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Note

Separation of nucleic acid bases, nucleosides and nucleotides on strong cation-exchange thin layers

IX*. Separation of cyclic nucleotides

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The separation of cAMP and cGMP** from each other and from the parent base, nucleoside, as well as nucleoside 5′-mono-, -di- and -tri-phosphate, respectively, is of crucial importance in cyclic nucleotide research. Anion-exchange TLC on PEI-cellulose chromatoplates in aqueous salt solutions of different concentrations or, sometimes, in dilute organic acids, as developing solution, is one method that has acquired widespread application[1]. Subsequently we pointed out that cation-exchange TLC, on commercially available chromatoplates (Fixion 50-X8) containing Dowex 50-X8 resin in the hydrogen form and silica gel, in deionized water as developing solvent, is an equally useful tool for the separation of cAMP from a mixture of adenine, Ado, 2′(3′)-AMP, 5′-AMP, ADP and ATP[2]. At the same time, our attempt to employ the same TLC system in the separation of cGMP from the analogous compounds has met with failure, because cGMP spread on the chromatoplates during development, resulting in the formation of diffuse spots with strong pre-tailing. The present paper reports that this shortcoming can be eliminated and the applicability of the system extended by using 0.05 *M* aqueous oxalic acid as the developing solution instead of water.

EXPERIMENTAL

Fixion 50-X8 (H^+) chromatoplates were obtained by developing the commercial Fixion 50-X8 (Na^+) chromatoplates (Chinoin, Nagytétény, Hungary) with 2.0 *M* HCl for at least 16 h using the continuous ascending technique[3]. Excess HCl was

* For Part VIII, see ref. 4.

** The abbreviations used are: cAMP = adenosine 3′,5′-cyclic phosphate; cGMP = guanosine 3′,5′-cyclic phosphate; cCMP = cytidine 3′,5′-cyclic phosphate; cUMP = uridine 3′,5′-cyclic phosphate; Ado = adenosine; 2′(3′)-AMP = adenosine 2′(3′)-phosphate; 5′-AMP = adenosine 5′-phosphate; ADP = adenosine 5′-diphosphate; ATP = adenosine 5′-triphosphate; Guo = guanosine; 2′(3′)-GMP = guanosine 2′(3′)-phosphate; 5′-GMP = guanosine 5′-phosphate; GDP = guanosine 5′-diphosphate; GTP = guanosine 5′-triphosphate; PEI = polyethyleneimine; TLC = thin-layer chromatography.

removed from the layer by a stream of cold air (30–60 min). Stock solutions were prepared from the compounds to be separated (Sigma, St. Louis, Mo., U.S.A., and Reanal, Budapest, Hungary) in deionized water (10 mg/ml). 1–2 μl of each solution was applied to a chromatoplate as round spots. Compounds were visualised under a short-wave UV lamp (Uvis; Desaga, Heidelberg, G.F.R.).

RESULTS AND DISCUSSION

As shown in Fig. 1, all the four ribonucleoside 3′,5′-cyclic phosphates derived from major ribonucleosides give compact spots with well-defined outlines after development on Fixion 50-X8 (H^+) chromatoplates in 0.05 *M* aqueous oxalic acid and are cleanly separated. At the same time, as can be seen in Fig. 2, both cAMP and cGMP are well separated from the respective nucleoside and nucleoside mono-, di- and triphosphates.

This cation-exchange TLC system, consisting of a Fixion 50-X8 chromatoplate in the hydrogen form and 0.05 *M* aqueous oxalic acid as developing solution, com-

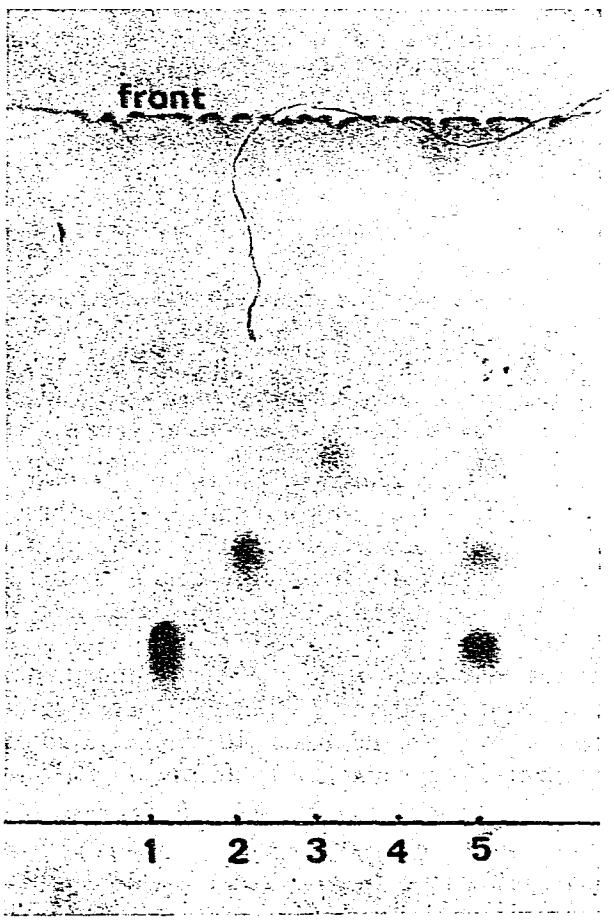

Fig. 1. Separation of ribonucleoside 3′,5′-cyclic phosphates on Fixion 50-X8 (H^+) chromatoplates in 0.05 *M* aqueous oxalic acid. Development time, 90 min for 10 cm. 1 = cAMP; 2 = cCMP; 3 = cGMP; 4 = cUMP; 5 = 1 + 2 + 3 + 4.

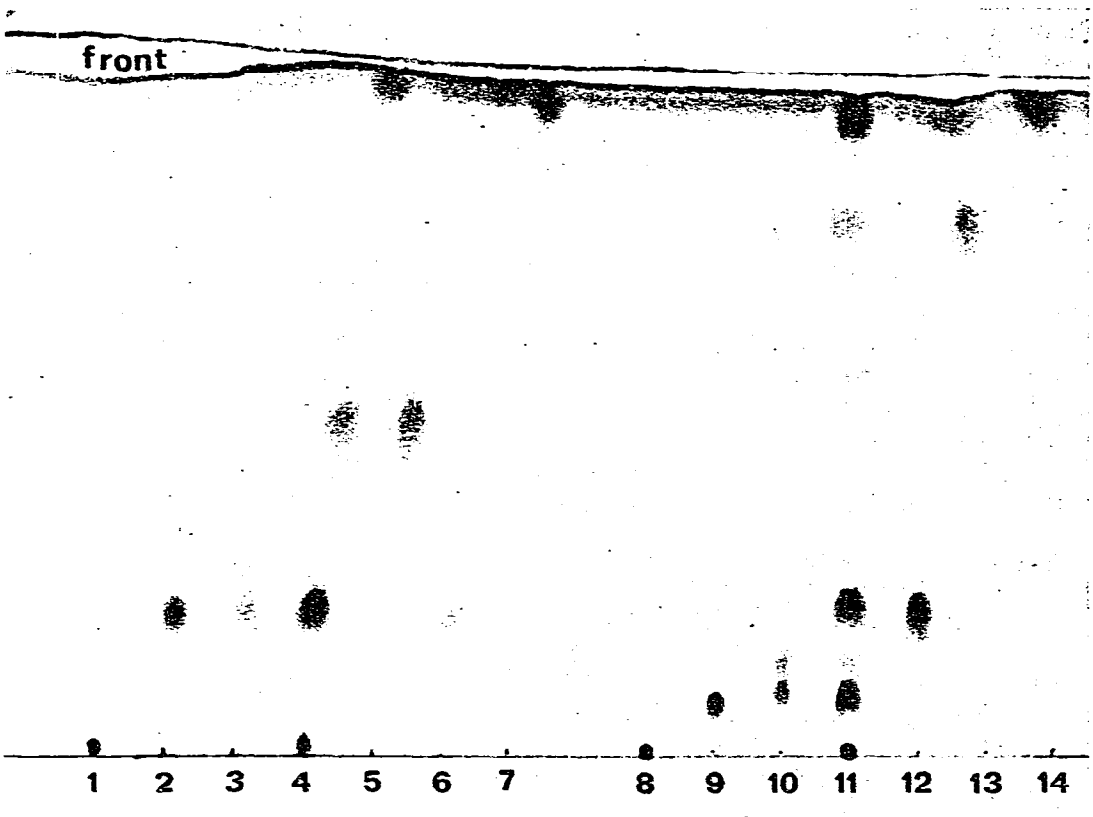

Fig. 2. Separation of adenosine and guanosine nucleotides on Fixion 50-X8 (H^+) chromatoplates in 0.05 *M* aqueous oxalic acid. 1 = Guo; 2 = 5′-GMP; 3 = 2′(3′)-GMP; 4 = 1 + 2 + 3 + 5 + 6 + 7; 5 = cGMP; 6 = GDP; 7 = GTP; 8 = Ado; 9 = 5′-AMP; 10 = 2′(3′)-AMP; 11 = 8 + 9 + 10 + 12 + 13 + 14; 12 = cAMP; 13 = ADP; 14 = ATP. Commercial GDP, GTP and ADP (Reanal) contained some monophosphate impurities. Among 2′(3′)-phosphates, the 2′-isomer had the higher R_F value.

plements the anion-exchange PEI-cellulose TLC systems and offers an alternative and useful tool for cyclic nucleotide separation. In the cation-exchange system bases and nucleosides remain at or near the origin during development, while triphosphates move with the solvent front. The opposite is observed with anion-exchange systems, bases and nucleosides being found at the greatest distance from the origin and triphosphates migrating the slowest. The relative order of 3′,5′-cyclic phosphates and 5′-phosphates is the same in both systems. Ribonucleoside 2′,3′-cyclic phosphates and deoxyribonucleoside 5′-phosphates (except thymidine 5′-phosphate) are hydrolysed to the corresponding 2′(3′)-phosphate isomers and bases, respectively, under the acidic conditions of the cation-exchange TLC system[4]. Bases do not move from the origin.

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