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CORRELATION OF THE DIFFERENCES IN CONFORMATION BETWEEN 2′–5′ AND 3′–5′ DINUCLEOSIDE MONOPHOSPHATES WITH THEIR BEHAVIOUR ON A SEPHADEX LH-20 COLUMN

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SUMMARY

The separation of 2′–5′ dinucleoside monophosphates from their 3′–5′ isomers on Sephadex LH-20 column is described. In addition, the CD spectra of all the different dinucleoside monophosphates are given. The correlation of the differences in conformation between the 2′–5′ and the 3′–5′ dinucleoside monophosphates with their behaviour on the Sephadex LH-20 column is discussed. It is shown that nucleotides do not behave on the Sephadex LH-20 column according to the rules of gel filtration and it is suggested that the separation of the different dinucleoside monophosphates is dictated by the conformation, which affects the adsorption of the nucleotidic material to the column.

INTRODUCTION

Dinucleoside monophosphates represent the smallest diester units of nucleic acids, and were used as model compounds for chemical, physicochemical and enzymatic studies. Most of the studies were done with dinucleoside monophosphates containing the natural 3′–5′ diester linkage. Brahms *et al.*[1] Kondo *et al.*[2] and Warshaw and Cantor[3] studied the influence of the phosphodiester linkage on the conformation of CpC, ApA, ApC and GpA. From the results of nuclear magnetic resonance and circular dichroism studies, the authors concluded that the conformation of A2′–5′A, A2′–5′C, C2′–5′C and G2′–5′A is different from the corresponding 3′–5′ isomers.

In 1968 Ukita *et al.*[4] reported on the separation between 2′–5′ and 3′–5′ dinucleoside monophosphates on a Dowex-1 X2 (formate form) column at acidic pH. Recently we showed that a similar separation between the two isomers can be achieved by chromatography on a DEAE-Sephadex (bicarbonate) column at pH 8.6 (ref. 5). It was suggested that the different elution pattern of the 2′–5′ and the corresponding

Abbreviations: A, adenosine; C, cytidine; G, guanosine; U, uridine. ApN, CpN, GpN, UpN, dinucleoside monophosphates where N stands for A, C, G or U. Pu, purine base; Py, pyrimidine base.

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3′–5′ dinucleoside monophosphates is due to conformational differences[5]. In order to exclude the influence of charges on the separation between the two isomers on the ion exchange columns, the behaviour of the different dinucleoside monophosphates on a Sephadex LH-20 column was studied and the results are reported in the present communication. Moreover, we discuss the correlation of the differences in conformation between 2′–5′ and 3′–5′ dinucleoside monophosphates (as concluded from the CD spectra) with their behaviour on the Sephadex LH-20 column.

MATERIALS AND METHODS

Sephadex LH-20, particle size 25–100 μm, (Lot No. 2772) was purchased from Pharmacia, Uppsala, Sweden. 2′–5′ and 3′–5′ dinucleoside monophosphates were prepared as described elsewhere[5].

The chromatography apparatus consisted of a 1.5 cm × 75 cm column (K 15/90, Pharmacia, Uppsala, Sweden), (except for the experiments involving organic solvents and heating where a water-jacketed column 2.4 cm × 55 cm was used), an ultraviolet flow-through cell 254 nm (LKB Uvicord I), recorder (LKB 6520), and an LKB fraction collector. Samples were applied in 0.1 ml of 0.01 M cacodylate buffer, pH 7.0, at a concentration of about 10 mg/ml. The flow rate was 80 ml/h and fractions of 3.6 ml were collected. The void volume was determined by the use of bulk tRNA (from *Escherichia coli*). The identification of the peaks was done by high voltage ionophoresis on DEAE-cellulose paper[6]. In the experiments involving heating of the column, a Forma thermostatically regulated heater was used.

Spectroscopic measurements

Ultraviolet absorption was measured on a Cary 14 spectrophotometer at 25 °C. CD spectra in the region of 220–300 nm were measured on a Cary 60 equipped with CD attachment 6001, using a pen period of 3 s and a full range sensitivity of 0.04 degrees. Before the measurements, samples were heated to 60 °C for 5 min, and measurements were taken at 26 °C.

When not noted otherwise, samples were dissolved in 0.01 M cacodylate buffer, pH 7.0. The solution of GpG contained 0.04 M urea because it was prepared by diluting a hot concentrated solution in 8 M urea with the 0.01 M cacodylate buffer. In all cases the concentration of the bases was about $1 \cdot 10^{-4}$ M. The concentrations were calculated from the absorption at 260 nm.

RESULTS

The behaviour of all the sixteen dinucleoside monophosphates, containing the natural 3′–5′ linkage and the unnatural 2′–5′ linkage, on a Sephadex LH-20 column is summarized in Table I. The elution pattern of the different dinucleoside monophosphates is dictated by the nature of the phosphodiester bond, (2′–5′ or 3′–5′), by the base composition and base sequence (UpA *versus* ApU, GpA *versus* ApG, *etc.*). In all cases where the two isomers separate from each other, the dinucleoside monophosphate containing the 2′–5′ linkage is eluted before the 3′–5′ isomer. In one case, CpU, the two isomers are eluted together.

The extent of the separation between the 3′–5′ and the 2′–5′ isomers of a given

TABLE I

CHROMATOGRAPHY OF DINUCLEOSIDE MONOPHOSPHATES ON SEPHADEX LH-20 COLUMN

Fluent: 0.01 M cacodylate buffer, pH 7.0. Unfractionated tRNA was used as marker to indicate the void volume and came out in Fraction No. 11. For details, see Materials and Methods.

Dinucleoside phosphate	*Peak position (fraction No.)*	
	2′–5′ linkage	*3′–5′ linkage*
ApA	26	34
ApC	26	29
ApG	24	31
ApU	24	29
CpA	25	26
CpC	18	19
CpG	19	21
CpU	20	20
GpA	23	29
GpC	19	22
GpG	22	26
GpU	21	25
UpA	23	25
UpC	20	22
UpG	23	25
UpU	19	21

dinucleoside monophosphate depends on the base composition and on the sequence of the bases. Dinucleoside monophosphates containing only purines (ApA, ApG, GpA, or GpG) give better separation between the two isomers as compared to dinucleoside monophosphates containing only pyrimidines (UpU, UpC, CpC or CpU). In dinucleoside monophosphates containing a purine and a pyrimidine, the separation between the two isomers depends on the order of the bases. When the base in the 5′ end is a purine, the separation is much better than in the case where pyrimidine

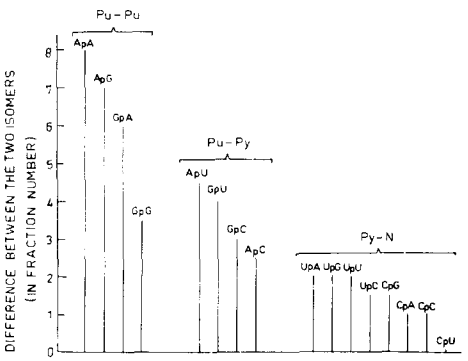
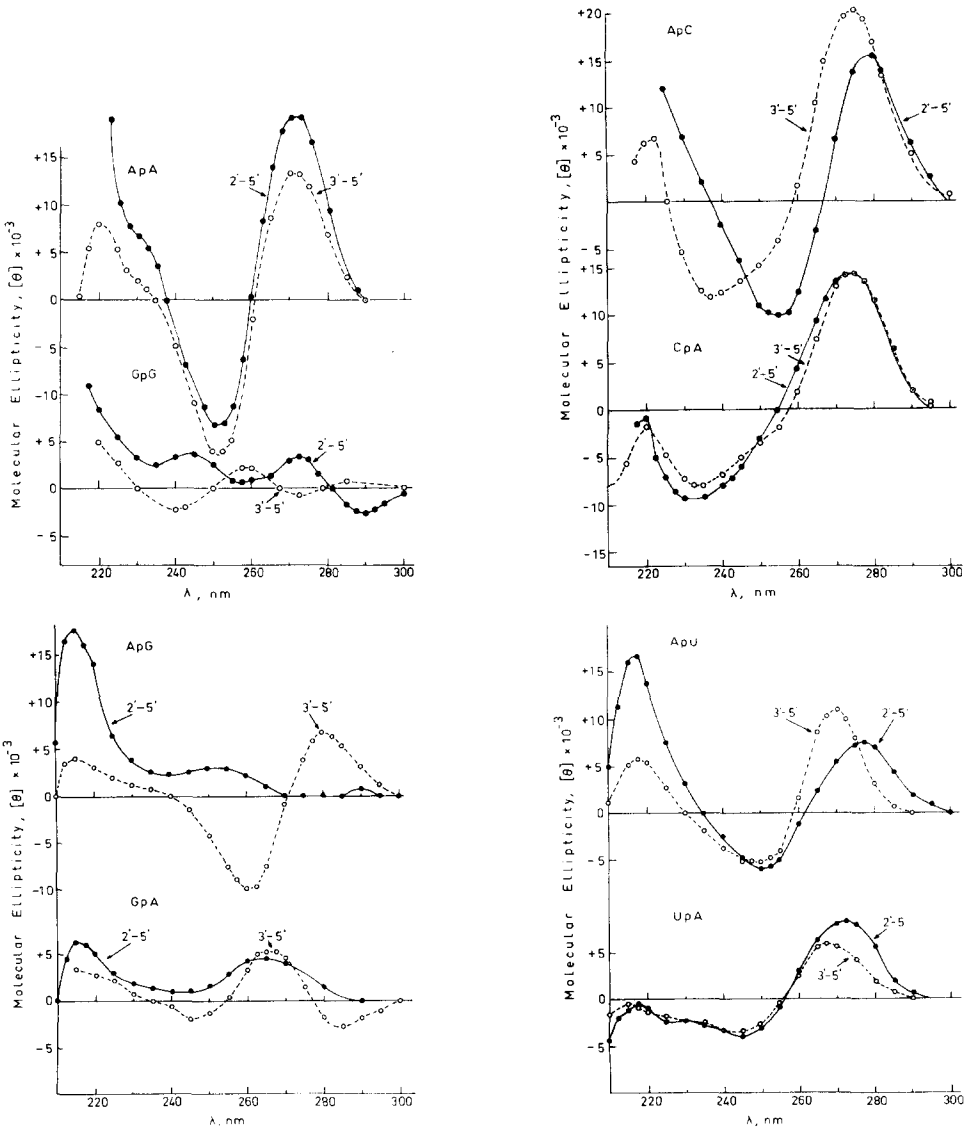


Fig. 1. Separation of 2′–5′ and 3′–5′ isomers of dinucleoside monophosphates containing the same base sequence on a Sephadex LH-20 column, under the conditions described in Table 1. The ordinate corresponds to the difference in fraction number of the peak of the 3′–5′ isomer *minus* the fraction number of the peak of the 2′–5′ isomer.

is in the 5′ end. Thus, the two isomers of ApU are separated better than those of UpA; the isomers of GpU are separated better than those of UpG, *etc.* Fig. 1 summarizes the separation between the two isomers as function of base composition. From Fig. 1 a simple rule can be derived, showing the extent of separation between the two isomers Pu–Pu ≧ Pu–Py > Py–*N*. The only exception is GpG, in which the two isomers do not separate as well as those of ApU or GpU.

In order to study the correlation of the differences in the elution pattern of the different dinucleoside monophosphates on the Sephadex LH-20 column and the differences in their conformation, the CD spectra of all the 2′–5′ and the 3′–5′ isomers were measured. As can be seen in Fig. 2, the two isomers of a given dinucleoside



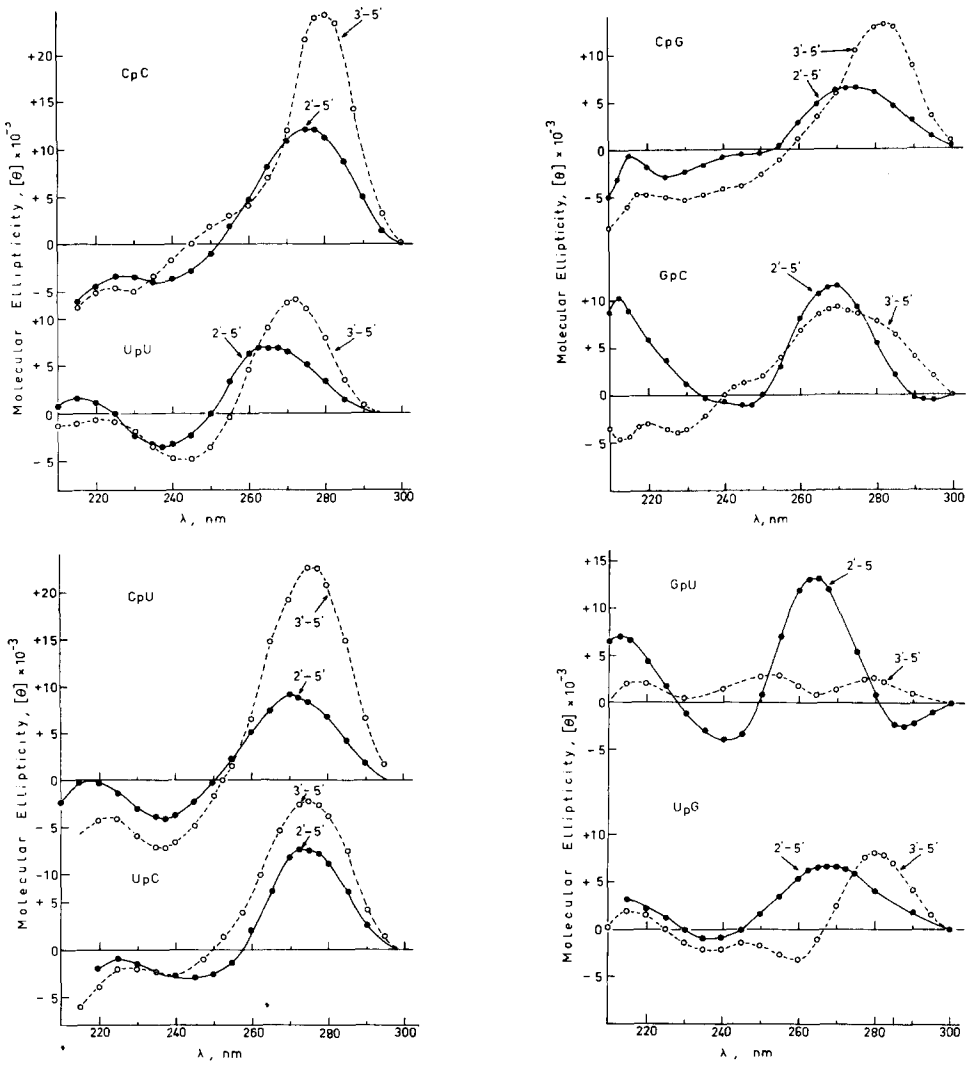


Fig. 2. CD spectra of 2′–5′ and 3′–5′ dinucleoside monophosphates. For details, see Materials and Methods.

monophosphate differ significantly from each other in the amplitude as well as in the position of the peaks. Fig. 2, for example, gives the CD spectra of four dinucleoside monophosphates containing A and U. In the shorter wavelength region ApU have high peaks, while UpA have a small net ellipticity. The A2′–5′U peak at 215 nm is about three times larger than that of A3′–5′U. In the long wavelength region there is a distinct difference in the peak positions: 268, 270, 273 and 277 nm for U 3′–5′A; A3′–5′U; U2′–5′A and A2′–5′U, respectively. There are also magnitude differences: A2′–5′U has a smaller peak as compared to A3′–5′U and the reverse is true for the UpA pair. Differences in peak position and magnitude between the CD spectra of 2′–5′ and 3′–5′ isomers are also found in all other dinucleoside monophosphates shown

in Fig. 2. The differences in the CD spectra between the different dinucleoside monophosphates indicates conformational differences. However, in the presence of 8 M urea or 50 % dimethylsulphoxide the CD spectra show partial conformational destruction of the dinucleoside monophosphates (results not shown).

If we assume that the separation of the two isomers of a given dinucleoside monophosphate on Sephadex LH-20 column is due to conformational differences, then the presence of 8 M urea, 50 % dimethylsulphoxide or high temperature should interfere with the separation between the two isomers. Fig. 3 shows the elution profile of three different pairs of isomers in the presence and absence of 8 M urea. U2′–5′A does not separate from U3′–5′A in the presence of 8 M urea (Fig. 3b), while in the absence of urea there is a good separation between the two isomers (Fig. 3a). A2′–5′U also does not separate from the 3′–5′ isomer in the presence of 8 M urea, and in the case of ApA the separation between the two isomers is very much diminished. Moreover, no separation was found between ApU, UpA and A2′–5′A in the presence of 8 M urea (Fig. 3b), while a good separation between these dinucleoside monophosphates was found in the absence of 8 M urea (Fig. 3a). In addition, in all examples given in Fig. 3, the dinucleoside monophosphates are less retarded on the column in the presence of 8 M urea.

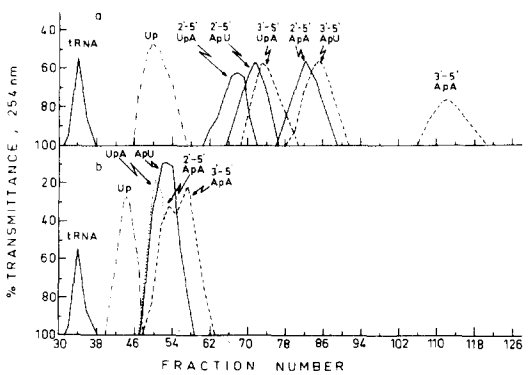


Fig. 3. Comparison of the chromatographic behaviour of dinucleoside monophosphates on a Sephadex LH-20 column in the presence and absence of 8 M urea. (a) Elution with 0.01 M cacodylate buffer, pH 7.0, and in the absence of urea. (b) Elution with 0.002 M cacodylate buffer, pH 7.0, in the presence of 8 M urea.

Fig. 4 shows the effect of high temperature and dimethylsulphoxide on the separation of A2′–5′U from A3′–5′U. The separation between the isomers at room temperature with 0.01 M cacodylate buffer, pH 7.0, is shown in Fig. 4a. When the elution was performed at 61 °C the two isomers were less retarded on the column, but still were separated from each other (Fig. 4b). Elution in the presence of 50 % dimethylsulphoxide at room temperature diminishes the separation between the two isomers (Fig. 4c); and when the elution was performed with 50 % dimethylsulphoxide at 61 °C, there was no separation and the retardation was very much diminished (Fig. 4d).

Sephadex LH-20 contains a small amount of carboxyl groups (about 0.02 mequiv/g) and the exclusion of anions or the retardation of cations on the column can

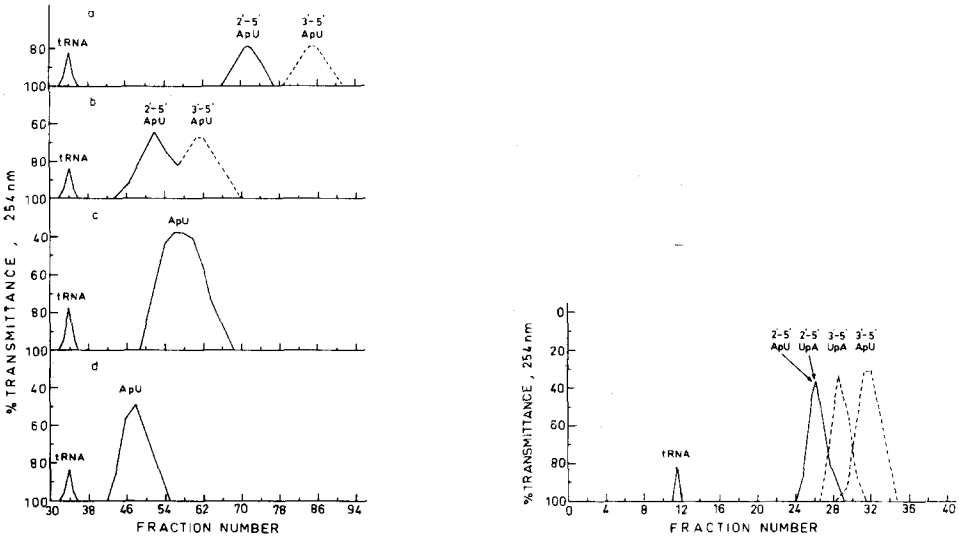


Fig. 4. Comparison of the chromatographic behaviour of 2′–5′ and 3′–5′ ApU on a Sephadex LH-20 column with and without the presence of 50 % dimethylsulphoxide at different temperatures. (a) Elution with 0.01 M cacodylate buffer, pH 7.0, at room temperature. (b) Elution as in (a) except for run at 61 °C. (c) Elution as in (a) except for an eluent which contains 50 % dimethylsulphoxide diluted with cacodylate buffer, pH 7.0 (final concentration 0.005 M). (d) Elution with 50 % dimethylsulphoxide diluted with cacodylate buffer, pH 7.0, (final concentration 0.005 M) at 61 °C.

Fig. 5. Chromatography of 2′–5′ and 3′–5′ isomers of ApU and UpA on a Sephadex LH-20 column. Eluent: 0.1 M cacodylate buffer, pH 7.0 (see Table 1 for the chromatography of ApU and UpA eluted with a lower salt concentration). For details, see Materials and Methods.

be eliminated at ionic strengths exceeding 0.05 M[7]. It does not seem to us that at pH 7.0 the existence of such charges have to do with the separation between the 2′–5′ dinucleoside monophosphates and the corresponding 3′–5′ isomers on Sephadex LH-20 column. But in order to exclude any influence of charges on the behaviour of dinucleoside monophosphates on the Sephadex LH-20 column, the elution of ApU and UpA was performed with 0.1 M cacodylate buffer. (The CD spectra of the dinucleoside monophosphates at 0.1 M cacodylate buffer do not differ from those at 0.01 M buffer.) As can be seen from Fig. 5 the separation of A2′–5′U (or U2′–5′A) from the corresponding 3′–5′ isomers at high ionic strength is similar to the separation performed at low ionic strength (Table I). It is interesting to note that at the high salt concentration both isomers move slower than at the low salt concentration. These results can be explained by a stronger adsorption of the nucleotidic material to the column at high ionic strength.

DISCUSSION

The separation of different molecules by gel chromatography proceeds mainly according to the differences in molecular size and shape. Usually, compounds with the higher molecular weight are eluted from the column before compounds with the lower molecular weight. It is clear from the results represented in the present communication that the separation of dinucleoside monophosphates on Sephadex LH-20

column does not proceed according to the conventional gel filtration. Mononucleotides, for example, are eluted from the column before dinucleoside monophosphates (Fig. 3) although the mononucleotides have a lower molecular weight. UpU with the lower molecular weight is eluted before ApA and CpC is eluted from the column before GpG (this finding is true for either isomer). On the other hand, there are cases where two dinucleoside monophosphates containing the same base composition (and therefore have the same molecular weight) but differ in the base sequence, have a different elution pattern on the Sephadex LH-20 column. Thus, for example C2′–5′A is eluted before A2′–5′C, or C3′–5′A is eluted before A3′–5′C. Finally, dinucleoside monophosphates having the same base composition and the same base sequence, but differ only in the nature of the phosphodiester bond, behave differently on the Sephadex LH-20 column. As a rule, dinucleoside monophosphates which contain the 2′–5′ linkage are eluted from the column before the corresponding 3′–5′ isomer (the only exception being CpU in which the two isomers are eluted together).

In all the cases where there are differences in the elution pattern on the Sephadex LH-20 column, there are also differences in the CD spectra, which indicates differences in conformation. Moreover, in the presence of 8 M urea, or 50 % dimethylsulphoxide (which causes partial conformational destruction) the differences in the elution pattern on the Sephadex LH-20 column is very much diminished (Figs 3, 4).

It seems to us, therefore, that another factor than gel filtration, most probably adsorption, has a strong effect on the elution pattern of nucleotides on Sephadex LH-20 column. The adsorption to the column is effected mainly by two factors: the chemical nature and the conformation of the dinucleoside monophosphate. Thus, the difference in the adsorption to the column between ApA and UpU, for example, can be explained by differences in the chemical nature of the two compounds, while the differences in the adsorption between A2′–5′A and A3′–5′A, for example, can be explained by differences in conformation. Kondo *et al.*[2] suggested that A2′–5′A and A2′–5′C are more compact than the corresponding 3′–5′ isomers. Therefore we would expect that the 3′–5′ isomers will interact with the Sephadex LH-20 stronger than the corresponding 2′–5′ isomers. And indeed, all the 2′–5′ dinucleoside monophosphates are less adsorbed to the column and therefore are eluted before the corresponding 3′–5′ isomers. A similar order of elution was also found on Dowex 1-X2 (formate) column[4], DEAE-Sephadex (bicarbonate) column[5], thin-layer ion-exchange chromatography and paper ionophoresis[6].

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REFERENCES

1 Brahms, J., Maurizot, J. C. and Michelson, A. M. (1967) *J. Mol. Biol.* 25, 481–495
2 Kondo, N. S., Holmes, H. M., Stempel, L. M. and Ts'o, P. O. P. (1970) *Biochemistry* 9, 3479–3498

3 Warshaw, M. M. and Cantor, C. R. (1970) *Biopolymers* 9, 1079–1103
4 Ukita, T., Irie, M., Imazawa, M., Furuichi, Y., Nishimura, H. and Sekiya, T. (1968) *Seikagaku (Japan)* 40, 363–371
5 Lapidot, Y. and Barzilay, I. (1972) *J. Chromatogr.* 71, 275–281
6 Lapidot, Y., Barzilay, I. and Salomon, D. (1972) *Anal. Biochem.* 49, 301–306
7 *Sephadex LH*-20, p. 6, Pharmacia, Uppsala, Sweden