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Solution Equilibria and Structures of Disulfhydryl-containing Peptide–Indium (III) and Gallium (III) Complex Species

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Solution equilibria of the In(III) and Ga(III) complex species of 2,3-dimercaptopropionylglycine (DMPG) and 2-mercaptopropionyl-L-cysteine (MPC) have been investigated by potentiometry in aqueous solution. The formation constants of various species and their distribution as a function of pH were evaluated with a non-linear least-squares treatment of the potentiometric data. The structural features and the complexation mechanisms were proposed through the interpretation of the results of the potentiometric and infrared spectral (in D_2O) studies. In the various protonated species formed in the In(III) solutions of DMPG and MPC, the carboxyl and sulfhydryl groups are protonated, respectively. In the 1: 1 DMPG–In(III) system, the stable In(OH)L species formed even in the alkaline solution. Ga(III) resists complexation with monosulfhydryl-containing ligands in contrast to In(III), but forms the complex with disulfhydryl-containing ligands in spite of the occurrence of the hydrolytic precipitation during part of the titration of the MPC–Ga(III) solution. Once formed Ga(III) complex is stable as well as In(III). Two sulfhydryl groups of peptides were required for complexation, even in the In(III) case. In all cases, no deprotonation of the peptide-amide proton was observed. The potentiometric calculations gave the large values of the equilibrium constants of the complexes, indicating that the disulfhydryl-containing peptides are remarkably effective towards the formation of the complexes of In(III) and Ga(III) in aqueous solution.

Keywords——sulfhydryl-containing peptide; In(III) complex; Ga(III) complex; radiopharmaceutical; potentiometric titration; IR spectra; non-linear least-squares computer calculation

A series of studies on the complex formation of In(III) and Ga(III) with some complexing agents in aqueous solution has been carried out previously,[2–4] in connection with the development of the radiopharmaceuticals labelled with ^{111}In, ^{113m}In, and ^{67}Ga for the imaging of various organs. As predicted from these studies, sulfhydryl-containing peptide is regarded as a kind of ligand of interest whose In(III) (or Ga(III)) complex may be stable enough to consider the selective tissue-localization. It has been indicated by the proton magnetic resonance (PMR) study[4] that some disulfhydryl-containing peptides such as 2,3-dimercaptopropionylglycine (DMPG) and 2-mercaptopropionyl-L-cysteine (MPC) form the 2: 1 peptides–In(III) or Ga(III) complexes involving a fused–chelate ring. On the contrary, monosulfhydryl–containing peptides such as 2(or 3)-mercaptopropionylglycine and N-mercaptoacetyl-L-histidine are inactive to In(III) and Ga(III).

In the present work, solution equilibria and structures of the In(III) and Ga(III) complexes of these disulfhydryl–containing peptides are discussed based on the results of the potentiometric and infrared (IR) spectral studies.

Experimental

Materials——Synthesis[4] of peptides and preparations[2] of the In(III) and standard KOH solutions have been reported previously. The 0.01 M Ga(III) solution was prepared from gallium metal (99.99%, Mitsuwa

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[1] Location: *Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto 606, Japan.*
[2] N. Kojima, Y. Sugiura, and H. Tanaka, *Bull. Chem. Soc. Jpn.*, **49**, 1294 (1976).
[3] N. Kojima, Y. Sugiura, and H. Tanaka, *Bull. Chem. Soc. Jpn.*, **49**, 3023 (1976).
[4] Y. Sugiura, N. Kojima, and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2263 (1977).

Chemicals) with the concentrated hydrochloric acid and standardized by 8-quinolinol method.[5] The concentrations of peptides and hydrochloric acid in the Ga(III) solution were determined potentiometrically. Indium sulfate, D_2O, DCl, and NaOD (99.5% isotopically pure) were used for the IR measurement. All the other reagents used were of a reagent grade.

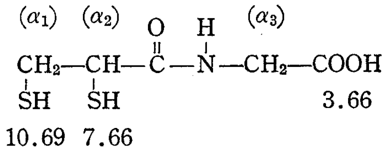
Infrared Spectra——The IR spectra on 0.1 M D_2O solution of the ligands with or without In(III) were measured in a CaF_2 cell with a light path of 0.1 mm. The value of pD was obtained by the addition of 0.44[6] to the value indicated by the pH meter. The details of the IR measurement were described previously.[3]

Potentiometric Titrations——The potentiometric study was performed in the presence of 0.1 M KNO_3 at 20 ± 0.05 °. The total volume was adjusted to 20.00 ml. The apparatus and procedure are given previously.[2] Formation constants ($\beta_{pqr}=[M_pH_qL_r]/[M]^p[H]^q[L]^r$) were refined from a non-linear, least-squares computer program[2] on a FACOM M-190 computer, Kyoto University. The values of $pK\text{w}=14.173$ and $f_{\pm}=0.84$ were obtained from the titration data for 0.0025 M HNO_3 ($I=0.1$ (KNO_3), 20°) and used in the calculation.

Results and Discussion

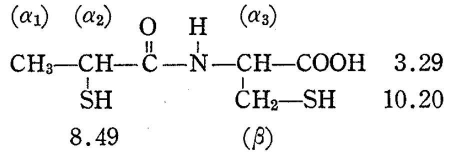
Proton–Peptide Systems

The proton–peptide systems were studied by titrating solutions containing the protonated ligands (0.0015 M) with standard KOH. The results of several titrations were averaged, and the evaluation of the dissociation constants was accomplished by an iterative least squares method which accepts a crude first approximation of the constants to be evaluated. The resulting pKa values for DMPG and MPC were assigned by the PMR study[7] as shown in Charts 1 and 2, respectively. Estimated standard deviations for each value were less than 0.01.



$$\begin{array}{llllll} (\alpha_1) & (\alpha_2) & O & H & (\alpha_3) & \\ CH_2- & CH- & \overset{\|}{C}- & \overset{|}{N}- & CH_2- & COOH \\ \overset{|}{SH} & \overset{|}{SH} & & & & 3.66 \\ 10.69 & 7.66 & & & & \end{array}$$

Chart 1



$$\begin{array}{lllllll} (\alpha_1) & (\alpha_2) & O & H & (\alpha_3) & & \\ CH_3- & CH- & \overset{\|}{C}- & \overset{|}{N}- & CH- & COOH & 3.29 \\ & \overset{|}{SH} & & & \overset{|}{CH_2}- & SH & 10.20 \\ & 8.49 & & & (\beta) & & \end{array}$$

Chart 2

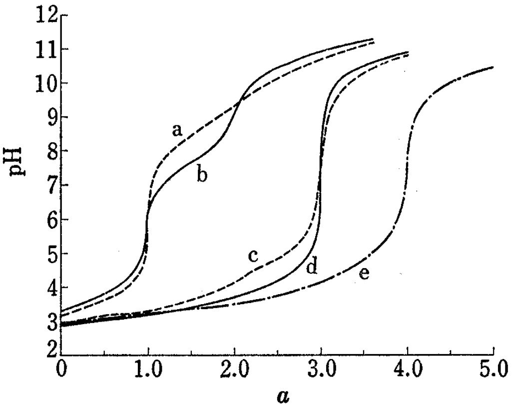


Fig. 1. Titration Curves of 2-Mercaptopropionyl-L-cysteine (MPC) and 2,3-Dimercaptopropionylglycine (DMPG) with or without In(III) (0.0005M)

(a), MPC(0.0015M); (b), DMPG(0.0015M); (c), MPC (0.001M) and In(III); (d), DMPG(0.001M) and In(III); (e), DMPG(0.0005M) and In(III).

In(III)–Peptide System

Figure 1 shows the titration curves of 1:1 and 2:1 peptides to In(III) (0.0005 M). The moles of total proton liberation in the 2:1 solution were 3, indicating the formation of the final species, InL_2. The formation of the complex species, InL_3, was not recognized even in the 4:1 solution. In the MPC: In(III)=1:1 system, precipitation of the indium hydroxide occurred from the second buffer region (pH>4), consistent with the usual observation under the ligand-deficient condition. In the DMPG: In(III)=1: 1 system, in

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[5] Lószló Erdey, "Gravimetric Analysis," Part II, Pergamon Press, New York, N.Y., 1965.

[6] K. Mikkelsen and S.O. Nielsen, *J. Phys. Chem.*, **64**, 632 (1960).

[7] The α_2 proton chemical shifts of both peptides are affected in somewhat lower pH range than the α_1 (DMPG) or β (MPC) proton.

contrast, hydrolytic precipitation was not observed and 4 protons were liberated by the complexation. This additional proton liberation suggests the formation of the stable hydrolyzed species, In(OH)L(L=DMPG), or the deprotonated species of the peptide-amide proton, In(L–H). These two possible species are indistinguishable each other from the computer calculation of the constants. Calculation of the stability constants of the InL and InL_2 species was attempted by the Irving–Rossotti method,[8] and the resulting values were used as the initial estimates of the unknown constants for the complexes. The other estimates were obtained by the method described elsewhere.[2] The best fit to the experimental results was sought for by the use of the computer program with an automatic refinement of the formation constants.

DMPG–In(III) Complexes

In the 1:1 DMPG–In(III) solution, calculation based on the assumption that probable complex species were InL and In(OH)L gave appreciably large standard deviation in titre(0.0066 ml)(Table I, Trial 1). The fit was improved by the introduction of the protonated species, InHL(Trial 2). No further improvement was obtained in consideration of the polynuclear species such as In_2L_2, In_3L_3, and $In_2(OH)_2L_2$, *etc.*

TABLE I. Values (Logarithmic Units) of the Formation Constants in the Successive Trials for the In(III) (0.0005 M)–2,3-Dimercaptopropionyiglycine System[a]

Trial	Complex species						Std. dev. in titre (ml)
	InL	InL_2	In(OH)L	InHL	In(HL)L	$In(HL)_2$	
(L/In=1/1)							
1	16.733 (0.033)		11.980 (0.060)				0.0066
2	17.082 (0.038)		12.444 (0.049)	20.086 (0.076)			0.0020
(L/In=2/1)							
1	17.31 (0.10)	31.57 (0.10)					0.0141
2	16.881 (0.019)	30.949 (0.024)	13.183 (0.015)				0.0034
3	17.104 (0.048)	31.245 (0.059)	13.620 (0.071)	19.79 (0.11)			0.0022
4	17.249 (0.036)	31.462 (0.041)		19.722 (0.083)	35.571 (0.050)		0.0013
5		32.54 (0.34)		21.27 (0.35)	36.86 (0.35)	40.46 (0.33)	0.0015
6	17.273 (0.059)	31.54 (0.15)		19.90 (0.31)	35.66 (0.17)	38.36 (0.94)	0.0014
7	17.241 (0.039)	31.435 (0.056)	12.33 (0.57)	19.719 (0.085)	35.543 (0.070)		0.0014

a) Standard deviations in parentheses.

In the 2:1 solution, on the other hand, calculations concerning Trials 1—3 gave the large values of the standard deviation in titre. The chemical model of InL, InL_2, and InHL failed to converge. Although the standard deviations in titre were good in Trials 5—7, those in each constant were fairly large. Trial 4 gave the acceptable data fit. In this case, the In(OH)L species was considered to be absent in the 2:1 (L to In(III)) solution. Figure 2 shows the distribution diagram based on the constants of Trial 4. The two protonated species, InHL and In(HL)L, exhibit maximum abundances at $pH<3$ and 4, respectively, where the carboxyl group ($pKa_1=3.66$) is not fully ionized. An eight-membered chelate

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[8] H. Irving and F.J.C. Rossotti, *J. Chem. Soc.*, **1953**, 3397.

complex in which the sulfhydryl group at 2-position and the terminal carboxyl group are coordinated to the metal ion is regarded to be of low stability. In the protonated species, InHL and In(HL)L, therefore, it is reasonably assumed that the carboxyl group of the coordinating ligand is protonated. This assumption is supported by the following discussion. The stability constant $K^{\mathrm{In}}_{\mathrm{InHL}}$ in the reaction

$$\mathrm{In^{3+} + HL^{2-} \rightleftharpoons InHL^{+}}$$

is calculated by the following equation:

$$K^{\mathrm{In}}_{\mathrm{InHL}} = \frac{[\mathrm{InHL}]}{[\mathrm{In}][\mathrm{HL}]} = \frac{[\mathrm{InHL}]}{[\mathrm{In}][\mathrm{H}][\mathrm{L}]} \cdot \frac{[\mathrm{H}][\mathrm{L}]}{[\mathrm{HL}]}$$
$$= \beta \cdot K\mathrm{a}$$

where β and Ka are the formation constant of InHL and the dissociation constant of the carboxyl group defined by the next reactions, respectively.

$$\mathrm{In^{3+} + H^{+} + L^{3-}} \overset{\beta}{\rightleftharpoons} \mathrm{InHL^{+}}$$
$$\mathrm{H^{+} + L^{3-}} \overset{K\mathrm{a}}{\rightleftharpoons} \mathrm{HL^{2-}}$$

Using the calculated values of log β=19.72 and pKa=3.66, log $K^{\mathrm{In}}_{\mathrm{InHL}}$ was obtained as 16.06. On the other hand, the stability constant log K_1=17.14±0.02 of the In(III) complex with 2,3-dimercapto-1-propanol (p$K\mathrm{a}_1$=8.79±0.01, p$K\mathrm{a}_2$=10.81±0.01) was determined from the titration data for the 0.001 M ligand and 0.0005 M In(III) solution. The fairly good agreement between the values of $K^{\mathrm{In}}_{\mathrm{InHL}}$ and K_1 reveals that In(III) in the protonated species is coordinated with the two sulfhydryl groups in a similar fashion to the 2,3-dimercapto-1-propanol–In(III) complex. The polynuclear species were not detected in the system.

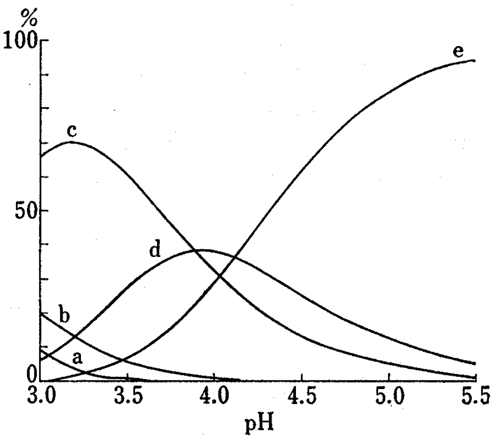

Fig. 2. Variation with pH of the Percentage of In(III) Bound in the Form of the Various Complexes in the System 2,3-Dimercapto-propionylglycine (0.001 M)–In(III) (0.0005 M)

(a), $\mathrm{In^{3+}}$; (b), InHL; (c), InL; (d), In(HL)L; (e), $\mathrm{InL_2}$.

IR spectra for ligand itself, 2:1, and 1.3:1 DMPG–In(III) systems in $\mathrm{D_2O}$ were measured in the carbonyl absorption region (Table II). Two characteristic bands at 1715 and 1635 cm^{-1} (metal-free system, a=0) are assigned to be due to the protonated carboxyl and peptide (amide I) groups. Amide II band which is located at 1550 cm^{-1} in the spectrum of ligand

TABLE II. Infrared Bands of Carboxylates and Peptide Amides in $\mathrm{D_2O}$ Solutions of 2,3-Dimercaptopropionylglycine and Its In(III) Complexes

System	a value	Absorption bands, cm^{-1}			
		–COOH	Amide I	–COO$^-$	Amide II′
L	0	1715	1635		1458
	1		1635	1587	1458
	2		1625	1583	1440
	3		1620	1580	1430
L/In=2/1	0	1715	1625	1598	1453
	1	1715	1620	1595	1450
	2		1620	1590	1450
	3		1620	1590	1450
L/In=1.3/1	(pD 6.99)		1620	1590	1450

recorded by Nujol mul method shifts, on deuteration, to 1450 cm^{-1} (amide II′).[9] Along with the disappearance of the band at 1715 cm^{-1}, a new band (1587 cm^{-1}) assigned to the deprotonated carboxyl group appears at $a=1$, and shifts slightly towards lower frequency as the sulfhydryl groups release their protons. Considering the frequency shift during titration of glycine peptides,[10] the large shift in amide I band can be also attributed to the deprotonation of the sulfhydryl groups. In the 2:1 DMPG–In(III) system, the coordination of the sulfhydryl groups to In(III) is evident from the amide I band shift from 1635 cm^{-1} (free ligand) to 1625—1620 cm^{-1} in the pD region of 1.49 ($a=0$), 3.77 ($a=1$), and 5.94 ($a=2$) where the shift is not yet observed in the metal-free system. Appearance of the carboxylate ion band (1598 cm^{-1}) at $a=0$ indicates the coordination of the carboxyl group to In(III), because the group is protonated in the pD region in the metal-free system. The formation of an eight-membered chelate ring is probably stabilized by the coordination of the disulfhydryl groups. In addition, the InL complex is considered to be soluble under the condition of the concentrated 0.1 M solution in spite of zero charge, because of flexible coordination of the carboxyl group. The amide II′ band is known to disappear as a result of participation of the peptide amide nitrogen in the coordination to metal ion.[11] In the 2:1 DMPG–In(III) system, the amide II′ band is observed over the range of the a-values. Therefore, it is reasonable that the In(L–H) species is not formed. This is consistent with the result of the potentiometric study for the 2:1 solution. In the 1:1 solution, the precipitation was occurred under the condition of 0.1 M on the contrary to that of 0.001 M. The IR spectra were recorded at pD 6.99 for the 1.3:1 (L to In(III)) solution in which no precipitation occurred and the InL$_2$ species is considered to be almost absent. The amide II′ band is also observed in this case. The results support that the species formed at the end of the titration for the 1:1 solution is not In(L–H) but In(OH)L(Chart 3). These two species were indistinguishable each other from the potentiometric calculations. On the basis of these results, the coordination process in the 2:1 DMPG–In(III) solution is proposed as shown in Chart 4.

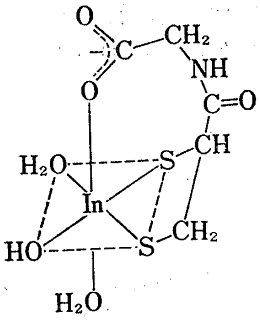

Chart 3

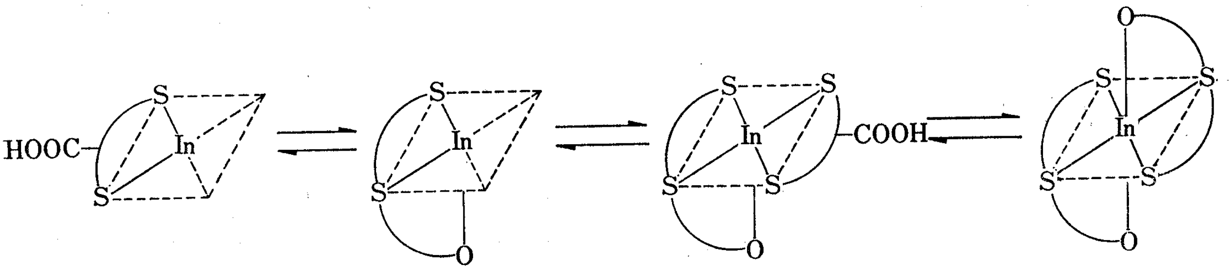

Chart 4

MPC–In(III) Complexes

A similar analysis of the titration curve for the 2:1 system of MPC: In(III) gave the two possible chemical models (Table III, Trials 5 and 6). The model of InL, InL$_2$, InHL, In(HL)L, and In(HL)$_2$ was non-converged. No polynuclear species were detected by the calculation in this case too. In the concentrated solution (0.1 M) an insoluble complex (probably non-charged InL complex) was formed over the a-values range of 0.1—2.5. This

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9) T. Miyazawa, T. Shimanouchi, and S. Mizushima, *J. Chem. Phys.*, **24**, 408 (1956).
10) M.K. Kim and A.E. Martell, *J. Amer. Chem. Soc.*, **85**, 3080 (1963).
11) M. Tasumi, S. Takahashi, T. Nakata, and T. Miyazawa, *Bull. Chem. Soc. Jpn.*, **48**, 1595 (1975).

Table III. Values (Logarithmic Units) of the Formation Constants in the Successive Trials for the In(III) (0.0005 M)–2-Mercaptopropionyl-L-cysteine (0.001 M) System[a]

Trial	Complex species						Std. dev. in titre (ml)
	InL	InL_2	In(OH)L	InHL	In(HL)L	$In(HL)_2$	
1	16.289 (0.018)	29.030 (0.035)					0.0079
2	16.278 (0.019)	28.946 (0.051)	11.00 (0.22)				0.0079
3	16.378 (0.024)	29.150 (0.035)		19.084 (0.096)			0.0060
4	16.437 (0.010)	29.060 (0.015)	11.693 (0.028)	19.398 (0.029)			0.0021
5		29.277 (0.014)		19.568 (0.026)	34.323 (0.018)	38.750 (0.009)	0.0015
6	16.454 (0.008)	29.260 (0.012)		19.444 (0.022)	33.814 (0.025)		0.0014

[a] Standard deviations in parentheses.

observation suggests that the chemical model of Trial 6 is better than that of Trial 5. In fact, Trial 6 gave a slight improvement over Trial 5. Figure 3 shows the distribution diagram of the complexes obtained from Trial 6.

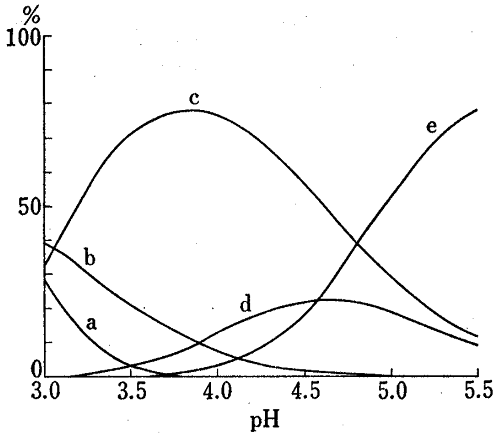

Fig. 3. Variation with pH of the Percentage of In(III) Bound in the Form of the Various Complexes in the System 2-Mercaptopropionyl-L-cysteine (0.001 M)–In(III)(0.0005 M)

(a), In^{3+}; (b), InHL; (c), InL; (d), In(HL)L; (e), InL_2.

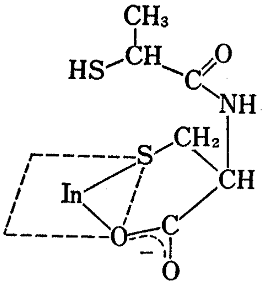

Chart 5

The IR spectrum measured at $a=0$ (pD 1.84) in the 2:1 solution was substantially the same as that of the DMPG–In(III) solution under similar condition. The protonated carboxyl, amides I and II′ bands are respectively observed at 1720, 1635, and 1460 cm^{-1}. The deprotonated carboxyl band (1598 cm^{-1}) appears even at $a=0$. This observation suggests the imvolvement of the carboxyl oxygen atom in the protonated complex species, InHL. In order to clarify this point, the stability constant of the InHL complex was calculated in the same manner as the case of DMPG, using the values of log $\beta=19.44$ and pKa$=8.49$ (the sulfhydryl group at 2-position), as follows:

$$K^{\mathrm{In}}_{\mathrm{InHL}} = \beta \cdot K\mathrm{a}$$
$$= 10^{19.44}/10^{8.49}$$
$$= 10^{10.95}$$

The value of 10.95 is comparable to the stability constant, log K_1=10.24±0.02 of the In(III) complex with N-acetyl-L-cysteine (pKa_1=3.20±0.01 and pKa_2=9.74±0.01, see Fig. 4). This result indicates that the sulfhydryl group at 2-position does not coordinate to In(III) in the protonated species, InHL(Chart 5) and In(HL)L. For the formation of the eight-membered chelate ring $\mathrm{In}{<}^{\mathrm{S}}_{\mathrm{S}})$ in the case of MPC, the proposed coordination mechanism is shown in Chart 6.

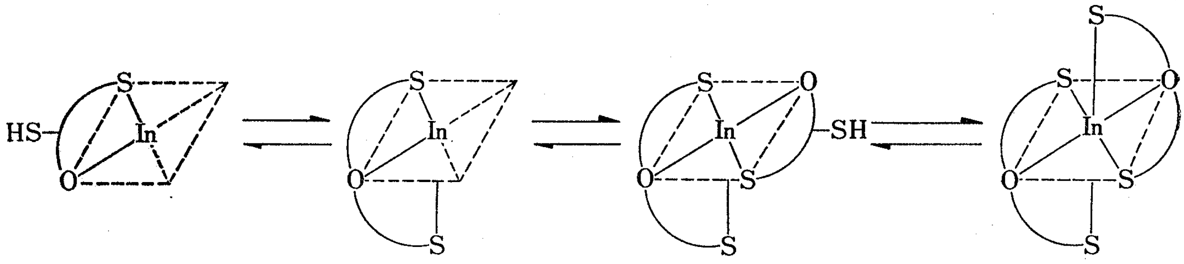

Chart 6

Ga(III)-Peptide Systems

Complicated features of the hydrolytic reaction of Ga(III) have been studied by various methods, such as NMR by the use of ^{17}O,[12] ^{1}H, and ^{71}Ga,[13] the light-scattering technique,[14,15] and potentiometric titration,[16] *etc.* Ga(III) is so hydrolyzable that hydrolyzed precipitation was not observed in dilute aqueous solution, or in highly alkaline solution on account of

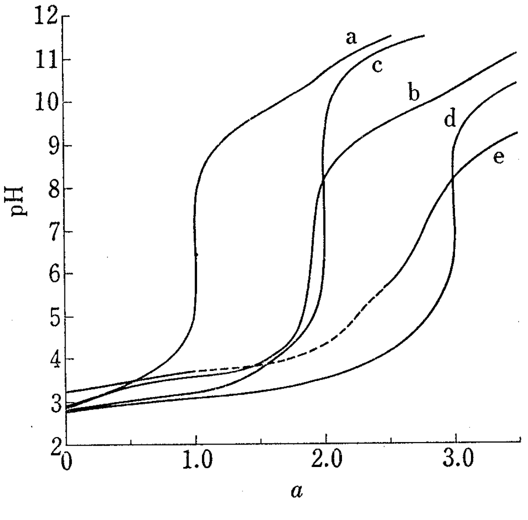

Fig. 4. Titration Curves of the Solutions Containing Ligands and Ga(III)

(a), N-acetyl-L-cysteine(0.003M); (b), N-acetyl-L-cysteine (0.003M) and Ga(III)(0.001M); (c), 2,3-dimercapto-1-propanol(0.002M) and Ga(III)(0.001M); (d) 2,3-dimercaptopropionylglycine(0.001M) and Ga(III)(0.0005M); (e), 2-mercaptopropionyl-L-cysteine(0.001M) and Ga(III)(0.0005M). Dotted line indicates the precipitation.

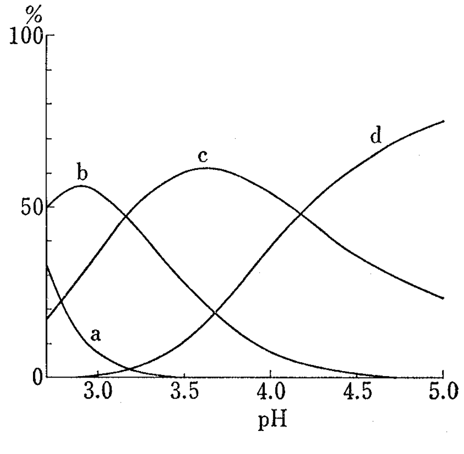

Fig. 5. Variation with pH of the Percentage of Ga(III) Bound in the Form of the Various Complexes in the System 2, 3-Dimercaptopropionylglycine (0.001 M)–Ga(III) (0.0005 M)

(a), Ga^{3+}; (b), GaL; (c), Ga(OH)L; (d), GaL_2.

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[12] D. Fiat and R.E. Connick, *J. Amer. Chem. Soc.*, **90**, 608 (1968).

[13] J.D. Glickson, T.P. Pinter, J. Webb, and R.A. Gams, *J. Amer. Chem. Soc.*, **97**, 1679 (1975).

[14] H.R. Craig and S.Y. Tyree, Jr., *Inorg. Chem.*, **8**, 591 (1969).

[15] F.G.R. Gimblett, *J. Inorg. Nucl. Chem.*, **33**, 2887 (1971).

[16] H. Gamsjäger, K. Aeberhard, and P. Schindler, *Helv. Chim. Acta*, **52**, 2315 (1969).

formation of the tetrahedral $Ga(OH)_4^-$ complex.[17] The studies on the complexes of Ga(III) have been almost limited to those in organic solvent,[18] because of their hydrolyzability and complexity, and little is known on the complex formation of Ga(III) in aqueous solution.

The titration curve of the 3 : 1 N-acetyl-L-cysteine–Ga(III) (0.001 M) is shown in Fig. 4(b). The pH inflection at $a=2$ seems to reflect the 3 : 1 complex formation. However, the curve in the a-value region of 2—3 can be superimposed on that of the ligand itself (Fig. 4(a)) in the region of 1—2. This result strongly indicates that the sulfhydryl group does not coordinate to Ga(III). The other monosulfhydryl-containing chelating agents were found to be unable to form the Ga(III) chelate. On the other hand, the disulfhydryl-containing ligands, such as 2,3-dimercaptosuccinic acid, 2,3-dimercapto-1-propanol, DMPG, and MPC (Fig. 4), can disrupt the hydration sphere of Ga(III) and bind to the metal ion. Because the precipitation occurred in the a-value range of 1—2.5 in the MPC–Ga(III) solution, calculation of the formation constants is impossible. Analysis of the titration data for the DMPG–Ga(III) system by analogy with the In(III) system gave the formation constants, 17.24±0.02(GaL), 31.91±0.05(GaL_2), and 14.07±0.02(Ga(OH)L) on the assumption that Ga(III) is present in the free ion form. As far as calculation was made on the assumption, minor species were not taken into account on the analysis. The equilibrium constants of the Ga(III) complexes were comparable to those of the In(III) complexes. Hence, once formed Ga(III) complexes are rather stable. Figure 5 shows the distribution diagram based on the obtained constants. In conclusion, the present potentiometric study shows that the disulfhydryl-containing peptides are remarkably effective ligands towards the complexation of In(III) and Ga(III).

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18) A.J. Carty, *Can. J. Chem.*, **45**, 345 (1967).