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Note

Separation of purine 3',5'-cyclic nucleotides and nucleosides by thin-layer chromatography on PEI cellulose

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One of the main problems in cyclic nucleotide research is the separation of adenosine 3',5'-cyclic monophosphate (cAMP) from guanosine 3',5'-cyclic monophosphate (cGMP) and the separation of these cyclic nucleotides from their respective 5'-nucleotides and nucleosides. Previously, alumina and silica gel thin-layer sheets have been used to separate cAMP and cGMP from other adenine and guanine nucleotides[1–5]. Although thin-layer chromatographic (TLC) techniques using polyethyleneimine (PEI) cellulose have been used extensively for purine and pyrimidine separation[6–8], no simple technique has been developed for the separation of cAMP, cGMP, adenosine (ADO) and guanosine (GUO). This paper describes a simple and highly reproducible system for separating these purines.

MATERIALS AND METHODS

Precoated thin-layer PEI impregnated cellulose (0.1 mm thick) 20 × 20 cm plastic sheets (MN-300) without fluorescent indicator were obtained from Brinkmann (Westbury, N.Y., U.S.A.). The following compounds were purchased from Boehringer (Mannheim, G.F.R.): adenosine, adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), adenosine 3',5'-cyclic monophosphate, guanosine, guanosine 5'-triphosphate (GTP), guanosine 5'-diphosphate (GDP), guanosine 5'-monophosphate (GMP), and guanosine 3',5'-cyclic monophosphate. Ammonium acetate and ammonium hydroxide (30%) were obtained from J. T. Baker (Phillipsburg, N.J., U.S.A.) and Fisher Scientific (Pittsburgh, Pa., U.S.A.), respectively. Undenatured 95% ethanol was purchased from U.S. Ind. Chem. (New York, N.Y., U.S.A.).

The PEI cellulose sheets were used directly, as commercially supplied, and not pre-washed. The nucleotides and nucleosides were prepared in glass-distilled water, and 1-μl aliquots containing approximately 10 nmoles were spotted at 1-cm intervals along an origin line 2.5 cm from the bottom of the sheets. Spots containing more than one of the nucleotides and nucleosides were made by sequential spotting of 1-μl aliquots of each of the compounds.

The mobile phase was prepared by adjusting a 1 *M* ammonium acetate solution

to pH 9.0 using concentrated ammonium hydroxide, and combining it with 95% ethanol in a ratio of 7:13[2].

The final step in this technique was the ascending development of the sheets in a glass tank containing the mobile phase to a level of 1 cm. The tank was covered and the solvent allowed to rise at room temperature until the solvent front reached the top edge of the sheets after 3–4 h. The sheets were dried completely and the separated compounds visualized under ultraviolet (UV) light. Spots were outlined with a soft pencil. Tracings of the chromatograms were made on clear plastic film placed over the sheets. The tracings were then accurately transferred to paper for photography.

RESULTS AND DISCUSSION

Fig. 1 shows that cAMP and cGMP were separated from each other. In this system, ADO and GUO were also resolved from each other as well as from the two cyclic nucleotides. The R_F values for cGMP, GUO, cAMP, and ADO were 0.20, 0.57, 0.41 and 0.73, respectively. The purine 5′-monophosphates, diphosphates, and triphosphates migrated only a few millimeters, if at all, from the origin line.

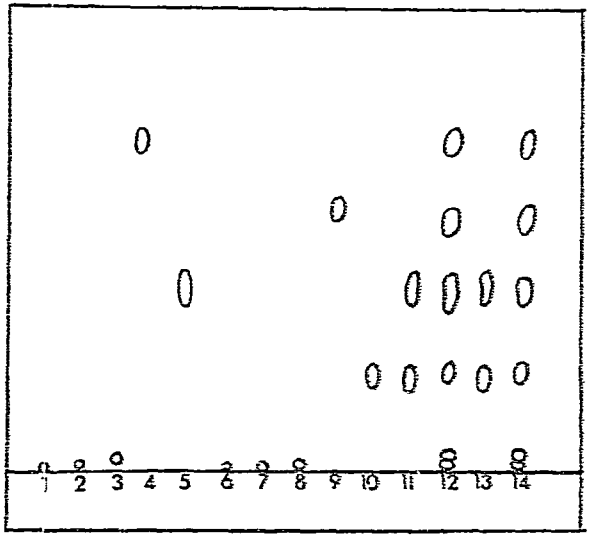

Fig. 1. Tracing of a typical chromatogram showing the separation of the purine 3′,5′-cyclic nucleotides and nucleosides on a PEI cellulose thin-layer sheet. The numbers refer to the following compounds spotted on the origin line: 1 = ATP, 2 = ADP, 3 = AMP, 4 = ADO, 5 = cAMP, 6 = GTP, 7 = GDP, 8 = GMP, 9 = GUO, 10 = cGMP, 11 = cAMP and cGMP, 12 = all of the above nucleosides and nucleotides, 13 = 11, 14 = 12. For abbreviations see text.

Further experience with this system has shown that separation of picomolar amounts of cAMP and cGMP is possible. Tritiated cAMP and cGMP aliquots containing approximately 0.3 and 4 picomoles respectively, were spotted at the origin line and the sheets developed as usual. Sequentially ascending 1.0 × 1.5 cm areas were scraped above the origin line, and radioactivity determined by conventional scintillation spectrometry. The tritium label was found to be located at areas on the

chromatograms corresponding to the known R_F values for the UV–visible cAMP and cGMP standards.

This one-step TLC system appears to be a useful inexpensive method for both qualitative and quantitative separation of purine 3',5'-cyclic nucleotides and their respective nucleosides and nucleotides.

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