The Complexing of Indium(III) by Cysteine: Evidence for Sulfur-bridged and Protonated Complexes

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The complex formation of the cysteine–In(III) system has been investigated by potentiometric titration and by PMR, IR, and UV spectroscopic measurements. The InL, InL_2, and InLH species are predominantly formed at the metal concentrations from 0.00025 to 0.0005 M. At higher concentrations of the metal ion (0.0025—0.005 M), however, the InL species is negligible and the InL_2, InL_3, InL(LH), and $In(LH)_2$ species are predominant. The formation constants calculated with a non-linear, least-squares program were $\log \beta_{110}=14.12$, $\log \beta_{120}=27.26$, $\log \beta_{130}=32.20$, $\log \beta_{11-1}=18.46$, $\log \beta_{12-1}=31.78$, and $\log \beta_{12-2}=35.74$ for InL, InL_2, InL_3, InLH, InL(LH), and $In(LH)_2$ respectively. The IR and UV spectra revealed that the amino group of the coordinating cysteine was protonated. The $[In_3L_4(OH)(H_2O)]$ complex was isolated.

The study of the complex formation of In(III) with various ligands is needed for the development of new radiopharmaceuticals for clinical diagnosis by the use of ^{113m}In or ^{111}In, which are favorable radioisotopes and which can effectively be used with several chelating agents. We have studied the complex formation of In(III) with penicillamine.[1] In this paper, we will discuss the complex formation of In(III) with cysteine in comparison with that with penicillamine.

Experimental

Materials. All the details of the preparation and standardization of the metal and KOH solutions for pH titration have been reported in a preceding paper.[1] Indium sulfate, $In_2(SO_4)_3 \cdot 9H_2O$, was used for the PMR and UV measurements and for the isolation of the complex. L-Cysteine hydrochloride was dried under a vacuum over diphosphorus pentoxide, and the solution was standardized potentiometrically. The D_2O, DCl, and NaOD (99.5% isotopically pure) were obtained from E. Merck. All the other reagents used were of a reagent grade.

IR Spectra. The IR spectra of the solid were measured in a KBr disk by means of a Hitachi grating infrared spectrophotometer, Model 215. The IR measurements were also made on a D_2O solution of the 0.1 M ligand with or without 0.05 M In(III), in a KRS-5 cell with a light path of 0.1 mm. All the pD values were obtained by the addition of 0.44[2] to the pH values determined using a Hitachi-Horiba pH meter, Model F-7.

PMR Spectra. The PMR spectra were recorded with a Varian A-60 NMR spectrometer at an ambient probe temperature of 33 °C. The concentration of the In(III) ion was 0.052M, and the chemical shifts were measured with sodium 3-(trimethylsilyl)propionate-2,2,4,4-d_4 as the internal standard.

Optical Spectra. The optical spectra were measured in an aqueous solution by means of a Shimadzu recording spectrophotometer, Model Double-40R. Sample solutions were composed of cysteine hydrochloride (0.00016M) and a small amount of 1 M hydrochloric acid, with or without In(III) (0.00008 M), and were neutralized with 1 M potassium hydroxide.

Potentiometric Titrations and Calculations. The potentiometric titrations were performed with standard carbonate-free potassium hydroxide solutions(1.002 M and 0.1001 M) at 21.00±0.05 °C in the presence of 0.1 M potassium nitrate. The total volume was adjusted to 20.00 ml. The apparatus and procedure have been described.[1]

The titrations were carried out for solutions with a wide range of total metal concentrations in order to investigate the formation of hydroxo, protonated, and/or polynuclear species. The system containing polynuclear species does not give coincident formation curves. If hydroxo and/or protonated species are formed, complicated formation curves are to be expected since the free ligand concentration [L] calculated by the usual manner is incorrect. In such cases, a non-linear, least-squares computer program was used for the calculation of the stability constants, β_{lmn}:

$$\beta_{lmn} = [M_lL_mH_{-n}]/[M]^l[L]^m(H^+)^{-n}$$

where n is positive for a hydrolyzed species and negative for a protonated species, and where the parentheses denote activity. The calculations were carried out in a manner previously described[1] by the use of a FACOM 230-75 computer, Kyoto University. This program was also used for the calculation of the dissociation constants of the ligand. The values of $pK_w=14.130$[3] at 21 °C and $[H^+]=10^{-pH}$ were used in the calculation.

Results

Elemental Analysis and IR Spectra of an Isolated In(III) Complex. When a solution containing cysteine hydrochloride (0.1 M) and In(III) (0.052 M) in the ligand-to-metal ratio of 1.9: 1 was titrated with 8 M KOH, a large quantity of a white precipitate which was different from simple In(III) hydrolysate was formed at pH 3.9 and which redissolved at pH 5.2, whereas no precipitation was observed in the system containing the ligand (0.1 M) and In(III) (0.05 M) in the ligand-to-metal ratio of 2: 1. The precipitate formed at pH 4.8 was isolated and dried under a vacuum over diphosphorus pentoxide. Elemental analysis showed that the ligand: metal ratio was 1.3: 1: Found: C, 15.54; H, 3.19; N, 6.12; In, 38.29%. Calcd for $[In_3L_4(OH)(H_2O)\cdot 3H_2O]$: C, 15.83; H, 3.19; N, 6.16; In, 37.85%.

Table 1 shows some significant IR absorption bands of the isolated complex, together with those of the free ligand and the In(III) hydrolysate. In the isolated In(III)-cysteine complex, the coordination of the ligand to In(III) *via* the thiol, amino, and carboxyl groups is assumed on the basis of the disappearance of the SH, NH_3^+, and COOH absorptions, and the presence of NH_2 and COO^- absorptions. The coordina-

Table 1. Infrared spectra of the isolated In(III)-cysteine complex, cysteine hydrochloride and simple In(III) hydrolysate (cm^{-1})[a]

In(III) Complex	Cysteine Hydrochloride	In(III) Hydrolysate	Assignments
3210 s			NH_2 str.
	2980 s, b		NH_3^+ str.
	2560 m		SH str.
	1745 s		C=O str.
1610 s			COO^- anti. str.
	1600 w		NH_3^+ deg. def.
1590 s			NH_2 bend.
	1520 m		NH_3^+ sym. def.
	1405 m		C-O str., OH bend.
1390 m			COO^- sym. str.
	1230 s		NH_3^+ rock.
	1205 s		OH bend., C-O str.
1120 m		1120 s	InOH bend.
1060 m		1060 s	InOH bend.
	935 w		SH bend.

a) Abbreviations: s=strong, m=medium, w=weak, b=broad.

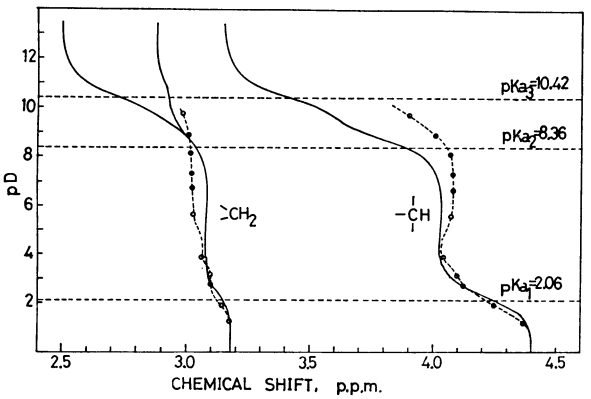


Fig. 1. Chemical shift data for cysteine (0.1 M) with (dashed lines) or without (solid lines) In(III) (0.052 M) at various pD values.

tion of the amino group indicates that the complex is not a non-charged protonated complex. In addition, the In(III) complex and the In(III) hydrolysate showed absorptions near 1100 cm^{-1}, which are assigned to the M-O-H bending mode.[4]

PMR Spectra of the 1.9: 1 Cysteine-In(III) System. Figure 1 shows the chemical-shift values of the CH and CH_2 protons of cysteine *versus* pD.[5] The dashed lines represent the titration curves of the 1.9: 1 cysteine-In(III) system. The constant chemical-shift values in the pD region from 5.5 to 8 reflect the $\widehat{SNO}$ coordination of the InL_2 complex species. In the higher pD region, the chemical-shift data suggest that some coordinated groups are released from the coordination sphere. The values of the chemical shift at about pD 4, obtained from the supernatant of the sample, are similar to those of the ligand. This indicates that the free ligand is present predominantly in the supernatant.

Potentiometric Titrations of the Cysteine-In(III) Systems. The acid dissociation constant for the carboxyl group of cysteine was obtained from the potentiometric titration data on the 0.05 M cysteine hydrochloride solution. The two remaining ionization constants, $pK_{a2}=8.36$ and $pK_{a3}=10.42$, were determined from the titration data for the 0.0015 M ligand solution by the usual linear least-squares method. These three constants were then refined by the non-linear FORTRAN IV computer program. The final values, with the standard deviations, are $\log \beta_{01-1}=10.42\pm0.01$, $\log \beta_{01-2}=18.78\pm0.01$, and $\log \beta_{01-3}=20.84\pm0.01$, at 21 °C and $I=0.1(KNO_3)$. The standard deviation in titre was 0.0029 ml. The pK_{a1} value of 2.06 was obtained from the $\log \beta_{01-3}$ and $\log \beta_{01-2}$ values. These constants are in good agreement with the reported values of $\log \beta_{01-1}=10.498$ and $\log \beta_{01-2}=18.833$ at 20 °C and $I=0.1(NaClO_4)$[6] and $pK_{a1}=1.96$ at 20 °C and $I=0.01$.[7]

The titration curve of the 4: 1 cysteine·HCl-In(III) system shows two distinct pH inflections, at $a=2$ and $a=2.75$, reflecting the formations of the InL_2 species and another species (probably InL_3) respectively; the latter was not observed in the penicillamine-In(III) system. Calculations of the stability constants for two simple species, InL and InL_2, were attempted by the Irving-Rossotti method,[8] but the consistency of the values obtained at each point was unsatisfactory. The pH titrations were carried out in the systems with various metal concentrations and metal-to-ligand ratios. The total metal concentrations $[In]_T$ were 0.00025, 0.0005, 0.0025, and 0.005 M, and the total ligand concentrations $[L]_T$ were 0.001, 0.001, 0.01, and 0.01 M respectively. The titration curves are shown in Fig. 2. Table 2 contains some data (94 points) relating to the four titration curves. The formation curves calculated in the usual manner, without assuming any hydroxo and protonated complexes, failed to be superimposed, and were not parallel to one another. The formations of the mononuclear hydroxo and protonated species were thus considered on the basis of the similarity to the penicillamine systems. These species were InL, InL_2, InLH, InL(LH), InL(OH), and $InL(OH)_2$. The best fit to the experimental results was sought for by the use of a non-linear computer program with an automatic

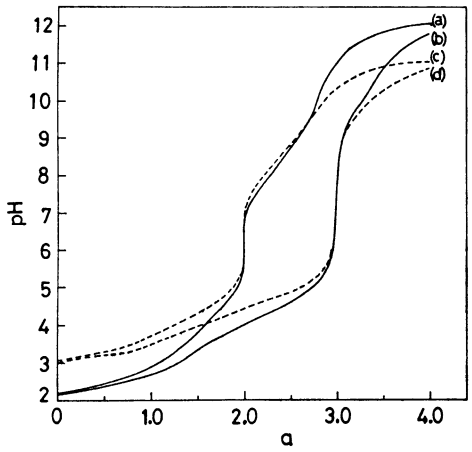


Fig. 2. Titration curves of the cysteine·HCl-In(III) systems. a=moles of KOH added per mole of the ligand. Experimental conditions for each curves ((a)—(d)) are given in Table 2.

TABLE 2. POTENTIOMETRIC DATA[a)]

Curve (a)			Curve (b)			Curve (c)			Curve (d)		
pH	V[b)]	Vc[c)]	pH	V[b)]	Vc[c)]	pH	V[b)]	Vc[c)]	pH	V[b)]	Vc[c)]
2.42	0.12	0.121	2.44	0.18	0.182	3.28	0.12	0.124	3.26	0.18	0.178
2.48	0.14	0.139	2.50	0.20	0.201	3.34	0.14	0.141	3.31	0.20	0.195
2.55	0.16	0.158	2.56	0.22	0.218	3.40	0.16	0.158	3.37	0.22	0.215
2.64	0.18	0.179	2.63	0.24	0.237	3.48	0.18	0.177	3.43	0.24	0.234
2.74	0.20	0.199	2.72	0.26	0.257	3.56	0.20	0.195	3.50	0.26	0.253
2.86	0.22	0.220	2.82	0.28	0.277	3.67	0.22	0.218	3.58	0.28	0.274
3.00	0.24	0.239	2.93	0.30	0.296	3.78	0.24	0.239	3.67	0.30	0.296
3.19	0.26	0.261	3.08	0.32	0.318	3.91	0.26	0.262	3.76	0.32	0.317
3.40	0.28	0.281	3.25	0.34	0.341	4.04	0.28	0.283	3.87	0.34	0.340
3.60	0.30	0.301	3.41	0.36	0.361	4.16	0.30	0.302	3.98	0.36	0.363
3.79	0.32	0.321	3.57	0.38	0.382	4.28	0.32	0.320	4.08	0.38	0.383
3.96	0.34	0.339	3.71	0.40	0.402	4.41	0.34	0.399	4.18	0.40	0.404
4.16	0.36	0.359	3.84	0.42	0.423	4.54	0.36	0.359	4.27	0.42	0.423
4.41	0.38	0.381	3.94	0.44	0.439	4.70	0.38	0.380	4.35	0.44	0.440
			4.05	0.46	0.459				4.44	0.46	0.460
			4.16	0.48	0.479				4.52	0.48	0.480
7.92	0.48	0.466	4.28	0.50	0.500	8.12	0.48	0.472	4.60	0.50	0.502
8.10	0.49	0.482	4.39	0.52	0.519	8.27	0.49	0.485	4.69	0.52	0.522
8.25	0.50	0.498	4.51	0.54	0.539	8.41	0.50	0.498	4.80	0.54	0.540
8.41	0.51	0.514	4.66	0.56	0.561	8.55	0.51	0.511	4.90	0.56	0.561
8.57	0.52	0.529	4.81	0.58	0.581	8.69	0.52	0.523	5.04	0.58	0.580
8.71	0.53	5.540	5.00	0.60	0.601	8.83	0.53	0.535	5.20	0.60	0.600
8.86	0.54	0.549	5.28	0.62	0.622	8.97	0.54	0.545	5.44	0.62	0.620
9.04	0.55	0.558				9.12	0.55	0.554			
9.25	0.56	0.566				9.28	0.56	0.563			
9.50	0.57	0.574				9.44	0.57	0.571			

a) About half of the data used for the calculation are shown. Composition of solutions: Curve (a): $[In]_T$= 0.0025 M, $[L]_T$=0.01 M, [HCl]=0.0013 M, [KOH]=1.002 M. Curve (b): $[In]_T$=0.005 M, $[L]_T$=0.01 M, [HCl]=0.0026 M, [KOH]=1.002 M. Curve (c): $[In]_T$=0.00025 M, $[L]_T$=0.001 M, [HCl]=0.00013 M, [KOH]=0.1001 M. Curve (d) $[In]_T$=0.0005 M, $[L]_T$=0.001 M, [HCl]=0.00026 M, [KOH]=0.1001 M. b) V=experimental titres (ml). c) Vc=calculated titres (ml).

TABLE 3. VALUES(LOGARITHMIC UNITS) OF THE FORMATION CONSTANTS IN THE SUCCESSIVE TRIALS FOR THE In(III) (0.005 M)-CYSTEINE HYDROCHLORIDE (0.01 M) SYSTEM[a)]

Trial	Complex species							Std. dev. in titre (ml)
	InL	InL_2	InLH	InL(LH)	$In(LH)_2$	InL(OH)	$InL(OH)_2$	
1	14.872 (0.025)	27.263 (0.027)	18.473 (0.024)					0.0047
2	14.759 (0.020)	27.049 (0.023)	18.521 (0.036)			10.255 (0.014)		0.0030
3	14.683 (0.022)	26.49 (0.36)	18.543 (0.011)			10.464 (0.034)	5.40 (0.23)	0.0023
4	14.662 (0.026)	27.197 (0.014)	18.548 (0.012)	31.548 (0.039)				0.0024
5		27.197 (0.014)	18.541 (0.013)	31.936 (0.015)	35.703 (0.033)			0.0023
6		27.150 (0.026)	18.536 (0.013)	31.916 (0.021)	35.728 (0.035)	9.05 (0.20)		0.0023
7	14.679 (0.026)	27.142 (0.025)	18.544 (0.012)	31.464 (0.078)		9.62 (0.21)		0.0023

a) Standard deviations in parentheses.

refinement of the stability constants. The approximate estimates, 14.92 and 28.12, obtained from the Irving-Rossotti method were used as the initial values of the unknown constants for the InL and InL_2 complexes respectively. The other estimates were obtained from the values of the corresponding penicillamine-In(III) complexes. The calculation was started in the system of 0.005 M In(III) and 0.01 M cysteine hydrochloride, assuming that three species, InL, InL_2, and InLH (Table 3, Trial 1), were formed. The agreement

between the experimental and calculated values is fairly good, as compared to the non-convergence in the chemical model of InL and InL_2. The fit was further improved by the introduction of InL(OH) into Trial 1 (Trial 2). However, the introduction of InL(OH) into the chemical model of InL and InL_2 was non-converged. The system was not consistent with the non-chelating complex, $InLH_2$. The further inclusion of the $InL(OH)_2$ species (Trial 3) increased the standard deviation of the log β_{120} value. The standard deviation in titre was appreciably improved by the introduction of the InL(LH) into Trial 1 (Trial 4). The further inclusion of the $In(LH)_2$ species in Trial 4, however, led to non-convergence, as had been expected. Trials 5, 6, and 7 gave good results, in agreement with the experimental and calculated values. The presence of the InL(OH) species was doubtful, because the constants obtained from Trials 2, 3, 6, and 7 varied considerably. Trial 5 gave a slight improvement over Trial 4. Under the condition of $[In]_T=0.05$ M (see Fig. 6), Trial 4 became non-converged. A similar investigation was carried out with various other polynuclear and hydrolyzed species: In_2L_2, $In_2L_2(LH)_2$, $InL_2(OH)$, $InL_2(OH)_2$, $In_2L_2(OH)$, $In_2L_2(OH)_2$, $In_2(LH)_4(OH)_2$, $In_3L_4(OH)$, $In_3L_4(OH)_2$, *etc.*, but the results showed the absence of these species. The same treatments on the other three curves indicated that the InL(LH) and $In(LH)_2$ species became negligible, and that the InL, InL_2, and InLH species were predominant, as the concentration of the metal ion decreased. A final refinement of all these constants over the range of experimental data (4 experiments, 166 points, <pH 6) yielded the constants listed in Table 4. The calculated and experimental titres agreed with each other within a standard deviation of 0.0059 ml. The constant of the InL_3 species is also shown in the table. This value was obtained from the higher pH regions (>pH 7) of the two 4:1 (ligand-to-metal) titration curves (2 experiments, 26 points). In these regions, the $InL_2(OH)$ species was not detected. The calculated titres are listed in Table 2. Figure 3 shows the distribution diagram of the InL_2, InL_3, InLH, InL(LH), and $In(LH)_2$ species, which was obtained from the system of 0.0025 M In(III) and 0.01 M cysteine hydrochloride.

TABLE 4. STABILITY CONSTANTS OF In(III) COMPLEXES WITH CYSTEINE HYDROCHLORIDE AT 21°C AND I=0.1 (KNO_3)[a]

InL	Log β_{110} =14.12±0.06
InL_2	Log β_{120} =27.26±0.02
InLH	Log β_{11-1}=18.46±0.02
InL(LH)	Log β_{12-1}=31.78±0.05
$In(LH)_2$	Log β_{12-2}=35.74±0.06
InL_3	Log β_{130} =32.20±0.09

a) The uncertainty intervals associated with all values are the standard deviations.

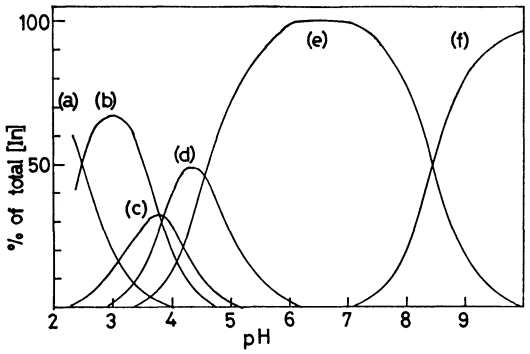


Fig. 3. Distribution diagram for the cysteine hydrochloride (0.01 M)-In(III) (0.0025 M) system: (a) In^{3+}; (b) InLH; (c) $In(LH)_2$; (d) InL(LH); (e) InL_2; (f) InL_3.

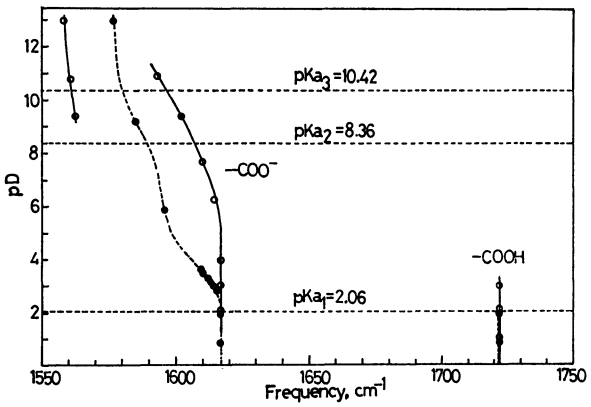


Fig. 4. IR data for cysteine (0.1 M) with (dashed line) or without (solid lines) In(III) (0.05 M) for various pD values.

IR and UV Spectra of the 2:1 Cysteine-In(III) System. Figure 4 shows the carboxylate absorption bands of cysteine (0.1 M) in a D_2O solution in the absence (solid lines) and in the presence (dashed lines) of In(III) (0.05 M). In the lower pD region, the free ligand gives two bands, at 1617 and 1722 cm^{-1}, which are assigned to dissociated and undissociated carboxyl groups respectively. The carboxylate band shifts from 1617 to 1560 cm^{-1} with an increase in the pD, and a splitting of the band at about pD 10.5 was observed. The shift ($\Delta\nu$=57 cm^{-1}) is attributed to the electrostatic effect on the basis of the dissociation of the amino ($\Delta\nu$=42 cm^{-1}) and thiol groups ($\Delta\nu$=15 cm^{-1}), as deduced from Table 5. The electron delocalization of the carboxylate ion, $-C\overset{O}{\underset{O}{<}}^{\ominus}$, could be more accelerated by the dissociation of these groups. In the solution containing the ligand and the metal ion, the carboxylate band appears

TABLE 5. CARBOXYLATE BANDS IN THE INFRARED ABSORPTION SPECTRA OF THE VARIOUS COMPOUNDS AT pD 7 AND 10 IN THE D_2O SOLUTION (cm^{-1})

Compounds	pD 7	pD 10	$\Delta\nu$[a]
α-Alanine[b]	1618	1570	48
β-Alanine[b]	1567	1567	0
S-Methyl-L-cysteine	1615	1573	42
N-Acetyl-L-cysteine	1592	1577	15
Cysteine	1617	1559	58

a) $\Delta\nu=\nu_{pD7}-\nu_{pD10}$. b) Ref. 16.

in a lower pD region in comparison with that of the ligand, indicating the coordination of the carboxyl group. The shift of the carboxylate band occurred at about pD 3, whereas about 40% of the ligand was already coordinated to the metal at pH 2, as is shown in Fig. 6. Consequently, the shift suggests the coordination of the amino group. In the pD region lower than 3, therefore, the presence of the $\widehat{S\ O}$-type complex is taken into account. The shift reaches its maximum at pD 6—7, where the InL_2 species is formed predominantly. The shift of the carboxylate band in the higher pD region (>pD 8) reveals the elimination of the carboxyl group from the coordination sphere, which is consistent with the PMR results.

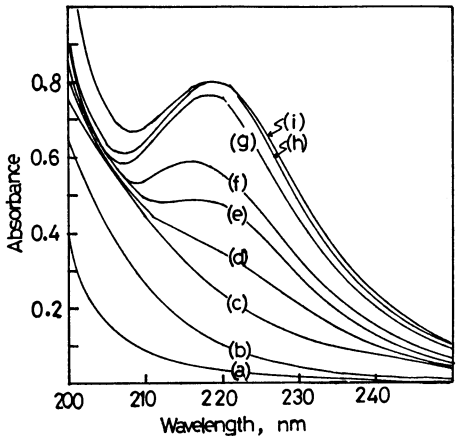
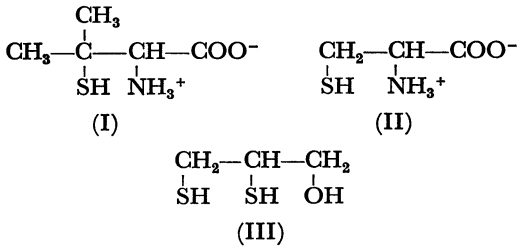


Fig. 5. Absorption spectra of the cysteine (0.00016 M)–In(III) (0.00008 M) system at various pH values: (a) pH 2.12; (b) pH 3.10; (c) pH 4.04; (d) pH 4.40; (e) pH 4.69; (f) pH 4.83; (g) pH 5.97; (h) 7.48; (i) pH 9.58.

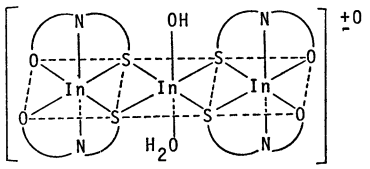
The UV absorption of cysteine in the 228—229 nm region is based on the form of the RS^- ion.[9] The absorption spectra of the cysteine–In(III) complex as a function of the pH (Fig. 5) exhibit a maximum at 217 nm, and they are quite different from those of the ligand. On the other hand, the In(III) ion(d^{10}) shows no absorption bands. The absorption band of the complex is attributed to $d\pi$ back-donation from the d^{10} metal ion to the empty d orbitals of the sulfur. In the pH region lower than 4.5, the absorption band at 217 nm does not appear, although the absorbance at about 200 nm increases with an increase in the pH. The absorption spectra of the In(III)-cysteine methyl-ester complex ($\widehat{S\ N}$ type) exhibit a maximum in the 214—219 nm region, whereas the absorption band of the In(III)-*N*-acetyl-L-cysteine complex ($\widehat{S\ O}$ type) appears in the wavelength region lower than 200 nm. Accordingly, the protonated cysteine complex InLH ($\widehat{S\ O}$ type) is formed in the pH region lower than 4.5. These findings also indicate that the charge-transfer bands based on the $In\begin{pmatrix}S\\N\end{pmatrix}$ and $In\begin{pmatrix}S\\O\end{pmatrix}$ bonds appear at about 217 nm and in the wavelength region lower than 200 nm respectively.

Discussion

The white precipitate which was not observed in the case of penicillamine(I) was formed during titration when the cysteine(II): metal ratio was smaller than 2: 1, as in 2,3-dimercapto-1-propanol(III).



The steric effect on the thiol group may be the cause of the difference among these ligands. Some sulfur-bridged polynuclear metal complexes have been reported[6,10–12] in the (II) and (III) ligands. The IR spectra suggest that the isolated cysteine–In(III) complex is hydrolyzed, but not protonated. The elemental analysis indicates that the ligand: metal ratio of the complex is 4: 3 and that the total charge of the complex is zero. The following structure can reasonably be postulated from these results:



A little excess of the metal ion causes the disruption of the equilibrium established because of the insolubility of the trimeric complex. Consequently, about 30% of the ligand will remain in the supernatant of the solution. In fact, the PMR spectra of the supernatant were similar to that of the ligand. When more of the base was added, the coordination of another hydroxide ion causes a change of the charge from zero to −1. Accordingly, the trimer is dissolved, and then the free ligand in the solution coordinates to the central metal in the trimer to form InL_2, together with the small amount of the metal hydrolysate. Based on this postulate, the sulfur-bridged trimer should have a *cis*-form. The In–S bond is stabilized by the $d\pi$ back-donation.[6,10,13–15] The higher stability constant of the sulfur-containing ligand than those of the corresponding oxygen- and nitrogen-containing ligands may be regarded as a reflection of this stabilization (cf. β-hydroxypropionic acid: log K_1=3.75, log K_2=3.04; β-aminopropionic acid: log K_1=2.72, log K_2=2.54, at 25 °C and I=0.1[13]). For the first molecule of the ligand which coordinates to In(III), the d_{xy} and d_{yz} orbitals of In(III) are used for the π bonding with the d orbital of the sulfur on the x coordinate. These orbitals of the metal ion may become less available for the bonding with the second molecule of the ligand on the $-x$ coordinate. Consequently, a *trans*-form may be unfavorable for the complex.

In the 2: 1 cysteine–In(III) system, the $In_3L_4(OH)$

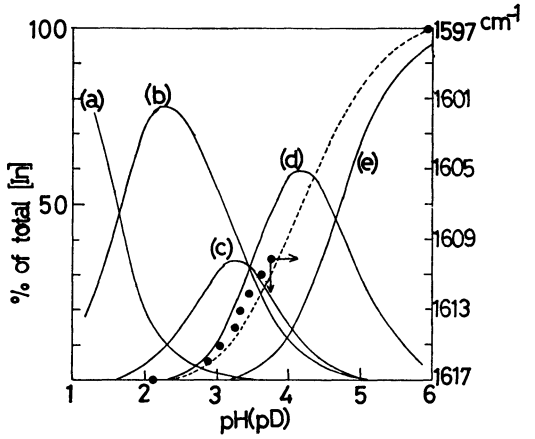
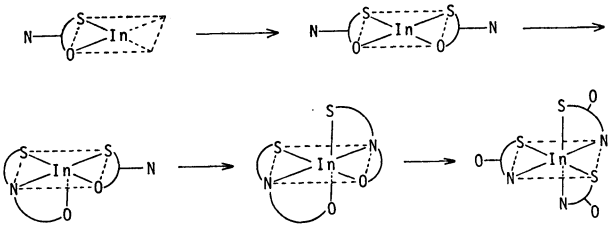


Fig. 6. Distribution diagram and IR data (●, in D_2O) for the cysteine hydrochloride (0.1 M)–In(III) (0.05M) system. Dashed line is given by $(1/2[InL(LH)]+[InL_2])/[In]_T$. (a) In^{3+}; (b) InLH; (c) $In(LH)_2$; (d) InL(LH); (e) InL_2.

and $In_3L_4(OH)_2$ trimer were not detected by the caluclation, as had been predicted. In the cysteine-In(III) system, the protonated complexes are more readily formed than in the penicillamine system, where the $In(LH)_2$ species is absent and InLH is not always formed. At the higher metal concentrations, therefore, the InL species is negligible in the cysteine system. These differences are due to the higher stability constants of the penicillamine complexes, *e.g.*, $\log \beta_{110}=15.330$ and $\log \beta_{120}=29.789$.

The results of the potentiometric and IR measurements support the formation of the three protonated species, InLH, InL(LH), and $In(LH)_2$. Figure 6 shows the distribution diagram of the solution containing 0.1 M cysteine hydrochloride and 0.05 M In(III). The dashed line represents the sum of the distribution ratios for the two species, InL(LH) and InL_2. This is obtained by $(1/2[InL(LH)]+[InL_2])/[In]_T$. The line reflects the coordination ratio of the amino group of the ligand to the metal. Each point in the figure is a plot of the absorption frequency of the carboxylate group against the pD obtained under the same conditions, assuming the complete formation of the InL_2 species at 1597 cm^{-1}. The shift of the absorption band is considered to be caused mainly by the coordination of the amino group. Each absorption point is approximately superimposable on the dashed line. The slight deviation is probably due to the small effect on the shift of the coordination of the thiol group. This finding indicates the presence of $In(LH)_2$ and the absence of InL under these conditions. The presence of the InLH species, and the conclusion that the amino group of the coordinating cysteine is protonated, are supported by the following observations: (1) the absence of the shift of the IR band in the pD region lower than 2.5 and (2) the absence of the band of the UV absorption based on the $In\begin{pmatrix}S\\N\end{pmatrix}$ bond in the pH region lower than 4.5. In the higher pH region, the final species, InL_3, is detected by the calculation. The IR and PMR spectra indicate that the carboxyl group does not coordinate to the metal in the complex. In the case of the penicillamine complex, the steric effect of the methyl groups of the ligand prevents the attack of the third ligand molecule. At the higher metal concentrations, the following coordination process is proposed:



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