Paper chromatography of diribonucleoside monophosphates*

A two-dimensional descending chromatographic method for the simultaneous separation of four common ribonucleosides, their 3′(2′)-monophosphates, and 3′(2′) 5′-diphosphates has previously been reported[1]. The solvent system used consisted of ethanol–1 *M* ammonium acetate, pH 5.0 (75:30, v/v) in the first direction and isobutyric acid adjusted to pH 3.7 with 0.5 *M* NH_4OH in the second direction.

It is now reported that this method, with merely a shift of pH of the first solvent from 5.0 to 3.7, can be extended to the direct separation of diribonucleoside monophosphates of all the possible base pairings (Fig. 1). Sequential isomers are not resolved but they all cochromatograph, thus lessening complication of the chromatogram. The compounds used were prepared by demonophosphorylation of mixed 5′-terminal dinucleotides with *E. coli* alkaline phosphatase (EC 3.1.3.1) (Worthington Biochem.

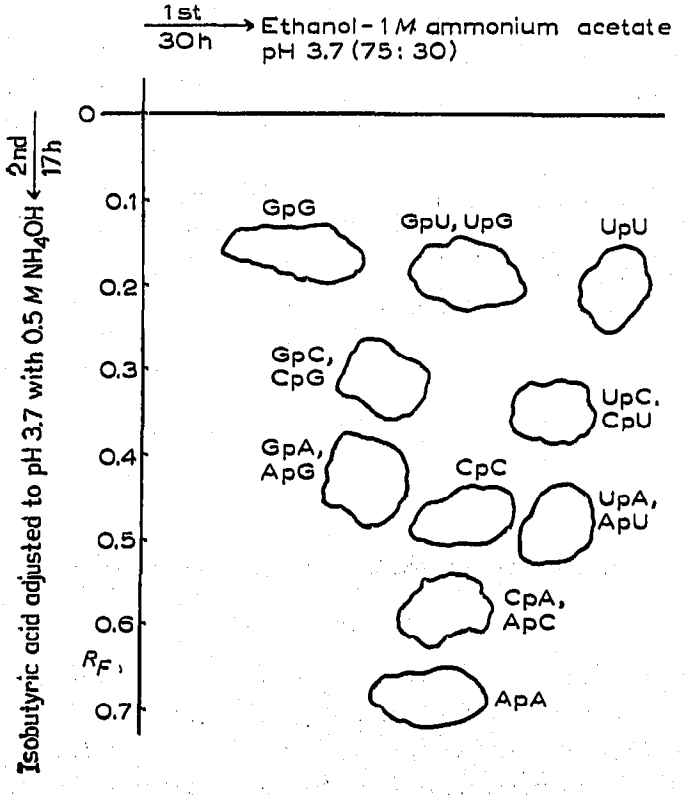

Fig. 1. Paper chromatogram of diribonucleoside monophosphates. The diribonucleoside monophosphate mixture, about 20 A_{270} units, was chromatographed using Toyo filter paper No. 51 A, 60 cm × 60 cm. In the first direction the solvent was allowed to drip from the serrated, lower edge of the paper, and the fastest moving UpU and the slowest GpG were about 22 cm and 7 cm from the origin after 30 h. In the second direction ApA and GpG moved about 33 cm and 8 cm in 17 h. Temperature, 22°. Each ultraviolet-absorbing spot was cut out, subjected to an elution concentration[2], eluted from the paper, and then digested to completion with 6 standard units (ref. 3) of purified ribonuclease$_{T_2}$ (EC 2.7.7.17) at pH 4.5 for 4 h at 37°. The digest was analysed for the constituent 3′-mononucleotides and nucleosides (3′-linked and 5′-linked nucleoside moiety, respectively) as mentioned in the text, and the original dinucleoside monophosphates were reconstructed therefrom. All the possible base-pairings, including sequential isomers, were found.

* The abbreviations of the derivatives of nucleic acids are those used by *Biochim. Biophys. Acta* according to IUPAC-IUB Commission on Biochemical Nomenclature (BBA Rules 4, *Biochim. Biophys. Acta*, 108 (1965) 1–4).

Corp.). The dinucleotides had been separated from a silkworm endonuclease digest of yeast RNA by DEAE-cellulose column chromatography with 7 *M* urea[4], and then desalted by a DEAE-cellulose bicarbonate method[5]. The demonophosphorylation brought about an increased mobility and a wider distribution of the nucleotides, and so improved the chromatogram greatly.

As far as is known, it has not been possible to separate dinucleosidic compounds of all the possible base pairings by a simple method. The present proposed method is also applicable to either 5'- or 3'-terminal dinucleotides or dinucleoside triphosphates since they all are easily and quantitatively converted to dinucleoside monophosphates enzymatically, and so may be widely used in the structural and enzymological work of nucleic acids.

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1 J.-I. MUKAI, *J. Chromatog.*, 21 (1966) 498.
2 F. DAVIS, C. A. DUBBS AND W. S. ADAMS, *Anal. Chem.*, 34 (1962) 175.
3 T. UCHIDA AND F. EGAMI, in G. L. CANTONI AND D. R. DAVIES (Editors), *Procedures in Nucleic Acid Research*, Harper and Row, New York, London, 1966, p. 46.
4 J. -I. MUKAI, *Biochem. Biophys. Res. Commun.*, 21 (1965) 562.
5 G. W. RUSHIZKY AND H. A. SOBER, *Biochim. Biophys. Acta*, 55 (1962) 217.

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Eine Trennkammer für die Hochspannungselektrophorese nach dem Michl'schen Prinzip

Das erste 1951 von MICHL[1] beschriebene Verfahren zur Hochspannungselektrophorese benutzt zur Abführung der im Papierstreifen auftretenden Joule'schen Wärme ein inertes organisches Lösungsmittel als Wärmeaustauscher. Da die Anordnung mit dem frei im Lösungsmittel hängenden Papierstreifen die Verwendung einfach gebauter Apparaturen erlaubt, hat diese Methode in der Folgezeit vielseitige Verbreitung gefunden (Zusammenfassungen Lit. 2 und 3). Dort, wo keine zu hohen Spannungsgefälle[4], zu lange Laufzeiten oder die Auftrennung von lipophilen Substanzen die Benutzung von Apparaten[5–7] mit Kühlflächen zur Wärmeableitung unumgänglich machen, ist die in Rede stehende Methodik mit Erfolg anwendbar.

Wir beschreiben nachstehend eine Kammer für das Papierformat 15 × 70 cm mit etwa 60 cm effektiver Trennlänge, die seit vielen Jahren von verschiedenen Arbeitskreisen[8–16] unseres Institutes erfolgreich benutzt wird.

Einfache Bauweise, leichte und gefahrlose Handhabung sowie gutes Trennvermögen sind die Vorteile dieser Kammer. Fig. 1 zeigt die Einzelteile der Apparatur. Der Tank (5) mit den Aussenmassen 80 × 27 × 13 cm ist aus 5 mm starkem,