Liquid Chromatography of Diastereoisomers on Neutral Tris- and Bis(L- α -amino acidato)chromium(III) Complexes

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Liquid chromatography (LC) has been much needed as a more quantitative analytical tool for geometrical isomers in the preparation of various metal complexes, and has also been recognized to be a useful method in kinetic studies for stereochemical rearrangement reactions of geometrical isomers, such as tris(β -diketonato)-ruthenium(III) complexes^{1,2}, cobalt(III) and chromium(III) complexes containing bis(tridentate)amino acidato ligands.^{3,4}

In previous papers⁵⁻⁷, we reported on the stereoselective preparation of *fac*-[Cr(AA)₃] or [Cr(AA)₂(AA-*N*)L], where AA, AA-*N* and L stand for a bidentate L- α -amino acidato ligand, a monodentate L- α -amino acidato ligand coordinating through a nitrogen atom and a solvent molecule as a ligand, respectively. However, the purities of these complexes have been checked optically by measuring only the optical rotations. In addition, we have recently observed structural changes involving isomerization for chromium(III) amino acidato complexes in non-aqueous solvents, such as EtOH or *N,N*-dimethylformamide (DMF).^{7,8} Therefore, more quantitative analyses for these complex solutions have come to be needed. Diastereomeric neutral *fac*-[Cr(AA)₃], which was soluble in polar solvents, DMF or Me₂SO, but insoluble in EtOH, could be isolated by column chromatography on an acidic alumina column with a DMF/MeOH (2:3, v/v) mixture as an eluent.⁹ While, neutral [Cr(AA)₂(AA-*N*)L] complexes were soluble in not only DMF or Me₂SO, but also EtOH, they could be isolated less completely on an acidic alumina column with pure DMF.⁹ However, the rapid separation and simultaneous determination of these four types of complexes by LC have not yet been attempted.

In this communication, we describe the results of our successful LC system for the simultaneous determination of diastereoisomers of *fac*-[Cr(AA)₃] and [Cr(AA)₂(AA-*N*)L], and briefly discuss their retention behavior based on the structural differences among four types of com-

plexes.

Experimental

Chemicals

Fac- Δ, Δ -[Cr(AA)₃] (abbreviated as tris- Δ, Δ) and Δ, Δ -[Cr(AA)₂(AA-*N*)DMF] (abbreviated as bis- Δ, Δ) were prepared by published methods with L-isoleucine, L-leucine, L-norvaline, and L-valine.⁵⁻⁷ The predictable structures of four types of chromium(III) amino acidato complexes examined in this study are shown in Fig. 1 (1)–(4), whose definite crystal structures have not yet been determined by X-ray analysis. DMF and MeCN were purchased from Nacalai Tesque and distilled over CaH₂ once prior to use.

Fig. 1 Schematic geometries of (L- α -amino acidato)chromium(III) complexes. (1) bis- Δ ; (2) bis- Λ ; (3) tris- Δ ; (4) tris- Λ : L and R stand for a solvent molecule as a ligand and an alkyl chain on amino acidato, respectively.

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Liquid chromatography conditions

The LC system comprised a TOSOH Model HLC-803D and a UV-8010 detector; the chromium(III) complexes eluted were spectrophotometrically detected at 520 nm and at a flow rate of 0.5 or 1.0 ml/min. A CHEMCOSORB 7NH₂ column (4.6 mm i.d.×150 mm, Chemco, Japan) was employed at room temperature and 20 µl aliquots of samples were injected into the column for analyses. Alumina (Merck aluminium oxide 90 acidic, neutral or basic, activity I) was also used for LC as a packing material, where it was packed in a stainless-steel column (4.6 mm i.d.×150 mm) by a slurry method.

Results and Discussion

At first, LC behavior on a home-made alumina column was tested. However, no species could be retained on neutral or basic alumina with a DMF eluent. On only an acidic alumina column could bis-type species be separated with a DMF eluent; however, the tris-type species could not be eluted. Tris-type species remaining on a column could be eluted with a DMF/MeOH (2:3, v/v) mixture eluent and separated. Several commercial

Fig. 2 Typical chromatogram of the four types of (L-valinato)-chromium(III) complexes. LC conditions: CHEMCOSORB 7NH₂ column (4.6 mm i.d.×150 mm); mobile phase, DMF; flow rate, 0.5 ml/min; temperature, 23°C; detection, 520 nm; peaks: 1, bis-Δ; 2, bis-Δ; 3, tris-Δ; 4, tris-Λ (ca. 2 nmol each).

LC columns were subsequently tested for a simultaneous separation of these four species; only an aminopropyl-bonded silica-gel column (NH₂-column) could be consequently utilized with the DMF eluent. A typical chromatogram for four types of chromium(III) valinato complexes on an NH₂-column is given in Fig. 2. The height or area in each peak was proportional to the amount of complex injected, though each species was eluted with a peak tailing from an NH₂-column. The capacity factors (k') for four types of species and the separation factors ($\alpha=k'_A/k'_B$) were determined with respect to four kinds of amino acidato ligands, and are summarized in Table 1. Four types of complexes cannot be eluted in the order of their solubilities (e.g., the order of solubilities is bis-Δ>tris-Δ>bis-Λ>tris-Λ for valinato complexes in DMF), because the solubility depends upon not only the solute-solvent interaction, but also the intermolecular interaction in crystals. Also, the difference in the retentions attributable to the kinds of amino acidato ligands could not be interpreted based on the solubility data (e.g., the order of solubilities is Cr(leu)₃>Cr(ile)₃>Cr(val)₃>Cr(norval)₃ for tris-Λ in DMF). However, the retentions for the complexes were enhanced by the addition of a less polar solvent, such as MeCN, into the DMF eluent, because of a decrease in the solubilities of complexes as well as a reduction of the eluting ability. Figure 3 shows a correlation of the log k' values for valinato complexes with the amount of MeCN in the DMF eluent. The use of a DMF/MeCN (1:4, v/v) mixture eluent enabled the separation of four types of species, even in an unmodified silica-gel column; however, in such a case only a dilute sample can be applied because of the insolubility of tris-type species in the eluent.

The order of elutions for four types of complexes from an NH₂-column was the same as that obtained for the acidic alumina column. On the acidic alumina column, the hydrogen-bond was considered to be the most effective interaction for a neutral solute in various intermolecular forces between the solutes and adsorbents. However, the most important factor in chromatographic separation with an NH₂-column, which has often been used in LC analyses of sugars, also seemed to be a hydrogen-bond interaction between the solutes and the stationary phase. Accordingly, it is presumed that the amino-hydrogen of aminopropyl group fixed on a silica adsorbent for the NH₂-column or the hydroxyl-hydrogen at the surface of acidic alumina interacts with

Table 1 Capacity factor (k') and separation factor ($\alpha=k'_A/k'_B$) for (L-α-amino acidato)chromium(III) complexes

Ligand	$k'_{\text{bis-}\Delta}$	$k'_{\text{bis-}\Lambda}$	α_{bis}	$k'_{\text{tris-}\Delta}$	$k'_{\text{tris-}\Lambda}$	α_{tris}
L-Isoleucinato	0.23	0.32	1.42	0.67	2.28	3.38
L-Leucinato	0.25	0.38	1.52	0.68	3.07	4.50
L-Norvalinato	0.35	0.50	1.44	0.92	2.88	3.12
L-Valinato	0.28	0.42	1.53	0.94	3.11	3.30

Chromatographic conditions are the same as those in Fig. 2.

Fig. 3 Dependence of $\log k'$ on the MeCN content in the DMF eluent. Flow rate, 1.0 ml/min; other LC conditions are the same as those in Fig. 2.

donor atoms on ligands. The tris-type species, having three strongly electronegative donor oxygen atoms, can form three hydrogen-bonds with the adsorbent, whereas the bis-type species can form only two hydrogen bonds. Consequently, the hydrogen-bond acceptor ability of bis-type species is weaker than that of tris-type species. A similar concept was proposed to account for the reason why the *fac*-[Co(AA)₃] was more strongly adsorbed to silica-gel and exhibited a lower R_f value than did *mer*-[Co(AA)₃] in thin-layer chromatographic separation.¹⁰ On the other hand, in order to explain that the hydrogen-bond acceptor ability of the tris- Δ species is slightly inferior to that of tris- Λ species, we must consider the steric effect on the difference in the geometrical configuration between two species, which had also been pointed out in the polarographic reduction of tris-type species in Me₂SO.¹¹ The schematic geometries of tris-type species shown in Fig. 1 (3) and (4) reveal a significant difference in molecular crowding on the site of nitrogen

atoms around the three-fold (C_3) axis of the octahedral structure. That is, in the case of the tris- Δ species, three nitrogen atoms are surrounded by side chains of amino acids residues bonded to a carbon atom (β -position) and shielded from the solvents. In contrast, the interaction between a polar solvent and amino-hydrogen can be sufficiently expected for tris- Λ species, which must increase the electronegativity of oxygen atoms opposite the nitrogen atoms. Consequently, the hydrogen-bond acceptor ability of tris- Λ increases and the tris- Λ species is retained more strongly than is tris- Δ . The separation behavior between the bis- Λ and bis- Δ species may not be simply explained by a similar reason, since the influence of a free carboxyl group of a monodentate ligand cannot be ignored. The difference in the retentions attributable to the kind of amino acidato ligand must also be interpreted based on a steric effect by side chains of the amino acidato ligand.

In order to obtain more information concerning the steric effect of the geometrical configuration or side chain of the amino acidato ligand on various solution behavior of these complexes, we plan to look more closely at the chromatographic or electrochemical behavior.

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