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Note

Metal chelate affinity chromatography

II. Group separation of mono- and dinucleotides

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In a recent communication[1] we investigated the influence of various parameters such as pH, buffer composition and ionic strength on the retention of various nucleotides and related compounds in copper chelate affinity chromatography. In the present paper, we report an extension of our studies to illuminate further the potential of our method to separate nucleotides according to their metal affinity.

EXPERIMENTAL

The gel used was a bis-carboxymethylamino Sepharose 6B. It was prepared according to the published procedure[1,2] by activation of Sepharose 6B with epichlorohydrin, followed by treatment with sodium iminodiacetate. The adsorbent was packed into columns (diameter 1 cm, V_t = 2–5 ml), loaded with the desired metal ion (20 mM aqueous solution of $CuSO_4 \cdot 5\ H_2O$ or $NiSO_4 \cdot 7\ H_2O$), washed with distilled water and equilibrated with the desired buffer until loosely bound metal was completely eluted.

Artificial mixtures to be tested were prepared from aqueous solutions (2.5 mg/ml) of different nucleotides. The elution profile was recorded at 280 nm. Identification of the peaks was made from the reduced elution volumes, V_e/V_t, of the compounds chromatographed as pure samples.

Specific conditions used in each separation experiment (composition of the nucleotide mixture, V_t of the column, elution buffer) are indicated in the legends to the figures.

RESULTS AND DISCUSSION

Group fractionation of pyrimidine and purine mononucleotides can easily be achieved on a copper chelate adsorbent, as shown in Fig. 1. Since all the pyrimidines show little interaction with the bound metal[1] they are eluted together in a single peak at the void volume. The stronger complexation of purine mononucleotides allows their separation from pyrimidine mononucleotides and also provides sufficient selectivity to differentiate AMP from GMP.

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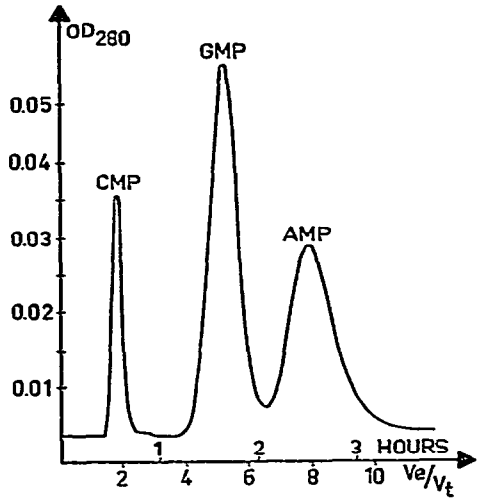


Fig. 1. Fractionation of CMP (62.5 μg per 25 μl) + GMP (62.5 μg per 25 μl) + AMP (250 μg per 100 μl). Copper column: V_t = 4.8 ml. Flow-rate: 15 ml/h. Buffer: 0.1 *M* ethylmorpholine-acetic acid (pH 6.5)–0.2 *M* $MgSO_4$.

The same behaviour was observed with the deoxy derivatives (Fig. 2). However, the fractionation of AMP or GMP from their respective deoxy homologues could not be achieved (results not shown), indicating a minor contribution of the 2′-OH group in the retention process.

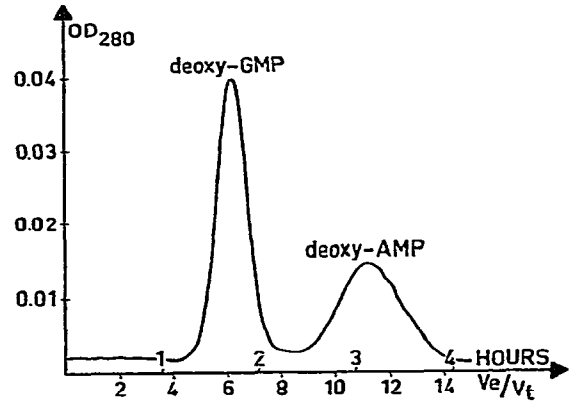


Fig. 2. Fractionation of deoxy-GMP (100 μg per 40 μl) + deoxy-AMP (300 μg per 120 μl). Copper column: V_t = 4.5 ml. Other details as in Fig. 1.

Copper chelate affinity chromatography affords excellent separation of dinucleotides. However, with this metal the dipurine compounds are generally strongly adsorbed (ApA: $V_e/V_t \approx 45$ in 0.05 *M* Tris–HCl (pH 7.0)–1 *M* NaCl) and cannot be eluted within a reasonably short time. To overcome this difficulty, the ligand exchange capacity of the gel can be decreased by raising the pH, the molarity of the elution buffer or /and its nucleophilic character[1]. Alternatively, we performed the group experiment on a gel loaded with Ni^{2+}, a less potent chelating ion under our conditions. Fig. 3 shows that isocratic elution of a mixture of dinucleotides allows total fractiona-

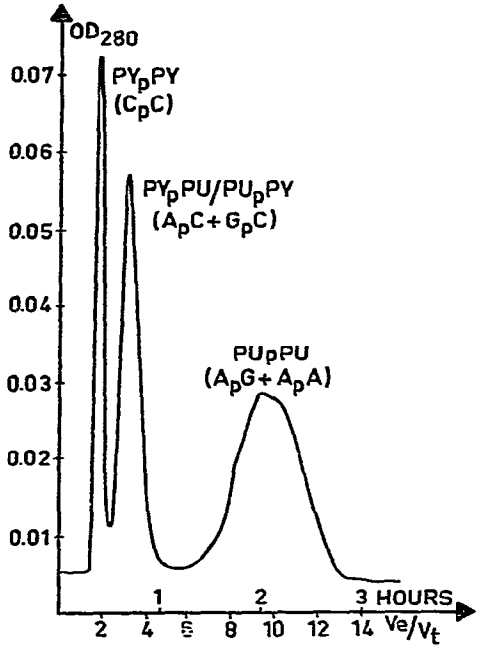


Fig. 3. Group fractionation of CpC (17.5 μg per 7 μl) + GpC (25 μg per 10 μl) + ApC (37.5 μg per 15 μl) + ApC (75 μg per 30 μl) + ApA (125 μg per 50 μl). Nickel column: V_t = 3 ml. Flow-rate: 14 ml/h. Buffer as in Fig. 1.

tion into three peaks on the basis of the purine content of the dinucleotide molecules: PUpPU > PUpPY, PYpPU≫ PYpPY. Fractionation within the PUpPY/PYpPU group can be obtained when the experiment is performed on the copper chelate gel (Fig. 4).

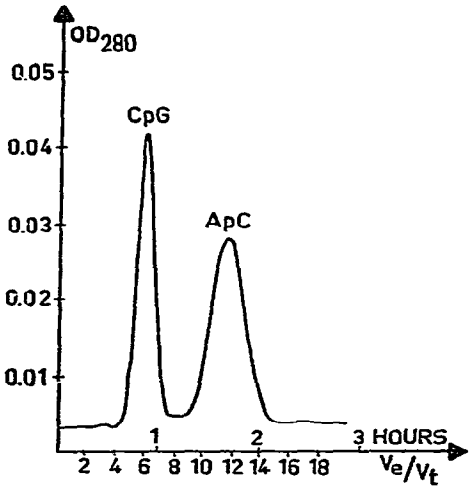
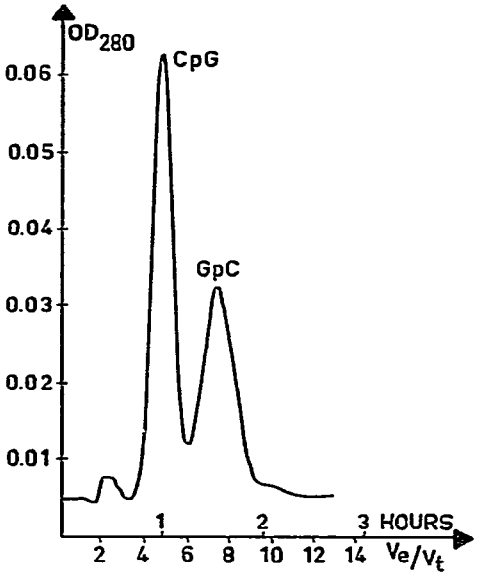


Fig. 4. Fractionation of CpG (62.5 μg per 25 μl) + ApC (250 μg per 100 μl). Copper column: V_t = 2.45 ml. Flow-rate: 17 ml/h. Buffer as in Fig. 1.

The high selectivity of metal chelate affinity chromatography is further demonstrated by the total separation afforded of a mixture of dinucleotides belonging to the PUpPY/PYpPU group, on the basis of the position of the purine moiety in the molecule. Figs. 5 and 6 show, respectively, the separation of CpG from GpC and CpA from ApC in an isocratic elution process.



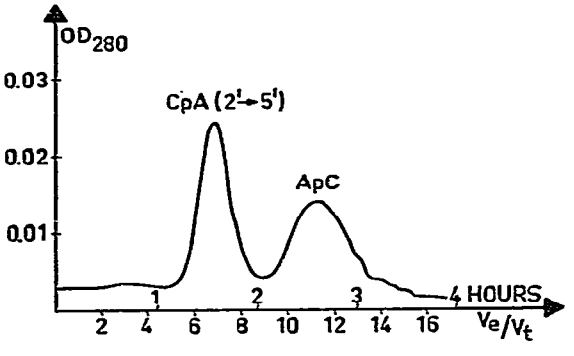


Fig. 5. Fractionation of CpG (87.5 μg per 35 μl) + GpC (87.5 μg per 35 μl). Copper column: V_t = 2.75 ml. Flow-rate: 13.2 ml/h. Buffer as in Fig. 1.

Fig. 6. Fractionation of ApC (87.5 μg per 35 μl) + $CpA_{(2'\rightarrow 5')}$ (87.5 μg per 35 μl). Copper column: V_t = 4.5 ml. Flow-rate: 19.2 ml/h. Buffer as in Fig. 1.

The retention of nucleotides on metal chelate affinity chromatography is due to complex phenomena. The abundance of potential binding sites (nitrogen and oxygen atoms on the bases, hydroxyl groups on the ribose and negatively charged oxygen atoms in the phosphate residue) account for this complexity, although our data seem to rule out any major contribution of either the phosphate or the ribose moieties to the binding.

The dominant role played by the bases in the coordination mechanism, as suggested by our results, has already been demonstrated by crystallographic studies as well as experiments in solution[3]. The nature of the bases present in an oligonucleotide is obviously very important (Figs. 1–4), but the conformation of the molecule should also be taken into account. In the case of ApC and CpA for instance, the examination of models as well as theoretical calculations[4] indicates the existence of very small differences, concerning mainly the orientation of the heterocyclic rings. These differences presumably affect the overlapping of the orbital systems of the two bases and modify also the environment of the most strongly interacting centre, *i.e.*, purine N_7 (ref. 3). This different environment of the N_7 purine binding site, along with the fact that the immobilization of the metal on a rather rigid matrix may favour the approach

of molecules having a certain type of conformation, may explain the surprisingly good separation obtained. Alternatively, the occurrence of a fifth or even sixth copper coordination bond[5] and the participation of the pyrimidine ring in a weak binding could also be responsible for the effect observed, due to a slight difference (between the two derivatives) in the base–metal–base spatial arrangement. These differences and their reflection in the chromatographic behaviour should be further explored in order to develop particularly efficient methods for fractionation of nucleotides on a preparative scale.

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REFERENCES

1 P. Hubert and J. Porath, *J. Chromatogr.*, 198 (1980) 247.
2 J. Porath and L. Sundberg, *Protides Biol. Fluids*, 18 (1970) 401.
3 H. Sigel (Editor), *Metal Ions in Biological Systems*, Vol. 8, Marcel Dekker, New York, 1979.
4 C. H. Lee, E. Charney and I. Tinoco, *Biochemistry*, 18 (25) (1979) 5636.
5 A. F. Wells, *Structural Inorganic Chemistry*, 3rd ed., Oxford University Press, London, 1967.