Chromatography of Nucleosides on Copper(II)-Complexed β-Cyclodextrin Polymer in Alkaline Medium

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A newly prepared copper(II)-complexed β-cyclodextrin polymer cross-linked with epichlorohydrin was used as a packing material with a strongly alkaline eluate for the liquid chromatography of nucleosides. Nucleosides such as adenosine and cytidine were retained on the column because of their strong interaction with copper(II) ions. On the other hand, 2′-deoxyadenosine was retained on the copper(II)-free and copper(II)-complexed columns because of the inclusion property of β-cyclodextrin. These findings indicate that chromatographic separations can be achieved by the inclusion effect and copper(II)-complexation.

Key words ligand-exchange chromatography; β-cyclodextrin polymer; nucleoside

Copper(II) ions are known to form soluble, blue-colored, coordination complexes in an alkaline solution by interaction with the dissociated hydroxyl groups of various saccharides including cyclodextrins.[1—4] ESR, ^{13}C-NMR and circular dichroism spectra indicate that the principal copper(II)-binding sites on carbohydrate molecules are pairs of vicinal hydroxyl groups, and that the degree of binding is markedly influenced by the dihedral angles of the two hydroxyls.

Copper(II)-complexed carbohydrate polymer gels, commercially available as Cellulofine and Sephadex, were used for the liquid chromatography of adenosine and 2′-deoxyadenosine.[5]

In addition, copper-free β-cyclodextrin polymers have been used for the gel-inclusion chromatography of various nucleotides and nucleosides,[6] as well as racemates of indoles[7] and mandelic acid.[8]

This paper deals with the use of a newly prepared copper(II)-complexed β-cyclodextrin polymer support for liquid chromatography, which has inherent inclusion ability and also an additional ligand-exchange function due to the copper(II)-carbohydrate bonding.

Chromatography on a Copper(II)-Free β-Cyclodextrin Polymer Column The copper(II)-free β-cyclodextrin polymer column is considered to function chiefly as a chromatographic sorbent for the selective separation of nucleosides based on its ability to form inclusion complexes. The elution order of solutes reflects clearly a difference in strength of the host–guest interaction in the cyclodextrin cavity of the polymer matrix. The results of the chromatography of some nucleosides shown in Fig. 1 and Table 1 indicate that adenine nucleosides, especially 2′-deoxyadenosine, are the most strongly retained on β-cyclodextrin polymer, which is consistent with the findings by Hoffman *et al.*[6]

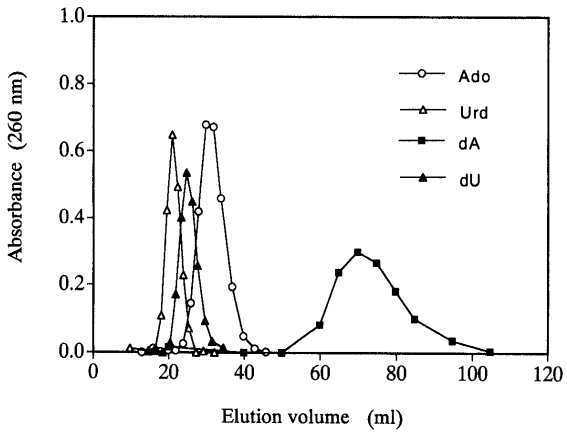

Fig. 1. Chromatography on a Copper(II)-Free β-Cyclodextrin Polymer Column

Dimensions, 12×246 mm (column A); flow rate, 0.5 ml/min. For abbreviations of nucleosides, see the footnote to Table 1.

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Chromatography on a Copper(II)-Complexed β-Cyclodextrin Polymer Column The results of chromatography on a copper(II)-complexed β-cyclodextrin polymer column are shown in Fig. 2 and Table 1.

Table 1 indicates that cytidine, guanosine and uridine which scarcely formed inclusion complexes with β-cyclodextrin, chromatographed on the copper(II)-complexed matrix, had elution volumes ranging from 35 to 46 ml. This elusion order is the same as that obtained for a copper(II)-complexed Sephadex column.[5] Consequently, the slow elution of such ribonucleosides may be largely attributed to the strong interaction of the saccharide with immobilized copper(II) ions.

Table 1. Elution Volumes from Cu(II)-Complexed and Cu(II)-Free β-Cyclodextrin Polymer (CDP) Columns

Nucleoside	Elution volume (ml)		
	Cu-complexed CDP[a]	Cu-free CDP	
		Column A[b]	Column B[c]
Ado	56.4, 62.3	30.7	31.2
Guo	36.9	22.6	—
Cyd	46.4	23.3, 24.0	—
Urd	35.1	20.9	21.0
dA	60.4, 59.4	68.8	84.1
dU	24.7, 24.8	24.7	26.5
Thd	23.5, 23.8	22.3	—
araA	27.4, 28.1	—	29.1
araU	22.5, 22.1	20.8	20.8

a) Column size, 12×240 mm; Cu(II) content, 0.079 mmol/g wet gel; flow rate, 0.5 ml/min. *b*) Column size, 12×246 mm; flow rate, 0.5 ml/min. *c*) Column size, 12×220 mm; flow rate, 0.3 ml/min.

Different batches of cyclodextrin polymer preparation were packed into column A and B, respectively.

Abbreviations: adenosine (Ado), guanosine (Guo), cytidine (Cyd), uridine (Urd), 2′-deoxyadenosine (dA), 2′-deoxyuridine (dU), thymidine (Thd), 9-β-D-arabinofuranosyl adenine (araA), 1-β-D-arabinofuranosyl uracil (araU).

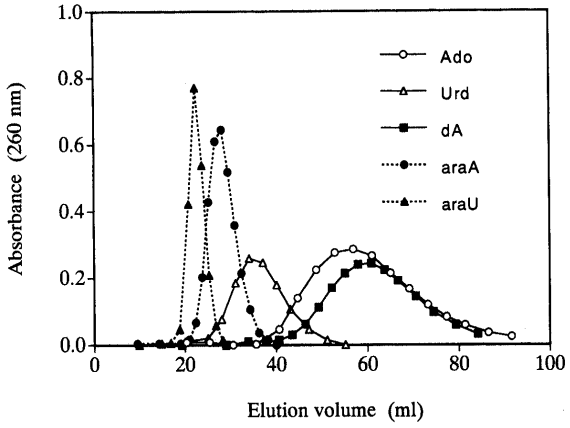

Fig. 2. Chromatography on a Copper(II)-Complexed β-Cyclodextrin Polymer Column

Dimensions, 12×240 mm; copper(II) content, 0.079 mmol/g wet gel; flow rate, 0.5 ml/min.

In contrast, the extremely slow elution of 2′-deoxyadenosine, which had no appreciable interaction with copper(II) ions, is entirely attributable to an inclusion effect.

In cases of adenosine chromatography employing the copper(II)-complexed matrix, elution volumes were around 60 ml. It is thought this was due to the combined effects of the inclusion ability and the copper(II)–carbohydrate interaction.

However, 2′-deoxyuridine, thymidine and two arabinose nucleosides had elution volumes not more than 28 ml, which were comparable with those observed using a copper(II)-free control column (A) of approximately the same bed volume.

It is concluded that a copper(II)–carbohydrate interaction could be introduced into a β-cyclodextrin polymer matrix without leading to serious impairment of its inclusion ability.

Experimental

Materials β-Cyclodextrin obtained from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan) was purified by double-recrystallization from distilled water. 9-β-D-Arabinofuranosyl adenine (araA) and 1-β-D-arabinofuranosyl uracil (araU) of high purity were purchased from Pfanstiehl Ltd. (U.K.). Other nucleosides used were high grade preparations from Sigma Chemical Co., Ltd. (U.S.A.). The source of copper(II) ions was $CuSO_4 \cdot 5H_2O$ from E. Merck (Darmstadt, Germany).

Preparation of a Copper(II)-Complexed Matrix and Packing of Chromatographic Columns β-Cyclodextrin polymer was prepared by a modification of the method developed by J. Solms *et al.*[9] The resulting dried cyclodextrin-polymer gel was crushed to a powder using a mortar and pestle, and sieved to obtain narrow size fractions from 48 to 100 mesh, which were used as chromatographic column matrices. Then, 5.5 g of this dry β-cyclodextrin polymer was treated with 25 ml 0.1 M $CuSO_4$ as already described.[5]

A copper-free control column was packed with untreated β-cyclodextrin polymer suspended in 1 N NaOH.

Chromatography on a Copper(II)-Complexed or Copper(II)-Free β-Cyclodextrin Polymer Equilibrated with 1 N NaOH 0.5 ml 1 N NaOH solution containing 0.1 mg nucleoside was placed on each column and eluted with 1 N NaOH at room temperature.

To determine the amount of nucleoside in each fraction, absorbance at 260 nm was measured.

Since repeated use of the copper(II)-loaded matrix caused a gradual release of copper(II) ions about 50 ml of copper(II) solution was applied to this column after every 300 ml of total eluate. The supernatant of the mixed solution of 50 ml 1 N NaOH and 0.5 ml 0.1 M $CuSO_4$ was used as the copper(II) solution.

The amount of copper(II) bound to the column matrix was determined by chelatometry and titrating with EDTA as described in a previous paper.[5]

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