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Separation of ribonucleoside 3′,5′-cyclic monophosphates by thin-layer chromatography on alumina

Previous chromatographic systems using cellulose media[1] do not permit a complete separation of the four ribonucleoside 3′,5′-cyclic monophosphates, and in these systems 2′,3′-cyclic mononucleotides and other mononucleotides often migrate similarly with the 3′,5′ cyclic mononucleotides[2]. In the course of developing a chromatographic assay system for adenosine 3′,5′-cyclic monophosphate, we found that all four ribonucleoside 3′,5′-cyclic monophosphates can be one-dimensionally separated on alumina thin-layer sheets. In this system, ribonucleoside 2′,3′-cyclic monophosphates as well as other ribonucleoside mono-, di-, and triphosphates do not migrate, thus allowing specific separation of the 3′,5′-cyclic mononucleotides.

Alumina thin-layer sheets without fluorescent indicator, obtained from Eastman Kodak Co. (Cat. No. 6062), were used without prior activation. The developer was prepared by adjusting 1 *M* ammonium acetate to pH 9.0 with concentrated ammonium hydroxide (30 %) and mixing it with ethanol in the ratio of 7:13, v/v. 5 µl of each nucleotide solution (0.005 *M*) were spotted on the alumina sheets, and without prior equilibration developed ascendingly in the ammonium acetate–ethanol solvent over-

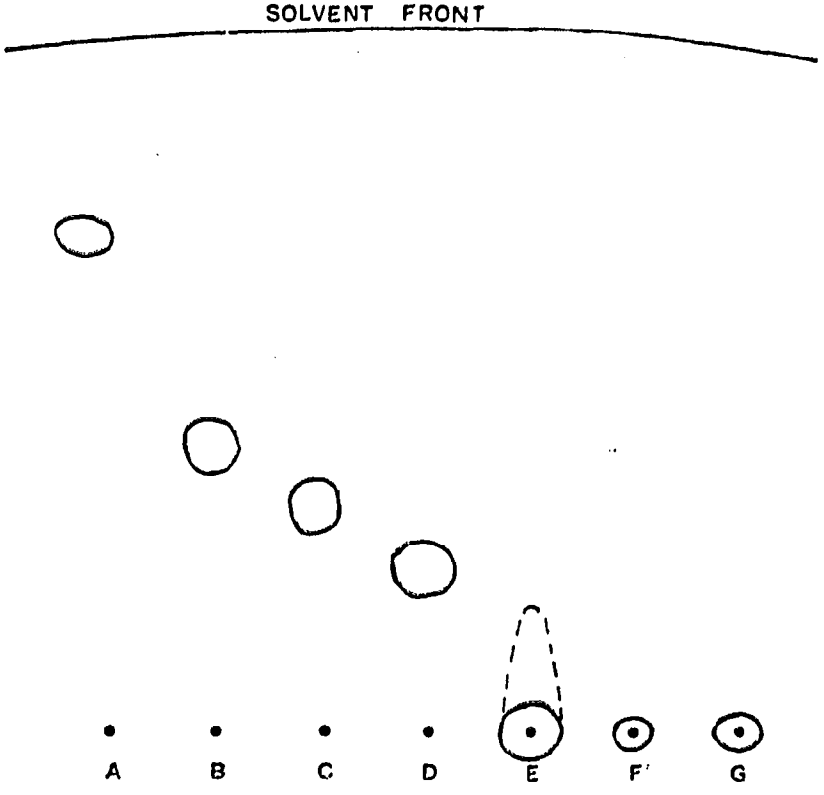

Fig. 1. Chromatogram showing the separation of ribonucleoside 3′,5′-cyclic monophosphates on an alumina thin-layer sheet. Solvent system: 1 *M* ammonium acetate (pH 9.0)–ethanol (7:13). A = adenosine 3′,5′-cyclic monophosphate; B = cytidine 3′,5′-cyclic monophosphate; C = guanosine 3′,5′-cyclic monophosphate; D = uridine 3′,5′-cyclic monophosphate; E = mixture of four ribonucleoside 2′,3′-cyclic monophosphates; F = mixture of four ribonucleoside 5′-monophosphates; G = mixture of four ribonucleoside 2′ and 3′-monophosphates. The closed circles indicate the origins, and the broken line indicates a faint tailing.

night (approximately 15 h) at room temperature. The chromatograms were dried at 100° for 5 min and the nucleotides were located by ultraviolet light.

Fig. 1 shows that the four ribonucleoside 3′,5′-cyclic monophosphates were well resolved from each other, whereas ribonucleoside 2′,3′-cyclic monophosphates and other ribonucleoside monophosphates did not migrate from their origins. In this system, ribonucleoside, mono-, di-, and triphosphates also did not move from their origins. Further experience with this system has shown that R_F values for the 3′,5′-cyclic mononucleotides significantly depend on the tightness of the developing chamber used — the R_F values tend to increase with a looser chamber without affecting the separation.

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1 M. SMITH, G. I. DRUMMOND AND H. G. KHORANA, *J. Amer. Chem. Soc.*, 83 (1961) 698.
2 Our unpublished observation.

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