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Note

Outer-sphere ligand exchange chromatography of nucleotides and related compounds on a modified polysaccharide gel

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In two recent papers concerning the study of new purification methods in biochemistry, Hubert and Porath[1,2] reported the fractionation of mono- and dinucleotides on a biscarboxymethylamino group-containing Sepharose gel loaded with a suitable metal. The dominant retention mechanism is considered to be an inner-sphere ligand exchange of the organic base moieties with the metal chelated on the support. On the other hand, a system with the support having a chemically bonded inert cationic complex has so far found less application in this field. In this instance outer-sphere complexing between the cationic complex and anionic sample species should control the retention mechanism. This approach was successfully exploited by Chow and Grushka[3] in the separation by high-performance liquid chromatography of nucleotides on a microparticulate siliceous support containing a $Co(en)_3^{3+}$ moiety.

In this paper the preparation of a cross-linked dextran gel (Sephadex G-75) with amino-cobalt(III) complex groups is described. This phase is obtained by reaction of an ω-aminobutyl-Sephadex derivative with $[Co(tetren)Cl]^{2+}$. The separation of some mono- and dinucleotides on this support has been achieved.

EXPERIMENTAL

Nucleotides and dinucleotides were obtained from Serva (Heidelberg, G.F.R.) and Sigma (St. Louis, MO, U.S.A.). All reagents were of analytical-reagent grade.

The ω-amino polysaccharide derivative support was prepared according to the method described by Cuatrecasas[4] using 4.0 g (dry gel) of Sephadex G-75 (particle size 40–120 μm) (Pharmacia), cyanogen bromide (6.18 g) and diaminobutane (10 g) (Fluka, Buchs, Switzerland). Its nitrogen content was 0.44%. Chlorotetraethylenepentaminecobalt(III) tetrachlorozincate(II), $[Co(tetren)Cl]ZnCl_4$, was synthesized as described by House and Garner[5].

A 6-g amount of $[Co(tetren)Cl]ZnCl_4$ was dissolved in 200 ml of 1 *N* hydrochloric acid and the solution was percolated through two columns (19 × 2.2 cm I.D.) packed with AG 1-X8 (Cl^-) anion-exchange resin (100–200 mesh) (Bio-Rad Labs.,

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Richmond, CA, U.S.A.) to make the eluate free from zinc as detected by flame atomic-absorption spectrophotometry on a Perkin-Elmer Model 372 instrument. The aminobutyl-Sephadex was added to the red-violet solution containing the cobalt(III) cation complex, adjusted to pH 7.0 with 1 *N* sodium hydroxide solution, then the mixture was stirred for 1 h at 80°C. The suspension was filtered through a sintered-glass Buckner funnel and the resulting red-orange gel was washed with a large amount of distilled water. The nitrogen and cobalt contents were 0.97% and 0.44%, respectively.

Chromatography was performed at room temperature using a glass column (16 mm I.D.), a peristaltic pump and a Varian Aerograph Variscan LC continuously variable wavelength detector to monitor the column effluent at 254 nm.

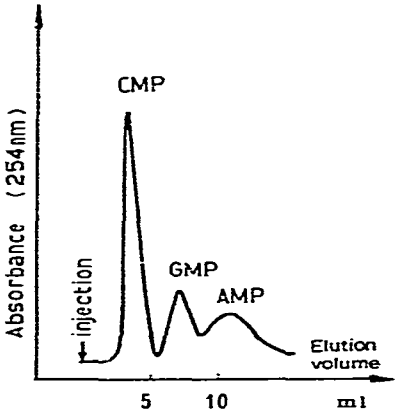

Fig. 1. Elution curve for the mononucleotides CMP, GMP and AMP. Column bed, 30 × 16 mm; buffer, Tris–0.01 *M* hydrochloric acid (pH 7.0)–0.01 *M* magnesium sulphate; flow-rate, 18 ml/h.

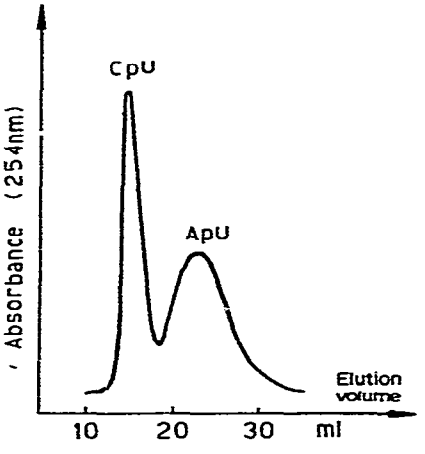

Fig. 2. Elution curve for the dinucleotides CpU and ApU. Column bed, 27 × 16 mm; buffer: Tris–0.01 *M* hydrochloric acid (pH 7.0)–0.003 *M* magnesium sulphate; flow-rate, 25 ml/h.

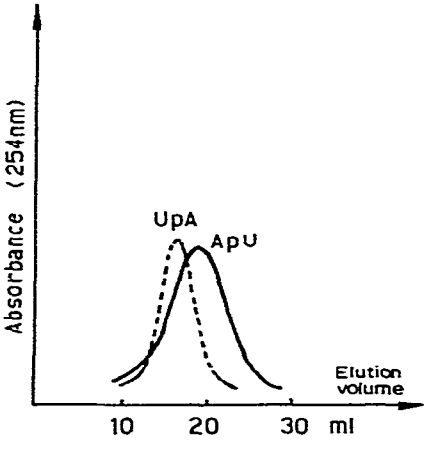

Fig. 3. Elution curve for the dinucleotides UpA and ApU eluted separately. Column bed and buffer as in Fig. 2; flow-rate, 24 ml/h.

RESULTS

Figs. 1–3 show respectively the isocratic elution of three mononucleotides (CMP, GMP and AMP) and two pairs of dinucleotides (CpU–ApU and UpA–ApU). Separations were achieved for CMP, GMP and AMP (Fig. 1) and CpU–ApU (Fig. 2), but the UpA–ApU mixture could not be resolved (Fig. 3). On the ω-aminopolysaccharide without the cobalt complex all of the above substances were strongly adsorbed and did not elute. It therefore seems that the separation effects obtained are due mainly to an outer-sphere complexing mechanism.

Further studies with this modified support are in progress in order to improve the separation of complex systems.

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