particularly well suited in this regard, e.g. solutions of MeHg(II) and 2-mercaptoethanol exhibit buffer regions in the pH range 3–5 in the presence of 0.1 *M* KI, due to the equilibrium

$$MeHgI + HSCH_2CH_2OH \rightleftharpoons MeHgSCH_2CH_2OH + I^- + H^+$$

The formation constant of MeHgI was obtained by pH titration of MeHg(II) in the presence of 0.1 *M* KI, giving log *K* 8.500(1) (lit. 8.60 (ref. 4), 8.7 (ref. 28)). Above pH 8 the equilibrium

$$MeHgI + H_2O \rightleftharpoons MeHgOH + I^- + H^+$$

is established. Similarly, formation constants for MeHgOH and $(MeHg)_2OH^+$ were evaluated by titration in 0.1 *M* KNO_3, giving log $\beta_{10\ -1} = -4.607$ (9) (lit. −4.50 (ref. 29), −4.59 (ref. 4), −4.67(3) (ref. 30), −4.70 (ref. 31), −4.40(2) (ref. 32), −4.686(15) (ref. 33)) and log $\beta_{20\ -1} = -2.234$ (18) (lit. −2.22 (constant calculated using values of K_w^c recorded in ref. 4), −2.53 (quoted (refs. 32, 33)), −1.725(30) (ref. 33), −2.33 (quoted (refs. 32, 33))).

The ternary MeHg(II)/thiolate/iodide/proton (or hydroxide) systems were treated using a recent version (34) of the program MINIQUAD 75 (35), which allows simultaneous refinement of formation constants and pH calibration parameters pH_{cal} and the analytical proton excess (H_{tot}) for each titration fit. Refinement of H_{tot} simultaneously with the formation constants by MINIQUAD 81 consistently indicated values 1–2 μmol greater than those calculated from anal-

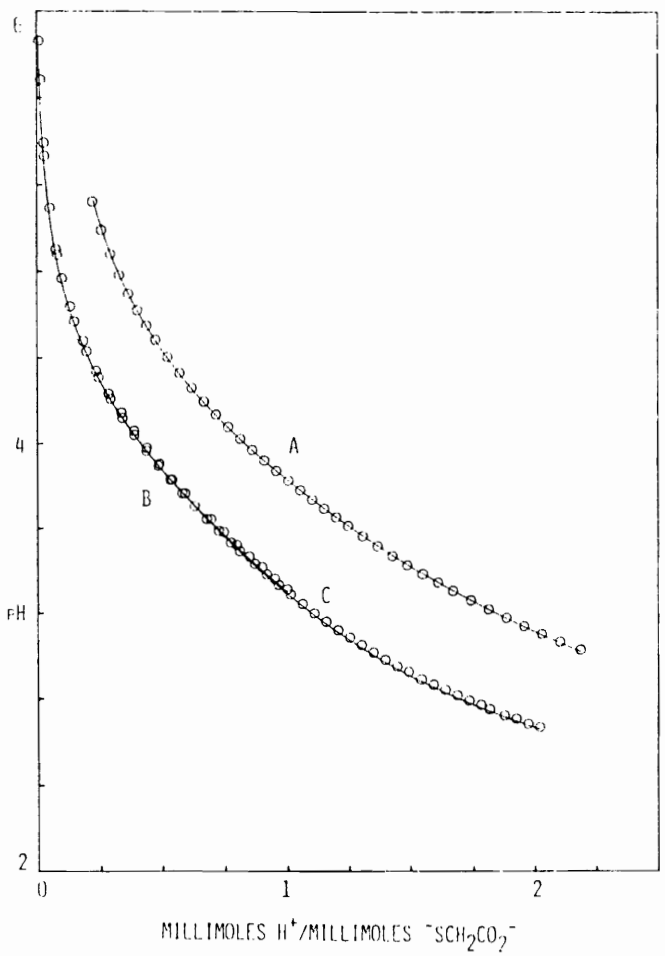

FIG. 1. Titration data for MeHg(II) and mercaptoacetic acid. Data for *A* were obtained with iodide competition, for *B* and *C* without iodide competition. For each titration initial amounts of MeHg(II), $SCH_2CO_2^-$, and H^+ are given in mmol followed by molarity of KOH and initial volume; *A*: 0.07703, 0.08244, 0.17977, 0.09947 *M*, 54.97 mL; *B*: 0.1895, 0.1895, 0.1895, 0.09649 *M*, 100.00 mL; *C*: 0.2051, 0.2051, 0.4144, 0.09837 *M*, 102.00 mL. For titration *A* 5.482 mmol of I^- were present.

[2]During this work the programs ACBA (27) and MUPROT (28) were reported. These serve the same purpose as TITRAT, but do not use the statistically rigorous weighting scheme necessary for titrations in which titrant volumes and pH have comparable uncertainties (26). TITRAT is written in OS/8 BASIC for implementation on a Dec PDC/8E minicomputer and uses 12 K core for 60 titration points and up to 6 parameters refined simultaneously. Listings of the program are available on request.

TABLE I. Acid dissociation constants for the ligands[a]

Ligand	pK (0.1 M KNO_3 or KI)				Literature values[b]					Reference
2-Mercaptoethanol	*9.58*				9.444				[≈0]	38
					9.72				[≈0]	39
					9.47				[0.3 M $NaClO_4$]	37
Mercaptoacetic acid	3.55	*10.16*			3.60	10.55			[≈0]	40
					3.58	9.78			[0.15 M KNO_3]	41
					3.48	9.92			[0.58 M $NaNO_3$]	42
					3.42	10.20			[0.1 M KCl]	43
					3.35(1)	9.32(2)			[0.3 M KNO_3]	9
O-Methylmercaptoacetate	*7.99*				7.95(2)					44
2-Mercaptosuccinic acid	3.12	4.59	*10.39*		3.64	4.64	10.37		[0.1 M KNO_3]	45
					3.30	4.94	10.64			46
					3.06(1)	4.56(1)	10.39(1)			47
L-Cysteine	1.98(1)	8.23	10.37		1.896(6)	8.178(2)	10.361(1)		[0.1 M KCl]	48
					1.97(1)	8.20(1)	10.87(1)		[0.1 M KNO_3]	49
					1.90	8.12(1)	10.15		[1.0 M $NaNO_3$]	5
						8.48	10.55		[0.15 M KNO_3]	41
						8.13	10.11		[0.1 M KNO_3]	50
D,L-Penicillamine	1.43(8)	8.00	10.59		2.44	7.97	10.46		[0.15 M KNO_3]	51
					1.94	7.93	10.39			52
					1.90(2)	7.932(4)	10.658(3)		[0.1 M KCl]	48
					1.95(1)	7.99(1)	10.56(1)		[0.1 M KNO_3]	53
					2.02(2)	7.92(1)	10.75(1)		[0.1 M KNO_3]	49
						7.88	10.43		[0.1 M KNO_3]	50
N-Acetyl-D,L-penicillamine	3.30	*10.14*			3.18	10.04				54
					3.69(5)	10.01(10)			[0.1 M $NaClO_4$]	55
Glutathione	1.98(2)	3.49	8.75	9.69	2.05	3.40	8.72	9.49	[0.2–0.5 M]	56
					2.09(2)	3.48(1)	8.67(1)	9.54(1)	[0.1 M KNO_3]	49
						3.59	8.75	9.65	[0.15 M KNO_3]	57
							8.74	9.62	[0.16 M KNO_3]	58
Thiocholine	*7.88*					7.7				59
						7.80			[≈0]	60
4-Mercapto-*N*-methylpiperidine	8.33	*10.35*								

[a] Macroscopic concentration constants obtained with TITRAT. Except for the lowest p*K* values for L-cysteine, D,L-penicillamine, and glutathione, calculated values were to three significant figures, e.g. 2-mercaptoethanol 9.583(2), but are reported to two figures here.

[b] Literature values at 25°C. Mixed constants have been converted to concentration constants by calculation of log γ_0, according to the Davies equation (61).

TABLE 2. Formation constants for methylmercury(II)–thiol complexes

Ligand	Formation constant	This work	Reid and Rabenstein[a]
2-Mercaptoethanol	log β_{110}	16.14(1)	[b]
Mercaptoacetic acid	log β_{110}	16.93(1)	16.92(1)
	log β_{111}	20.69(1)	*20.57(2)*[c]
O-Methylmercaptoacetate	log β_{110}	14.98(2)	
2-Mercaptosuccinic acid	log β_{110}	17.14(1)	17.31(6)
	log β_{111}	21.98(1)	*22.01(8)*[c]
	log β_{112}	25.11(1)	*25.39(11)*[c]
L-Cysteine	log β_{110}	16.46(1)	16.67(1)[d]
	log β_{111}	25.48(1)	*25.32(3)*[c,d]
D,L-Penicillamine	log β_{110}	16.60(1)	16.94(2)
	log β_{111}	25.34(1)	*24.93(3)*[c]
N-Acetyl-D,L-penicillamine	log β_{110}	16.51(1)	
	log β_{111}	19.80(4)	
Glutathione	log β_{110}	15.99(1)	
	log β_{111}	25.24(1)	
	log β_{112}	28.68(2)	
Thiocholine	log β_{110}	14.60(1)	
4-Mercapto-*N* methylpiperidine	log β_{110}	16.06(3)	
	log β_{111}	25.33(3)	

[a]At 25°C and 0.3 *M* ionic strength. Mixed constants involving protons have been converted to concentration constants using $-\log \gamma_H = 0.15$ from the Davies equation (61).

[b]Literature value 16.12 at 20°C and 0.1 *M* ionic strength (4).

Values in italics have been derived from measurement of p*K*'s, e.g. for the acid $MeHgSCH_2CO_2H$, log β_{111} = log β_{110} + (p*K* of $MeHgSCH_2CO_2H$).

[c]Jawaid and Ingman obtained log β_{110} 15.70 ± 0.12 and log β_{111} 24.96 ± 0.08 at 25°C and 1 *M* ionic strength (5).

ytical proton concentrations of the thiol and $MeHg^+/H^+/NO_3^-$ solutions. This may reflect the presence of minute quantities of weakly acid silicate or carbonate impurities in the ionic strength adjustment salt or water, or slow leaching of the borosilicate titration vessel, as has been noted elsewhere (36). Such impurities produce slight buffer action for solutions between pH 5 and 8 but do not significantly affect values outside this range. However, neglect of their presence, but not refining H_{tot}, leads to high values of *U* (residual sum of squares, $\sim 10^{-7}$ to 10^{-6}) and *R* (Hamilton *R* factor, ~ 0.003 to 0.010). Refinement of H_{tot} often improved the degree of fit indicators, e.g. $U \sim 10^{-12}$ to 10^{-9}, $R \sim 0.0003$ to 0.001, and formation constant error estimates, without significantly altering the constants themselves.

Formation constants of the species MeHgI, MeHgOH, and $(MeHg)_2OH^+$ and protonated forms of thiol were fixed in MINIQUAD 81 refinements of MeHg(II) thiolate formation constants. Ternary complexes of the type MeHgI(SR) were rejected by MINIQUAD 81.

A typical example of fit between experimental and calculated titration curves is shown in Fig. 1.

Results and discussion

Acid dissociation and formation constants

Acid dissociation constants (p*K*) for the ligands are given in Table 1, and agree well with previously reported values. Dissociation constants for the thiol groups are given in italics, but since the potentiometric method yields macroscopic constants the two highest values for L-cysteine, D,L-penicillamine, and glutathione are composite macroscopic constants due to simultaneous dissociation of NH_3^+ and SH groups over the same pH region.

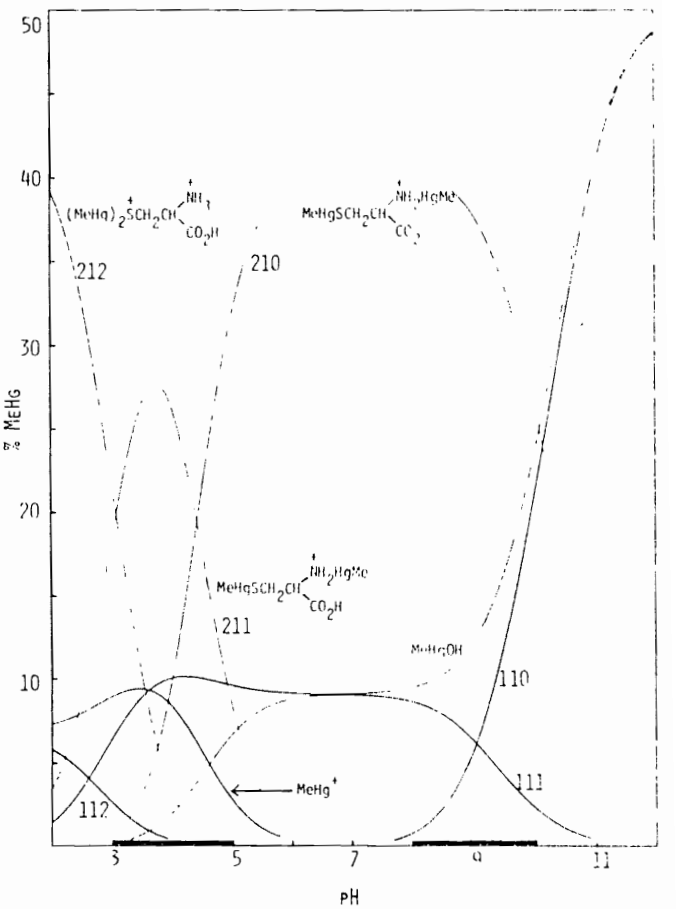

FIG. 2. Species distribution for 0.002 *M* MeHg(II) and 0.001 *M* L-cysteine. The heavy line indicates the pH range of MINIQUAD 81 data. Complex 112: $MeHgSCH_2CH(\overset{+}{N}H_3)(CO_2H)$, 111: $MeHgSCH_2CH(NH_3^+)CO_2^-$; 110: $MeHgSCH_2CH(NH_2)CO_2^-$.

Formation constants for the complexes are given in Table 2, and are in the form log β_{mlh} for equilibria [5], where RS^{n-} represents the fully deprotonated form of the thiol ligand (at pH ca. 12), e.g. 2-mercaptoethanol $^-SCH_2CH_2OH$, L-cysteine $^-SCH_2CH(NH_2)CO_2^-$, and glutathione $^-O_2C(NH_2)CHCH_2CH_2CONHCH(CH_2S^-)CONHCH_2CO_2^-$.

$$[5]\quad m\,MeHg^+ + l\,RS^{n-} + h\,H^+ \rightleftharpoons (MeHg)_m(RS)_l(H)_h^{m+h-nl}$$

$$[6]\quad \beta_{mlh} = \frac{[(MeHg)_m(RS)_l(H)_h^{m+h-nl}]}{[MeHg^+]^m[RS^-]^l[H^+]^h}$$

Thus, the equilibrium constant for equation [1] is described by β_{110}. Dissociation of a proton from a coordinated ligand is usually represented as p*K* of the protonated form of the complex, and may be expressed in terms of β_{mlh}, e.g. for the 2-mercaptoacetic acid complex the dissociation

$$MeHgSCH_2CO_2H \rightleftharpoons MeHgSCH_2CO_2^- + H^+$$

is described by $pK_{111} = \log \beta_{111} - \log \beta_{110}$. Values of log β_{110} are in excellent agreement with the results of Reid and Rabenstein (9), Schwarzenbach and Schellenberg (4), and Jawaid and Ingman (5); and thus support early determinations, involving other biological thiols, by Hughes (2) and Simpson (3).

Sulfhydryl-containing amino acids and peptides

Proton nmr spectroscopic studies indicate that the deprotonated sulfhydryl group of *N*-acetyl-L-cysteine, when coor-

dinated to MeHg(II), is able to coordinate to an additional MeHg(II) group (62); and Rabenstein and Fairhurst (63) obtained ^{1}H nmr evidence for similar binding by glutathione in the pH range 0–4. For glutathione at higher pH nmr spectra suggest binding of the second MeHg(II) group to the amine group (63). Several MeHg(II) and PhHg(II) complexes of L-cysteine and D,L-penicillamine with two organomercury groups have been isolated (8, 64, 65) and a MeHg(II) D,L-penicillamine complex isolated from an aqueous solution has been found from an X-ray crystallographic study to have sulfur and amine bound MeHg(II) groups, $MeHgSCMe_2CH(\dot{N}H_2HgMe)CO_2^-$ (64).

Since the ^{1}H nmr and crystallographic results indicate that complexes of 2:1 stoichiometry have structures that differ with pH, evidence was sought for complexes of these stoichiometries with a representative sulfhydryl containing amino acid, L-cysteine, using the potentiometric approach. Solutions containing L-cysteine with more than one equivalent of MeHg(II) were investigated by titration in the absence of iodide. Under these conditions, the sulfur atom may be regarded as "always coordinated", and equilibria were treated assuming a "ligand" of the form $MeHgSCH_2CH(NH_2)CO_2^-$. The data could be satisfactorily refined to give pK_{110} 8.936 (6) (c.f. 9.01 derived from log β_{110} and log β_{111} (Table 2)), pK_{111} 11.60 (2); and for log β'_{mlh} where l' = $MeHgSCH_2CH(NH_2)CO_2^-$ log β'_{110} 8.800 (7), log β'_{111} 13.221 (13), log β'_{112} 15.67 (12), giving derived values of log β_{210} 25.26 (2), log β_{211} 29.69 (3), and log β_{212} 32.13 (13). Thus, for the equilibrium of interest at low pH

$$MeHgSCH_2CH(\dot{N}H_3)(CO_2H) + MeHg^+ \rightleftharpoons (MeHg)_2\dot{S}CH_2CH(\dot{N}H_3)(CO_2H)$$

$$\log K = \log \beta_{212} - \log \beta_{112} \approx 4.1$$

and at higher pH

$$MeHgSCH_2CH(NH_2)CO_2^- + MeHg^+ \rightleftharpoons MeHgSCH_2CH(\dot{N}H_2HgMe)CO_2$$

$$\log K = \log \beta_{210} - \log \beta_{110} = 8.8$$

Formation constants for complexes of the type $(MeHg)_2\dot{S}R$ have not been previously reported, but for the proposed equilibrium at higher pH the value of 8.8 compares well with those of 7.88 (ref. 66) and 7.55 (ref. 33) for interaction of MeHg(II) with glycine to form $O_2CCH\dot{N}H_2HgMe$. A species distribution diagram (Fig. 2) calculated for a 2:1 ratio of MeHg(II):L-cysteine at 0.002 *M* MeHg(II) illustrates occurrence of these complexes in different pH ranges and, depending on pH, presence of appreciable proportions of the complexes of 1:1 stoichiometry.

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1. W. L. Hughes. Ann. N.Y. Acad. Sci. **65**, 454 (1957); J. T. McGregor and T. W. Clarkson. Adv. Exptl. Med. Biol. **48**, 64 (1974); A. J. Canty. Am. Chem. Soc. Symp. Ser. **82**, 327 (1978), A. J. Carty. Am. Chem. Soc. Symp. Ser. **82**, 339 (1978), D. L. Rabenstein. Acc. Chem. Res. **11**, 100 (1978).
2. W. L. Hughes, Jr. Cold Spring Harbour Symp. Quant. Biol. **14**, 79 (1950).
3. R. B. Simpson. J. Am. Chem. Soc. **83**, 4711 (1961).
4. G. Schwarzenbach and M. Schellenberg. Helv. Chim. Acta, **48**, 28 (1965).
5. M. Jawad and F. Ingman. Talanta, **28**, 137 (1981).
6. G. Schwarzenbach and U. Karlen. Unpublished results. H. Gross. Unpublished results.
7. G. Geier and I. W. Erni. Chimia, **27**, 635 (1973).
8. Y. Hoji, Y. Sugiura, and H. Tanaka. J. Inorg. Nucl. Chem. **38**, 641 (1976).
9. R. S. Reid and D. L. Rabenstein. Can. J. Chem. **59**, 1505 (1981).
10. D. L. Rabenstein, A. A. Isab, and R. S. Reid. Biochim. Biophys. Acta, **720**, 53 (1982).
11. F. Bakir, S. F. Damluji, L. Amin-Zaki, M. Murtadha, A. Khalidi, N. Y. Al-Rawi, S. Tikriti, H. I. Dhahir, T. W. Clarkson, J. C. Smith, and R. A. Doherty. Science, **181**, 230 (1973); J. Aaseth. Acta Pharmacol. Toxicol. **39**, 289 (1976); T. W. Clarkson, L. Magos, C. Cox, M. R. Greenwood, L. Amin-Zaki, M. A. Majeed, and S. F. Damluji. J. Pharmacol. Exp. Ther. **218**, 74 (1981).
12. J. Aaseth, A. Wannag, and T. Norseth. Acta Pharmacol. Toxicol. **39**, 302 (1976).
13. Aldrich Chemical Co. Ltd. Catalog of fine chemicals, 1981–2.
14. H. Barrera and R. E. Lyle. J. Org. Chem. **27**, 641 (1962).
15. I. W. Erni. Ph.D. Dissertation, ETH, Zurich, 1977.
16. G. Geier, I. W. Erni, and R. Steiner. Helv. Chim. Acta, **60**, 9 (1977).
17. G. Gran. Acta Chem. Scand. **4**, 559 (1950), Analyst, **77**, 661 (1952); A. Johansson and G. Gran. Analyst, **105**, 802 (1980); G. Gran and A. Johansson. Analyst, **106**, 231 (1981); L. Pehrsson, F. Ingman, and A. Johansson. Talanta, **23**, 769 (1976).
18. D. D. Perrin and I. G. Sayce. Chem. Ind. 661 (1966).
19. Pure Appl. Chem. **51**, 1 (1979).
20. H. M. N. H. Irving, M. G. Miles, and L. D. Pettit. Anal. Chim. Acta, **38**, 475 (1967).
21. W. A. E. McBryde. Analyst, **94**, 337 (1969); **96**, 739 (1971).
22. G. R. Hedwig and H. K. J. Powell. Anal. Chem. **43**, 1206 (1971).
23. G. Nowogrocki, J. Canonne, and M. Wozniak. Bull. Soc. Chim. Fr. **1**, 5 (1976); **1**, 1369 (1976).
24. H. S. Rosotti. Talanta, **21**, 809 (1974).
25. F. H. Sweeton, R. E. Mesmer, and C. F. Baes. J. Solution Chem. **3**, 191 (1974).
26. L. M. Schwartz and R. I. Gelb. Anal. Chem. **50**, 1571 (1978).
27. G. Arena, E. Rizzarell, S. Sammartano, and C. Rigano. Talanta, **26**, 1 (1979).
28. M. Wozniak and G. Nowogrocki. Talanta, **26**, 1 (1979).
29. T. D. Waugh, H. F. Walton, and J. A. Laswick. J. Phys. Chem. **59**, 395 (1955).
30. P. Zanella, G. Plazzogna, and G. Tagliavini. Inorg. Chim. Acta, **2**, 340 (1968).
31. S. Libich and D. L. Rabenstein. Anal. Chem. **45**, 118 (1973).
32. F. Ingman and D. H. Liem. Acta Chem. Scand. **28A**, 947 (1974).
33. M. Jawad, F. Ingman, and D. H. Liem. Acta Chem. Scand. **32A**, 333 (1978).
34. R. N. Sylva and M. R. Davidson. J. Chem. Soc. Dalton, 465 (1979).
35. P. Gans, A. Sabatini, and A. Vacca. Inorg. Chim. Acta, **18**, 237 (1976).
36. L. Ciacatta. Arkiv. Kemi. **20**, 417 (1963).
37. S. J. Backs and D. L. Rabenstein. Inorg. Chem. **20**, 410 (1981).
38. P. J. Antikainen and K. Tevanen. Suomen Kemi. **35B** 224 (1962).
39. R. J. Irving, L. Nelander, and I. Wadso. Acta. Chem. Scand. **18**, 769 (1964).
40. D. L. Leussing and I. M. Kolthoff. J. Am. Chem. Soc. **75**, 3904 (1953).
41. N. C. Li and R. A. Manning. J. Am. Chem. Soc. **77**, 5225 (1955).

42. D. L. LEUSSING and L. NEWMAN. J Am. Chem. Soc. **78**, 552 (1956).
43. D. L. LEUSSING. J. Am Chem Soc **80**, 4180 (1958).
44. J. L. KURZ and J. C. HARRIS. J Org. Chem **35**, 3086 (1970).
45. G. R. LENZ and A. E. MARTELL. Inorg. Chem. **4**, 378 (1965).
46. G. E. CHENEY, Q. FERNANDO, and H. FREISER. J. Phys. Chem. **63**, 2055 (1959).
47. L. J. PERRIN and D. D. PERRIN. Aust. J. Chem. **22**, 267 (1969).
48. J. H. RITSMA and F. JELLINEK. Rec. Trav. Chim. Pays-Bas, **91**, 923 (1972).
49. L. PILLAI, R. D. BOSS, and M. S. GREENBERG. J Solution Chem. **8**, 635 (1979).
50. G. R. LENZ and A. E. MARTELL. Biochemistry, **3**, 745 (1969).
51. E. J. KUCHINSKAS and Y. ROSEN. Arch. Biochem Biophys. **97**, 370 (1962).
52. E. W. WILSON and R. B. MARTIN. Arch. Biochem. Biophys. **142**, 445 (1971).
53. N. KOJIMA, Y. SUGIURA, and H. TANAKA. Bull. Chem. Soc. Jpn. **49**, 445 (1976).
54. D. A. DOORNBOS. Pharm. Weekblad. **102**, 269 (1967).
55. R. L. COATES and M. M. JONES. J Inorg. Nucl. Chem. **39**, 677 (1976); J. S. CASAS and M. M. JONES. J. Inorg. Nucl. Chem. **42**, 99 (1980).
56. D. L. RABENSTEIN. J. Am. Chem Soc **95**, 2797 (1973).
57. N. C. LI, O. GAWRON, and G. BASCUAS. J. Am. Chem Soc. **76**, 225 (1954).
58. R. B. MARTIN and J. T. EDSALL. Bull. Chem Soc Chim Biol **40**, 1763 (1958).
59. L. LARSSON and B. HANSEN. Svensk. Kem. Tidskr **68**, 521 (1956).
60. YA. L. KOSTYUKOVSKII, YU. A. BRUK, A. V. KOKUSHKINA, B. S. MIRKIN, N. M. SLAVACHEVSKAYA, L. M. PAVLOVA, and I. A. BELEN'KAYA. Zh. Obshch Khim **42**, 2104 (1972). Chem Abstr. **78**, 42615v (1972).
61. C. W. DAVIES. J Chem. Soc. 2093 (1938).
62. P. G. SIMPSON, T. E. HOPKINS, and R. HAQUE. J. Phys Chem **77**, 2282 (1973).
63. D. L. RABENSTEIN and M. T. FAIRHURST. J Am. Chem Soc. **97**, 2086 (1975).
64. Y. S. WONG, A. J. CARTY, and C. CHIEH. J. Chem. Soc Dalton, 1801 (1977).
65. A. J. CANTY and R. KISHIMOTO. Aust. J Chem. **30**, 669 (1977).
66. D. L. RABENSTEIN, R. OZUBKO, S. LIBICH, C. A. EVANS, M. T. FAIRHURST, and C. SUVANPRAKORN. J. Coord. Chem. **3**, 263 (1974).