*Journal of Chromatography*, 124 (1976) 415–417


CHROM. 9291

## Note

# Rapid resolution of 5'-mono-, -di-, and -triphosphate ribo- and deoxyribonucleoside mixtures by conventional anion-exchange chromatography*

J. X. KHYM

*Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830 (U.S.A.)*

(Received March 15th, 1976)

Ever since chromatographic techniques have been applied to the task of separating nucleic acid components (for reviews, see refs. 1–4), considerable thought and effort has been put forth, by investigators from many different laboratories, to solve the difficult problem of separating complex nucleotide mixtures. As a result of these efforts, several different chromatographic methods[5–13] are now available for the rapid separation of complex mixtures of either ribo- or deoxyribomononucleoside phosphates, but only limited success has been achieved in separating mixtures that contain all members of each class of nucleotide. For instance, simple mixtures of ribo- and deoxyribonucleoside monophosphates can be resolved from solutions containing borate[14] utilizing the techniques of paper[14], electrophoresis[7,14], ion-exchange[1,14,15], or thin-layer chromatography (TLC)[16]. Randerath and co-workers[6,17–19] have refined the latter technique and have developed two-dimensional TLC procedures to separate more complicated mixtures containing ribo- and deoxyribonucleotides at either the mono-, or di-, or triphosphate levels. But combinations of ribo- and deoxyribonucleotides at mixed phosphorylated levels are only partially resolved by the TLC procedures.

By refinements of the conventional ion-exchange method[13] used in this laboratory to separate nucleotides**, the resolution of mixtures that contain ribo- and deoxyribo-5'-nucleotides at the mono-, di- and triphosphate levels, or any combination of these, has been accomplished. This can be seen by the chromatogram shown in Fig. 1.

As noted in the legend to Fig. 1, a gradient delay and a lower initial pH are used to effect the separation shown. The gradient delay allows the solute bands of closely eluting species (*e.g.*, CDP, dCDP and GMP, or CTP and dCTP) to become somewhat separated on the column at low citrate concentration before the bands are removed from the column as sharp peaks by the increasing citrate concentrations of the gradient. The lower initial pH of 8.2 is necessary to resolve the UMP–dTMP pair; it also seems to effect the resolution of dTDP from CTP. The change to pH 8.6 (citrate concentration still at 25 m*M*) after the first 20 min is necessary to effect the separation

* Research supported by the Energy Research and Development Administration under contract with Union Carbide Corporation.

** Bases and nucleosides may be conveniently removed from nucleotide mixtures by polyacrylamide chromatography[20].

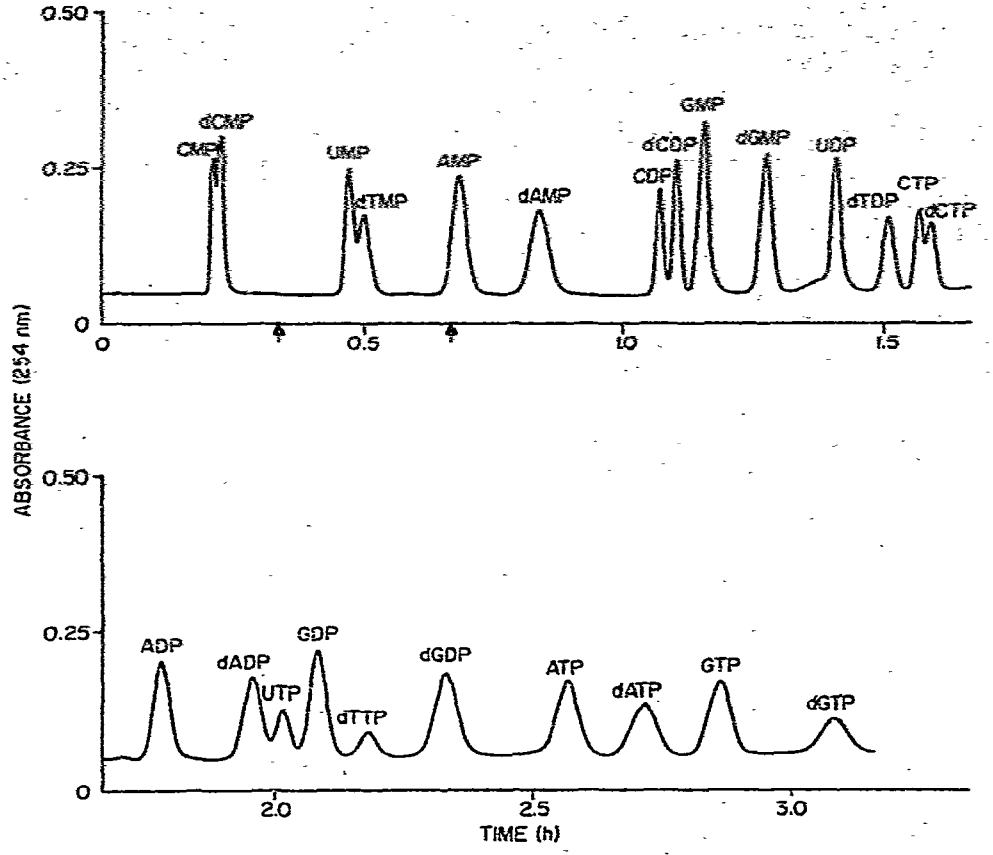


Fig. 1. Separation of a ribo- and deoxyribonucleoside phosphate mixture. Conditions: column, 20 × 0.62 cm I.D. of Aminex A-28 (citrate form); elution, 25 m*M* sodium citrate, pH 8.2 for the first 20 min (see first arrow) and 25 m*M* sodium citrate, pH 8.6 for the next 20 min, at which point (see second arrow) a convex gradient was started that was formed with a closed constant-volume mixing-container and an open reservoir vessel (see ref. 13). The former contained initially 50 ml of 25 m*M* citrate solution, and the latter 500 m*M* citrate solution, both at pH 8.6 (all citrate solutions contain 0.3 m*M* $NaN_3$ to prevent bacterial growth); flow-rate, 0.6 ml/min; column temperature, 70°; operating pressure, 160 p.s.i. at the beginning of the gradient, 175 p.s.i. at the end. The sorbed material was *ca.* 17 nmoles of each nucleotide shown contained in 50 $\mu$l of 25 m*M* sodium citrate, pH *ca.* 7.4; the sample was applied to the column by an off-column septum-type injector with a Hamilton syringe without interrupting the flow of eluent; recovery was quantitative. The column was monitored with a Model UA-5 ISCO UV analyzer (10-mm path-length flow-cell of 19-$\mu$l capacity).

of dADP from UTP; this increases slightly the retention time of UTP but that of dADP remains the same. This sensitivity to pH is because of the p*K* values of the base moieties in question; UMP, dTMP, and UTP have, respectively, p*K* values of 9.5, 10.0, and 9.5, while CTP, dCTP, and dADP have no acidic groups ionizing in this pH range.

Under study now is the application of a compound gradient to replace the simple elution mode described in Fig. 1. The use of a shallow gradient (both in pH and citrate concentration) at the beginning and increasing slightly in the middle, and

finally increasing strongly at the end of the chromatogram should improve the resolution of the CMP–dCMP and UMP–dTMP nucleotide pairs and also closely eluted nucleotide triplets such as CDP, dCDP and GMP, and dTDP, CTP and dCTP.

## REFERENCES


1 W. E. Cohn, in E. Chargaff and J. N. Davidson (Editors), *The Nucleic Acids*, Vol. I, Academic Press, New York, 1955, p. 211.
2 G. R. Wyatt, in E. Chargaff and J. N. Davidson (Editors), *The Nucleic Acids*, Vol. I, Academic Press, New York, 1955, p. 243.
3 J. D. Smith, in E. Chargaff and J. N. Davidson (Editors), *The Nucleic Acids*, Vol. I, Academic Press, New York, 1955, p. 267.
4 J. J. Saukkonen, *Chromatogr. Rev.*, 26 (1964) 53.
5 K. Randerath, *Thin-Layer Chromatography*, Verlag-Chemie, Weinheim/Bergstr. and Academic Press, New York, 1963.
6 K. Randerath and E. Randerath, in L. Grossman and K. Moldave (Editors), *Methods of Enzymology*, Vol. XIIA, Academic Press, New York, 1967. p. 323.
7 J. D. Smith, in L. Grossman and K. Moldave (Editors), *Methods of Enzymology*, Vol. XIIA, Academic Press, New York, 1967, p. 350.
8 C. Horvath, *Methods Biochem. Anal.*, 21 (1973) 79.
9 R. Singhal, *Sep. Purif. Methods*, 3 (1974) 339.
10 P. M. Rajcsanyi, M. Csillag and E. Kliskovics, *Sep. Purif. Methods*, 3 (1974) 167.
11 F. R. Brown, *High-Pressure Liquid Chromatography*, Interscience New York, 1973.
12 R. A. Hartwick and P. R. Brown, *J. Chromatogr.*, 112 (1975) 651.
13 J. X. Khym, *Clin. Chem.*, 21 (1975) 1245.
14 J. X. Khym, in L. Grossman and K. Moldave (Editors), *Methods of Enzymology*, Vol. XIIA, Academic Press New York, 1967, p. 93.
15 J. X. Khym and W. E. Cohn, *Biochim. Biophys .Acta*, 15 (1954) 139.
16 K. Randerath, *Biochim. Biophys. Acta*, 76 (1963) 622.
17 K. Randerath and E. Randerath, *J. Chromatogr.*, 16 (1964) 111.
18 E. Randerath and K. Randerath, *J. Chromatogr.*, 16 (1964) 126.
19 J. Neuhard, E. Randerath and K. Randerath, *Anal. Biochem.*, 13 (1965) 211.
20 J. X. Khym, *Anal. Biochem.*, 71 (1976) 231.