Chromatography of nucleotides on a new anion-exchange paper

Although it has been known for many years[1, 2] that polyethylenimine (poly-EI) and other basic polymer compounds are fixed substantively on cellulose fibers, it was not recognized that poly-EI-cellulose is an effective anion-exchanger which can be used in column[3], thin-layer[4], and paper chromatography.

In this paper the preparation of poly-EI-paper is described, and its usefulness for the separation of nucleotides is demonstrated.

Preparation of the anion-exchange paper

500 ml distilled water are added to 100 g of a 50% solution of poly-EI in water*. The solution is stirred until all the poly-EI has dissolved. It is then neutralized with concentrated hydrochloric acid and filled up with distilled water to 2,000 ml. Sheets of Whatman No. 1 chromatography paper**, previously washed with distilled water, are dipped for 2 sec in the poly-EI solution and then dried in a stream of warm air. Finally they are washed several times with distilled water and air-dried.

The nitrogen content of the paper can be raised (lowered) by raising (lowering) the concentration of the poly-EI solution.

Chromatography

The samples are applied from a pointed micropipette 2 cm from the edge of the paper sheet. After drying ascending chromatography is carried out in an open beaker, the paper being attached to a glass rod. The development is stopped after the solvent has travelled an appropriate distance. The chromatogram is dried immediately in a stream of warm air. The compounds are detected by means of an ultraviolet lamp (254 mμ).

TABLE I

R_F VALUES OF RIBONUCLEOTIDES ON POLY-EI-PAPER

Solvent: 1.0 *M* NaCl. Distance covered: 10 cm in 25–30 min. n = number of developments. F = compound moves with the solvent front.

Compound	R_F		
	$n = 1$	$n = 2$	$n = 3$
Adenosine-5′-phosphate	0.63	0.83	F
Adenosine-diphosphate	0.37	0.63	0.83
Adenosine-triphosphate	0.12	0.25	0.32
Guanosine-5′-phosphate	0.58	0.80	F
Guanosine-diphosphate	0.25	0.47	0.65
Guanosine-triphosphate	0.07	0.17	0.23
Cytidine-5′-phosphate	0.77	F	F
Cytidine-diphosphate	0.46	0.76	F
Cytidine-triphosphate	0.15	0.30	0.44
Uridine-5′-phosphate	0.86	F	F
Uridine-diphosphate	0.57	0.87	F
Uridine-triphosphate	0.21	0.40	0.59

* A 50% solution of poly-EI in water ("Polymin P") is obtainable from Badische Anilin- und Sodafabrik, Ludwigshafen a. Rh., Germany.

** H. Reeve Angel, London, E.C.4, England.

Nucleoside mono-, di-, and triphosphates are separated in 10–30 min with neutral electrolyte solutions on the poly-EI-paper, whereas on the commercial DEAE-, AE-, and ECTEOLA-papers* this separation cannot be obtained under these mild conditions.

In some cases repeated chromatography yields better separations: after the first chromatography the paper is washed with distilled water, dried and developed a second time with the same or with a stronger solvent. This procedure can be repeated a third time and so on.

Table I shows the R_F values of ribonucleotides on poly-EI-paper obtained with 1.0 *M* sodium chloride solution.

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Notes

Separation of the coproporphyrin isomers I and III by thin layer chromatography*

Ascending paper chromatography[1] was used for isomer analysis of coproporphyrin (COPRO), produced by a heme-requiring *Staphylococcus aureus* (JT/52)[2,3]. Though this method gave satisfactory results under standard conditions, it became desirable to develop a procedure that was more rapid, more sensitive, and less subject to disturbances due to slight temperature changes, contaminating salts or organic materials, and heavy sampling. An attempt was made therefore to adapt ERIKSEN'S method[1] to thin layer chromatography (TLC). While TLC was suggested for the separation of the tetramethyl esters of COPRO I and III[4], no mention of TLC applied to the chromatographic analysis of *free* porphyrins could be found in the literature.

Materials and methods

Only small glass plates (50 mm × 200 mm) in small, cylindrical developing chambers (58 mm × 230 mm) with plastic closures were used throughout this study. Thus, 3 or 4 samples could be conveniently accommodated while considerable amounts of materials were saved and the equilibration time in the chamber was reduced.

The plates were coated with silica gel G (30 g in 60 ml H_2O) using a 250 micron

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