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Proton Magnetic Resonance Study of Indium(III) and Gallium(III) Binding by Adjacent and Separated Disulfhydryl-Containing Peptides

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In (III) and Ga (III) binding by new ligands, 2,3-dimercaptopropionylglycine (DMPG) and 2-mercaptopropionyl-L-cysteine (MPC), which involve two adjacent and separated sulfhydryl groups, has been investigated with proton nuclear magnetic resonance spectra. The methylene protons of the 2:1 DMPG-In (or Ga) complex were observed as two signals at 3.28 and 3.02 (or 3.33 and 2.98) ppm, indicating a fused-chelate ring formation. On the other hand, MPC formed a polymer In species in addition to the 2:1 MPC-In (or Ga) complex which has a rigid configuration similar to the 2:1 DMPG-In (or Ga) complex. The coupling constants between the methylene and methine protons and the structure for these In and Ga complexes have been discussed.

Keywords——indium(III) complex; gallium(III) complex; disulfhydryl-containing peptide; 2,3-dimercaptopropionylglycine; 2-mercaptopropionyl-L-cysteine; proton magnetic resonance

Localization of the radioisotopes ^{111}In and ^{67}Ga in malignant tissue has been employed in the clinical detection of a broad range of tumors and in the imaging of various organs.[2] The behavior of In and Ga complexes in aqueous solution is important to the understanding of the tissue distribution and accumulation of the complexed species of the metals. Only a few studies have been reported,[3] however, because of the complexity of the aqueous chemistry of In and Ga. Our previous investigation on In (III) complexes of penicillamine[4] and cysteine[5] has revealed that the sulfhydryl group is an adequate donor toward In (III). Gallium has similar electron configuration ($3d^{10}4s^24p^1$), electronegativity (1.6 Pauling unit), and covalent radius (1.26 Å) to those ($4d^{10}5s^25p^1$, 1.7, and 1.44 Å) of indium. Herein, we would like to report a unique binding of In and Ga by the ligands, 2,3-dimercaptopropionylglycine (DMPG) and 2-mercaptopropionyl-L-cysteine (MPC), which involve two adjacent and separated sulfhydryl groups, respectively.

Experimental

DMPG (or MPC) was synthesized by the Schotten-Bauman reaction between 2,3-dibromopropionylchloride (or 2-bromopropionylchloride) and glycine (or L-cysteine) followed by a condensation with thiobenzoic acid and then hydrolysis in an ammonia solution.[6] The peptide ligands were determined by elemental analysis, iodometric titration, infrared, and proton nuclear magnetic resonance (^1H NMR) spectra. Indium sulfate and gallium nitrate were used for the ^1H NMR measurements. The D_2O, DCl, and NaOD (99.5% isotopically pure) were obtained from E. Merck, Japan. All other reagents used were of a reagent grade.

^1H NMR spectra were recorded at 100 MHz on a Varian HA-100 NMR spectrometer. Concentration of the ligand was 0.1 M in D_2O and chemical shifts were measured from internal TSP. The reading of the

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pH meter plus 0.4 was used to calculate pD according to the relationship: pD=pH+0.4. All pH measurements were performed using a Hitachi pH meter, model F-5, equipped with a Toko combination electrode CE-103.

Results and Discussion

^{1}H NMR Spectra

Figure 1 displays ^{1}H NMR spectra of DMPG only, 2:1 DMPG–In (III), 3:1 DMPG–In (III), 2:1 DMPG–Ga (III), and 3:1 DMPG–Ga (III) systems. The protons of DMPG only

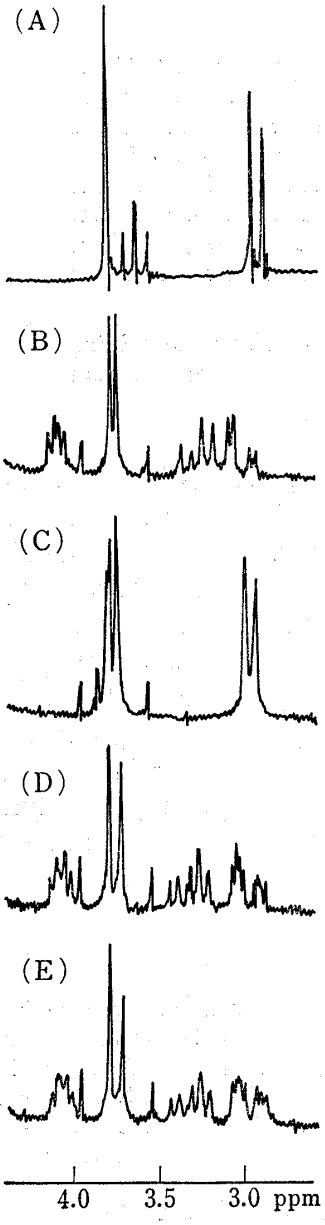

Fig. 1. ^{1}H NMR Spectra of 2,3-Dimercaptopropionyl Glycine (DMPG)(A), 2:1 DMPG-In (III)(B), 3:1 DMPG-In(III) (C), 2:1 DMPG-Ga(III)(D), and 3:1 DMPG-Ga(III)(E) Systems at pD 7.6

The NMR spectrum of the 1:1 DMPG–In(III) or Ga(III) system was not obtained, because of interference of precipitation.

Table I. Proton Chemical Shifts of Disulfhydryl-Containing Peptide-In(III) Complexes in Comparison with the Ni(II) and Sn(II) Complexes

Compound	Proton chemical shifts, ppm			
	α_1	α_2	α_3	β
$\overset{(\alpha_1)}{CH_2}\overset{(\alpha_2)}{CH}CONH\overset{(\alpha_3)}{CH_2}COOH$ (SH SH)	2.94(d)	3.65(t)	3.81(s)	—
In(III) complex(2:1)	3.28(q), 3.02(q)	4.10(q)	3.77(q)	—
In (III) complex(3:1)	2.98(d)	3.82(t)	3.79(q)	—
Ni(II) complex(2:1)	2.57(d)	3.37(t)	3.82(q)	—
Sn(II) complex(2:1)	3.31(d)	4.05(t)	3.78(q)	—
$\overset{(\alpha_1)}{CH_3}\overset{(\alpha_2)}{CH}CONH\overset{(\alpha_3)}{CH}\overset{(\beta)}{CH_2}SH$ (SH, COOH)	1.44(d)	3.53(q)	4.29(t)	2.93(d)
In(III) complex(2:1)	1.42(d)	3.81(q)	4.42(q)	3.23(q), 2.90(q)
Ni(II) complex(1:1)	1.42(d)	3.59(q)	4.13(d)	2.68(q), 2.28(d)
Sn(II) complex(2:1)	broadening			

^{1}H NMR measurements were carried out at pD 7.6.
signal designations: s, singlet; d, doublet; t, triplet; q, quartet

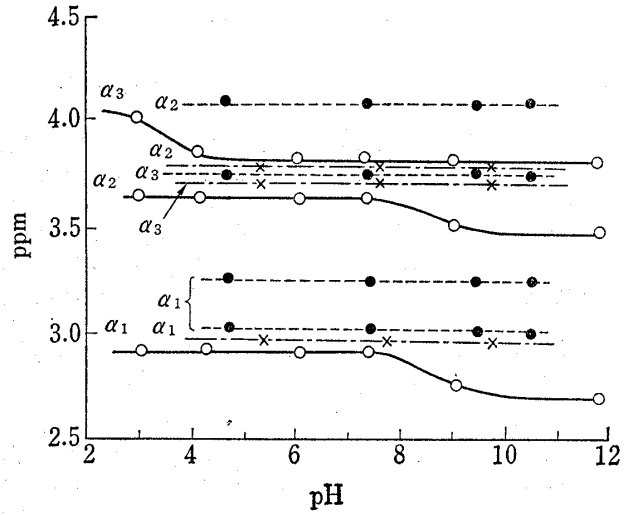

Fig. 2. Effect of pH on Proton Chemical Shifts of 2,3-Dimercaptopropionylglycine (DMPG) and Its In(III) Complexes

Solid and dotted lines represent DMPG only (○——○), 2:1 (●------●), 3:1 (×---×)DMPG-In(III) complexes, respectively.

exhibit signals at α_1=2.94(doublet), α_2=3.65(triplet), and α_3=3.81(singlet) ppm. On the other hand, α_1 methylene protons of the 2:1 DMPG–In (or the 2:1 DMPG–Ga) complex are observed as two different signals (each a quartet) at 3.28 and 3.02 (or 3.33 and 2.98) ppm, indicating the formation of a complex with a rigid conformation. From the spectra, the coupling constants between α_1 and α_2 protons were estimated as follows: J_{AB}=13.0, J_{AC}=6.5, and J_{BC} =4.5 Hz (In complex); J_{AB}=13.0, J_{AC}=6.0, and J_{BC}=4.5 Hz (Ga complex).[7] The numerous fine-splittings of the 2:1 DMPG–Ga complex are more attributable to the presence of two structurally analogous species, than to the presence of ^{69}Ga (natural abundance=60.2%, I=3/2) and ^{71}Ga (39.8%, I=3/2). In Fig. 1(D) for example α_1 methylene appears as quartets at 3.33 and 2.98 ppm. These are diastereotopic methylene groups. They are of clearly unequal intensity and cannot both arise from the same molecule. There must be two isomers (*cis* and *trans* with respect to the carboxyl position) in unequal amounts. The α_3 methylene in the complex must be also diastereotopic (as can be seen in the spectrum). The α_2 methine triplets overlap, thus the explanation based on the presence of ^{69}Ga and ^{71}Ga(I=3/2) seems to be unlikely. Resonances of the metal (^{69}Ga and ^{71}Ga) are generally too broad to be observed, indicating that quadrupolar relaxation is too rapid to permit effective scalar coupling of magnetic dipoles of protons and the metal.

The α_1 and α_2 protons of the 3:1 DMPG–In (III) complex appear at 2.98 (doublet) and 3.82 (triplet) ppm. The spectral characteristics reveal the formation of complex with an S_6 donor set and are similar to those of a diamagnetic 2:1 DMPG–Ni (II) complex and a 2:1 DMPG–Sn (II) complex. The α_1 and α_2 protons of the 2:1 DMPG–Ni (II) complex appear at 2.57 (doublet) and 3.37 (triplet) ppm, and the large high field shift is based on the formation of square-planar S_4–Ni (II) complex with π-bonding character.[6] The α_1 and α_2 proton signals of a tetrahedral 2:1 DMPG-Sn (II) complex shifted to lower field than those of the 2:1 DMPG–In (III) complex, indicating stronger σ-donating character of the sulfhydryl group toward Sn (II)(see Table I).

Figure 2 shows the effect of pH on the proton chemical shifts of DMPG and its In (III) complexes. The chemical shifts of the ligand only are remarkably affected by pH range of 3—4 and 8—11, where the carboxyl (pK_{COOH}=3.66) and two sulfhydryl groups (pK_{CHSH}=7.66 and pK_{CH_2SH}= 10.69) dissociate respectively. On the other hand, the pH independence of the chemical shifts in the In (III) complexes indicates the formation of the same complex species over the pH range of 4.5—10.5.

Figure 3 shows ^{1}H NMR spectra of MPC–In (III) and MPC–Ga (III) complexes. The protons of MPC only give the signals at α_1=1.44 (doublet), α_2=3.53 (quartet), α_3=4.29 (triplet), and β=2.93 (doublet) ppm. The expanded splitt-

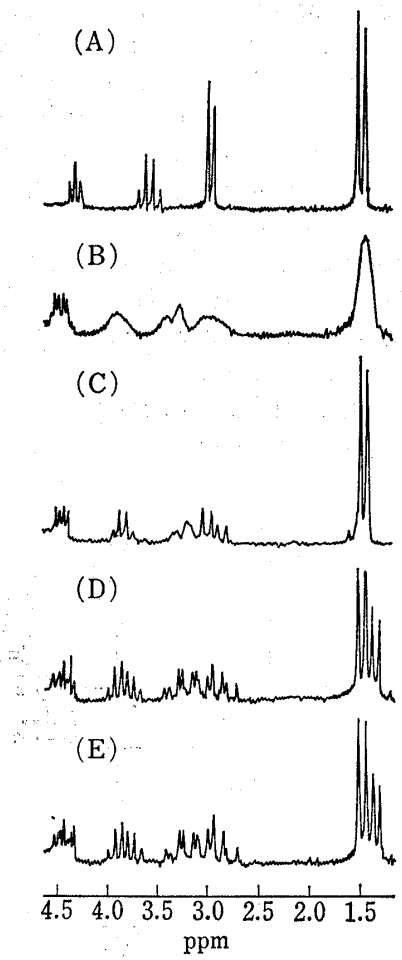

Fig. 3. ^{1}H NMR Spectra of 2-Mercaptopropionyl-L-cysteine (MPC) (A), 2:1 MPC-In (III) (B), 3:1 MPC-In(III)(C), 2:1 MPC-Ga(III)(D), and 3:1 MPC-Ga(III)(E) Systems at pD 7.6

7) The following abbreviations are used: $J_{AB,AC,BC}$= H_A, H_B, H_C, C, SH, SH (DMPG) and H_A, H_B, H_C, C, SH, COOH (MPC).

ing of the β methylene protons of the 2:1 MPC–Ga(III) complex suggests a rigid conformation similar to the 2:1 DMPG–Ga(III) complex. However, the four signals of the methyl protons reveal the presence of two species which are structurally analogous, presumably *cis* and *trans* isomers with the same donor set S_4O_2. There are clearly two species present in this case (again in unequal amounts). In the *cis*-(−)-[Co(L-alanine)$_3$] and *trans*-(−)-[Co(L-alanine)$_3$] complexes,[8] it has been observed that the methyl resonance is a simple doublet (111 cps from TMS) for the *cis* isomer and is three doublets (120, 113, and 105 cps) for the *trans* isomer. In addition, the α carbon atom protons have also given a little different chemical shifts between the *cis* and *trans* isomers.

The spectral broadening in the 2:1 MPC–In (III) system is probably due to polymer formation. Addition of excess MPC to the 2:1 MPC–In (III) system gives a depolymerization effect and narrows the line width (see 3:1 MPC–In system). In the presence of excess ligand, therefore, the 2:1 MPC–In (III) complex with a small molecular weight is probably formed. The 2:1 MPC–In (III) complex gave the following ^{1}H NMR signals and the coupling constants: α_1=1.42 (doublet), α_2=3.81 (quartet), α_3=4.42 (quartet), and 3.23 and 2.90 (each a quartet) ppm; J_{AB}=14.0, J_{AC}=3.5, and J_{BC}=8.0 Hz.[7]

In the dialysis experiment (Visking cellulose tube-type VT 351) of the 2:1 MPC–In (III) system, a considerable portion of In was retained in the membrane even after 12 hr dialysis, in contrast with result of the dialysis experiment using Visking tube of type VT 801. The result suggests the formation of a MPC–In (III) polymer species with molecular weight of about 3500—8000. Therefore, the polymer formation is at least in part the cause of ^{1}H NMR spectral broadening in the 2:1 MPC–In (III) system. Increased membrane permeability of the 3:1 MPC–In (III) system reflects the formation of small MPC–In (III) complex. Other evidence consistent with a polymer complex is the observed retention by an Amicon UM-2 membrane filter.

On the basis of the present results, the structure considered for the 2:1 DMPG (or MPC)–In (III) and –Ga (III) complexes is shown in Fig. 4.

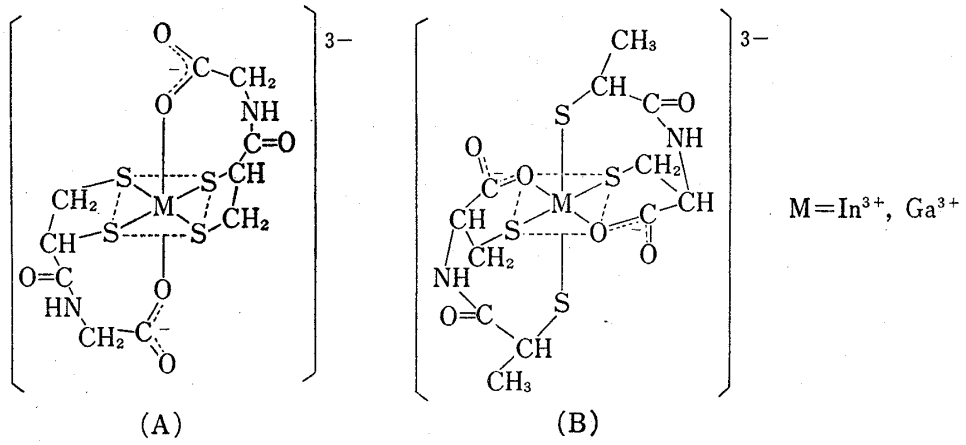

Fig. 4. Probable 2:1 DMPG-In(or Ga)(A) and 2:1 MPC-In(or Ga)(B) Complexes

Of special interest is the disulfhydryl-containing peptide as a new potential ligand for In (III) and Ga (III) ions. In addition, the small DMPG–In (or Ga) complex and polymer MPC–In species are considered to be promising potential scanning agents for kidneys and tumors, respectively. The investigation of radiopharmaceuticals is now under way.

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