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Note

Rapid separation of guanine-derived compounds using thin-layer chromatography on polyethyleneimine-impregnated cellulose

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The separation of guanine-derived compounds is of importance in the field of cyclic nucleotide research. Several methods have been developed to separate the nucleotides and nucleosides using column (anion-exchange and cation-exchange resins), thin-layer and adsorption chromatography[1–6]. Thin-layer chromatographic methods have concentrated upon polyethyleneimine (PEI)-impregnated cellulose. Bohme and Shultz[1] have reviewed these PEI chromatographic procedures utilizing both inorganic and organic solvent systems. The present paper investigates a PEI-cellulose thin-layer chromatographic separation procedure utilizing a single organic buffering system. The system possesses the advantage of rapidity, highly reproducible separation characteristics, and buffer volatility.

EXPERIMENTAL

Chromatographic procedures

PEI-impregnated cellulose MN 300 (0.1 mm thick) was utilized for all separation procedures. The plates were stored at 4° in a desiccated environment prior to use. The plates were cut from pre-formed 20 × 20 cm plastic backed sheets to a size of approximately 10 × 5 cm for chromatographic separations. The standard guanine-derived compounds were dissolved in water or dilute acid and placed on thin-layer plates as discrete spots measuring 3 mm in diameter. The spots were rapidly air-dried using a hot-air blower. The plates were subsequently placed in a rectangular, buffer-saturated chromatography chamber and developed in an ascending fashion until the solvent front had moved 8–9 cm. The plates were removed from the buffer chamber, air-dried and the standard compounds localized under ultraviolet light illumination (254 nm).

Materials

Triethylamine was purchased from Aldrich (Milwaukee, Wisc., U.S.A.) and redistilled prior to use. Guanosine 5′-triphosphate (GTP), 5′-diphosphate (GDP),

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5'-monophosphate (GMP), guanosine 3',5'-monophosphate (cyclic GMP) and guanosine were all purchased from Sigma (St. Louis, Mo., U.S.A.). Pre-coated plastic backed polygram CEL 300 PEI plates (0.1 mm PEI-cellulose MN 300) were purchased from Brinkman Instruments (Westbury, N.Y.).

Buffer preparation

The chromatographic plates were developed using 0.5 *M* triethylammonium bicarbonate (TEAB) buffer, pH 7.6. This buffer, made from a weak organic base, was routinely prepared every two weeks. A 140-ml volume of distilled triethylamine was diluted to 750 ml with triple deionized, distilled water. A stream of pure carbon dioxide was bubbled through the solution until a pH of 7.6 was reached. The TEAB solution was subsequently brought to 1 l (*i.e.*, 1 *M*) with water and stored in the dark at room temperature until use. For actual chromatographic separation, the 1 *M* TEAB stock was diluted to 0.5 *M* using triple deionized, distilled water. The pH of the buffer was always ascertained to be 7.6 before use.

RESULTS AND DISCUSSION

A representative chromatogram of assorted guanine-derived compounds may be seen in Fig. 1. The separation of the nucleotides and nucleosides is based upon pH-dependent charge interaction with the basic PEI[2]. The more highly anionic nucleotides (GTP and GDP) show very little migration while the lesser charged compounds traverse a greater distance. The separations in 0.5 *M* TEAB developed chromatograms were quite similar to that seen in chromatograms developed by multiple successive buffer systems which utilize acetic acid, water and two different molarities of LiCl[1].

The reproducibility of the separation may be seen in Table I. The R_F values have been determined from multiple independent chromatographic separations. The mean values were computed from samples which were either run singly or together

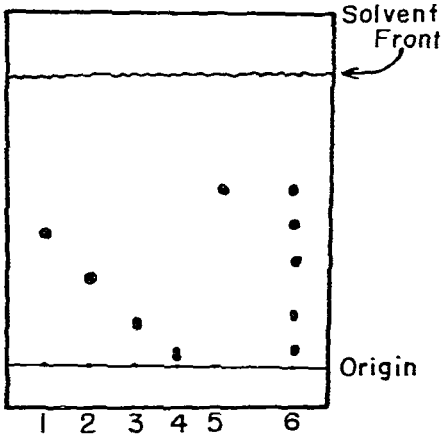

Fig. 1. Representative PEI separation of guanine-derived compounds 1 = cyclic GMP; 2 = GMP; 3 = GDP; 4 = GTP; 5 = guanosine; 6 = mixture of compounds 1–5. Running time with TEAB buffer, pH 7.6 was 40 min with the solvent front traversing 8 cm.

TABLE I

R_F VALUES OF GUANINE-DERIVED COMPOUNDS

The values presented represent the mean ± S.E.M. for multiple determinations. The individual determinations included samples run singly in one lane and multiple standards run in the same lane.

Compound	R_F (±S.E.M.)
GTP	0.05 ± .005 (n = 18)
GDP	0.18 ± .01 (n = 6)
GMP	0.33 ± .01 (n = 17)
Cyclic GMP	0.48 ± .01 (n = 26)
Guanosine	0.60 ± .01 (n = 7)

in a single chromatographic lane. The standard errors of the mean (*i.e.*, precision) were very small, indicative of the reproducibility of the separation. The average time for separation of the above compounds ranged around 40 min for 8 cm migration. Buffer evaporation from the plate following removal from the chromatography tank was extremely rapid due to the volatile nature of TEAB.

Recovery of cyclic GMP from the PEI-cellulose plates can be achieved with 0.15 *M* NaCl. This enables the rapid chromatographic separation to be utilized either directly (with labelled precursors) in a guanylate cyclase assay or as a preliminary step in further purification of cyclic GMP free of contamination. The use of this system adds to the armamentarium of existing thin-layer procedures presently available. It should be emphasized that this single 40-min separation achieves results comparable to the lengthy multiple buffer systems.

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