Preparation of Sephadex Derivatives with Optically Active Groups and Column-chromatographic Application to the Resolution of Some Cobalt(III) Complexes

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Synopsis. Optically active cation-exchangers were newly prepared as Sephadex derivatives derived from L-alanine, L-valine, L-aspartic acid, and L-threonine. They were applied to the column-chromatographic resolution of some cobalt(III) complexes, partial resolution being attained.

The chromatographic method using an SP-Sephadex column has been successfully applied in the last decade to resolve optical isomers and to separate geometrical isomers of metal complexes. The method is useful for resolution, but it requires a considerable amount of optically active eluent. Direct column-chromatographic resolution without optically active eluents could be attained with use of an optically active stationary-phase. However, natural chiral substances such as quartz, starch, or lactose have been found to be unsuitable for a highly efficient resolution.

On the other hand, an ion-exchanger with optically active groups on itself could be applied to the column-chromatographic resolution of metal complexes. Two types of TA-Sephadex, TA(ET)- and TA(ES)-Sephadex, in which L-tartrate groups are linked to form ether and ester, respectively, were prepared and successfully applied to completely resolve $[Co(en)_3]^{3+}$ by column-chromatography.[1]

In the present study, a series of Sephadex derivatives with optically active cation-exchanging groups derived from L-amino acids were newly prepared and a couple of cationic and non-electrolyte complexes were chromatographed on their columns.

Experimental

Sephadex cation-exchanger with optically active groups was prepared by the following reactions:

$$\underset{\mathbf{1}}{\text{L-}H_2N\text{-}CH(R)\text{-}COOH} \xrightarrow[NaCl+NaNO_2]{1M\ HCl} \underset{\mathbf{2}}{Cl\text{-}CH(R)\text{-}COOH}, \quad (1)$$

$$\underset{\mathbf{3}}{SD\text{-}OH} + Cl\text{-}CH(R)\text{-}COOH \xrightarrow[50\%\ NaOH]{benzene} \underset{\mathbf{4}}{SD\text{-}O\text{-}CH(R)\text{-}COONa}, \quad (2)$$

where SD-OH (**3**) denotes Sephadex framework. The method of preparation for reaction (1) is similar to that for (*S*)(+)-2-chlorosuccinic acid from D-(−)-aspartic acid by Tosa *et al.*[2] For the reaction (2), Flodin's method of the preparation of CM-Sephadex was used with a slight modification.[3]

A typical procedure is as follows: Eighty-nine grams of L-alanine and 35 g of sodium chloride were dissolved in 900 ml of 1 M (1 M=1 mol/dm^3) HCl by heating. To the cold solution (about 3 °C) was added 900 ml of a cold 4% $NaNO_2$ solution in small portions with stirring below 5 °C. The solution was then stirred for one day. Eight hundred milliliters of concd HCl was then added with stirring. The solution was filtered off from precipitated sodium chloride and extracted three times with ether. After concentrated extracts had been dried with anhydrous Na_2SO_4, 2-chloropropionic acid was obtained by distillation under reduced pressure: pale-yellow liquid (24 g), bp 105 °C/43 mmHg.

To the suspension of 60 g of dry Sephadex G-25 in 200 g of benzene, was added 90 g of 50% NaOH solution in small portions with stirring. Eighty-four grams of 2-chloropropionic acid was slowly poured into the mixture below 55 °C. The reaction mixture was kept standing at *ca.* 50 °C for 12 h, with constant stirring. This was filtered and washed throughly with water, methanol, and then water. This Sephadex derivative was converted into sodium form with sodium hydroxide.

The Sephadex derivative is denoted by PR-Sephadex (PR=propionate). By similar methods, three other types of Sephadex derivatives were prepared by using L-valine, L-aspartic acid, and L-threonine; they are denoted by IVA-, SU-, and HBU-Sephadex, respectively (IVA=isovalerianate, SU=succinate, and HBU=3-hydroxybutyrate).

Five kinds of complexes were subjected to column-chromatography on each column of the Sephadex cation-exchangers prepared and CM-Sephadex (R=H, available from Pharmacia). A sodium sulfate solution was used as an eluent except for the case of *mer*-$[Co(gly)_3]$ which was eluted with water. Each 3 ml of effluent was collected and measured for the maximum absorbance of the first absorption band and for the maximum CD intensity in the corresponding region of the CD spectrum. From the results, the ratio of $\Delta\varepsilon/\varepsilon$, the apparent dissymmetric factor, was obtained for each fraction and used as a measure of resolution.

Results and Discussion

Configuration around an asymmetric carbon atom is inverted in reaction (1).[4] Thus, L-amino acids of *S*-chirality give chloro derivatives (**2**) of D-form with *R*-chirality.

Ion-exchange capacity for $[Co(en)_3]^{3+}$ of the Sephadex

Table 1. Ion-exchange capacity and column size

Sephadex derivatives	Capacity[a]	Column size
PR-Sephadex	0.91	1.8 cm×130 cm
IVA-Sephadex	0.12	1.8 cm×90 cm
SU-Sephadex	0.12	1.8 cm×90 cm
HBU-Sephadex	0.53	1.8 cm×150 cm
CM-Sephadex	1.23	1.8 cm×130 cm

a) For $[Co(en)_3]^{3+}$ (in mmol/g).

Table 2. Apparent dissymmetric factors in the first and last fractions of elutions[a]

	PR-Sephadex	IVA-Sephadex	SU-Sephadex	HBU-Sephadex	CM-Sephadex
$[Co(en)_3]^{3+}$	—[b]	—[b]	—[b]	(+)5.8—(−)5.8 (26) (26)	(−)8.0—(+)9.2 (36) (41)
$[Co(gly)(en)_2]^{2+}$	(+)1.4—(−)2.5 (6.6) (12)	(+)0.8—(−)1.2 (3.8) (5.6)	(+)0.5—(−)0.5 (2.3) (2.3)	(+)6.0—(−)6.5 (28) (31)	(−)4.4—(+)5.5 (21) (26)
$[Co(ox)(en)_2]^{+}$	(+)2.3—(−)3.5 (9.6) (15)	(+)3.0—(−)2.9 (13) (12)	(+)2.0—(−)2.0 (8.4) (8.4)	(+)2.0—(−)3.5 (8.4) (15)	(+)11—(−)16 (46) (67)
c-$[Co(NO_2)_2(en)_2]^{+}$	—[b]	(+)2.0—(−)4.5 (11) (25)	(+)1.0—(−)1.0 (5.6) (5.6)	(+)0.8—(−)2.0 (4.5) (11)	(+)1.8—(−)2.4 (10) (14)
mer-$[Co(gly)_3]$	(−)1.6—(+)1.9	(−)3.2—(+)2.6	(−)2.2—(+)1.5	—[b]	(−)1.5—(+)1.7

a) Signs and figures denote $(\Delta\varepsilon/\varepsilon)\times 10^3$ values at the main CD peak. Figures in parentheses denote optical purity (%).
b) No significant resolution.

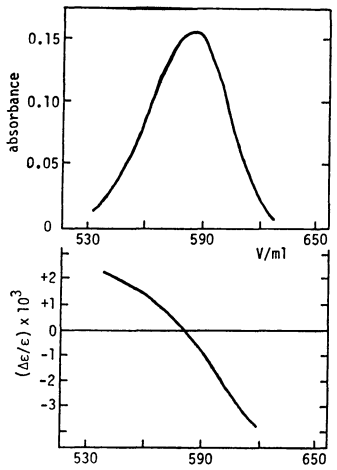

Fig. 1. The elution of $[Co(ox)(en)_2]^+$ on a PR-Sephadex column with a 0.07 M Na_2SO_4 solution.

derivatives prepared is given in Table 1 together with that of CM-Sephadex. Lower capacities of IVA- and SU-Sephadex might be attributed to lower yields of reaction (2).

The elution of $[Co(ox)(en)_2]^+$ from a PR-Sephadex column is shown as a representative example in Fig. 1. No clear-cut resolution is seen in the elution curve, but partial resolution is attained with a column of PR-Sephadex as well as other optically active Sephadex derivatives, as indicated by the change in dissymmetric factor from the top to the tail of the eluate.

The values of $\Delta\varepsilon/\varepsilon$ and optical purity in the first and the last fraction of each elution, together with the results on CM-Sephadex are summarized in Table 2. CM-Sephadex has no asymmetric centers on ion-exchange groups. Resolution with its column is, thus, due to the Sephadex skeleton which consists of dextran with a chirality similar to that of potato starch. With IVA- and SU-Sephadex, *mer*-$[Co(gly)_3]$ is resolved more efficiently than with CM-Sephadex, in spite of the fact that the ion-exchange capacities of the IVA- and SU-derivatives are only one-tenth of that of CM-Sephadex.

In the elution experiments of $[Co(gly)(en)_2]^{2+}$ with columns of Sephadex derivatives, the fast eluted enantiomer is the one with a negative CD peak. On the other hand, the enantiomer with a positive peak is eluted faster with a column of CM-Sephadex, as well as in the case of HBU-Sephadex for $[Co(en)_3]^{3+}$. Thus, optically active ion-exchanging groups on the Sephadex derivatives contribute to the resolution of the complexes. However, stereoselectivity for the complexes is opposite to that of Sephadex itself and the effect of optically active ion-exchanging groups for resolution is partially cancelled by the effect of the Sephadex skeleton.

References

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