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Note

Separation of arabinosyl, ribosyl, and deoxyribosyl purine nucleotides by thin-layer chromatography

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Studies on the cellular metabolism of endogenous nucleosides and structurally related analogs are facilitated by the thin-layer chromatographic (TLC) separation of nucleotides. Complex mixtures of ribosyl and deoxyribosyl nucleotides can be separated by ascending two-dimensional TLC on PEI-cellulose anion-exchange layers^{1–6}. Nucleoside mono-, di- and triphosphates are first separated in one dimension by stepwise development in solvents of increasing ionic strength^{4,5}. Ribosyl and deoxyribosyl nucleotides then are resolved in the other dimension using borate-containing solvent systems^{4–6}. Nucleosides and bases present in cell extracts interfere with resolution of nucleotides, however, and must be removed by an initial development of the chromatogram.

We report here a TLC system which resolves arabinosyl as well as ribosyl and deoxyribosyl nucleotides. Nucleosides and bases do not interfere with resolution of nucleotides and no prior development is required. The system has been used routinely to separate purine nucleotides in our studies of the cellular metabolism of 9- β -D-arabinofuranosyladenine, an active antiviral drug.

MATERIALS AND METHODS*

PEI-cellulose plates (20 × 20 cm) were obtained from Brinkmann Instruments (Des Plaines, Ill., U.S.A.). The 100- μ m MN300 cellulose (Macherey, Nagel and Co., Düren, G.F.R.) PEI-impregnated sheets were kept at 5° in the dark and used without prewashing.

Ara-AMP and ara-HMP were gifts of Dr. R. L. Tolman of ICN Nucleic Acid Research Institute (Irvine, Calif., U.S.A.). Ara-ADP and ara-ATP were purchased from Terra-Marine Bioresearch (La Jolla, Calif., U.S.A.). All other nucleotides used

* Abbreviations used: AMP, ADP, ATP = adenosine-5'-mono-, -di- and -triphosphates; dAMP, dADP, dATP = the corresponding deoxyadenosine nucleotides; ara-AMP, ara-ADP, ara-ATP = the corresponding arabinosyladenine nucleotides; GMP, GDP, GTP = the corresponding guanosine nucleotides; dGMP, dGDP, dGTP = the corresponding deoxyguanosine nucleotides; IMP, IDP, ITP = the corresponding inosine nucleotides; dIMP, dIDP, dITP = the corresponding deoxyinosine nucleotides; ara-HMP = arabinosylhypoxanthine-5'-monophosphate; PEI = polyethyleneimine.

as standards were obtained commercially from Sigma (St. Louis, Mo., U.S.A.) or from P-L Biochemicals (Milwaukee, Wisc., U.S.A.).

Ascending chromatography was carried out in the following solvent systems: (Ia) 0.1 *M* LiCl in 1 *M* acetic acid; (Ib) 1.0 *M* LiCl in 1 *M* acetic acid; (Ic) 1.5 *M* LiCl in 1 *M* acetic acid; (II) ethanol–2 *M* LiCl (1:1) saturated with H₃BO₃ (approx. 60 g/l), neutralized with ammonia (sp. gr. 0.90).

Aqueous solutions of nucleotide standards were spotted 1.5–2.0 cm from a corner of the plate and 10–25 nmoles of each of the nucleotides were applied. The chromatogram was developed at room temperature in solvent Ia for 15 min (6 cm above the origin). The plate was transferred without intermediate drying to solvent Ib for 45 min (13 cm above the origin) and subsequently to solvent Ic for 90 min until the solvent front had reached the top of the plate. The chromatogram was removed, transferred directly to a flat tray containing anhydrous methanol and slowly agitated for 1 min. It was removed and dried in a stream of cool air. The plate then was developed in its second dimension in solvent II, requiring 4.5–6.0 h for complete development. Standards were visualized under ultraviolet light (254 nm) after air drying.

RESULTS AND DISCUSSION

The system described resolved complex mixtures of purine nucleotides which differ in extent of phosphorylation on the 5'-carbon of the sugar, in the sugar moiety (ribose, deoxyribose or arabinose), and in the nature of the purine base (adenine, hypoxanthine or guanine). Purine nucleosides and bases did not interfere with the resolution of any nucleotides but ran to the far corner of the chromatogram after development in both dimensions.

The first dimension of the chromatographic system separated nucleoside mono-, di- and triphosphates utilizing a stepwise concentration gradient of LiCl in acetic acid; this system gave better resolution of nucleotides than systems employing LiCl in formic acid^{4,5}. An intermediate wash of the plate was needed to remove LiCl. Randerath and Randerath⁵ reported that less than 10% of nucleotides were removed by such a 10-min wash in anhydrous methanol. We determined radiochemically that there was no significant removal of ³H-labelled AMP from the chromatogram during the 1-min methanol wash employed in this study.

The second dimension of the chromatogram was utilized to separate nucleotides by virtue of the sugar moiety. A borate-containing solvent was used because ribonucleotides form borate complexes with the *cis*-glycol of the sugar whereas deoxyribonucleotides do not. Borate complexes of ribonucleotides have a greater affinity for the anion exchanger^{4–6} and consequently were separated from deoxyribonucleotides. Arabinonucleotides also were resolved having migration rates intermediate with those for ribonucleotides and deoxyribonucleotides (Fig. 1). This indicates that the *trans*-glycol structure of the arabinonucleotides may form a weak borate complex. A good resolution of arabinonucleotides and deoxyribonucleotides was obtained, however, only if the solvent system contained ethanol.

The extent of migration in both dimensions also was affected by the base moiety. As a result, all adenine nucleotides were resolved from either hypoxanthine nucleotides (Fig. 1a) or from guanine nucleotides (Fig. 1b). In fact, with the exception of overlap between hypoxanthine and guanine nucleoside monophosphates, the com-

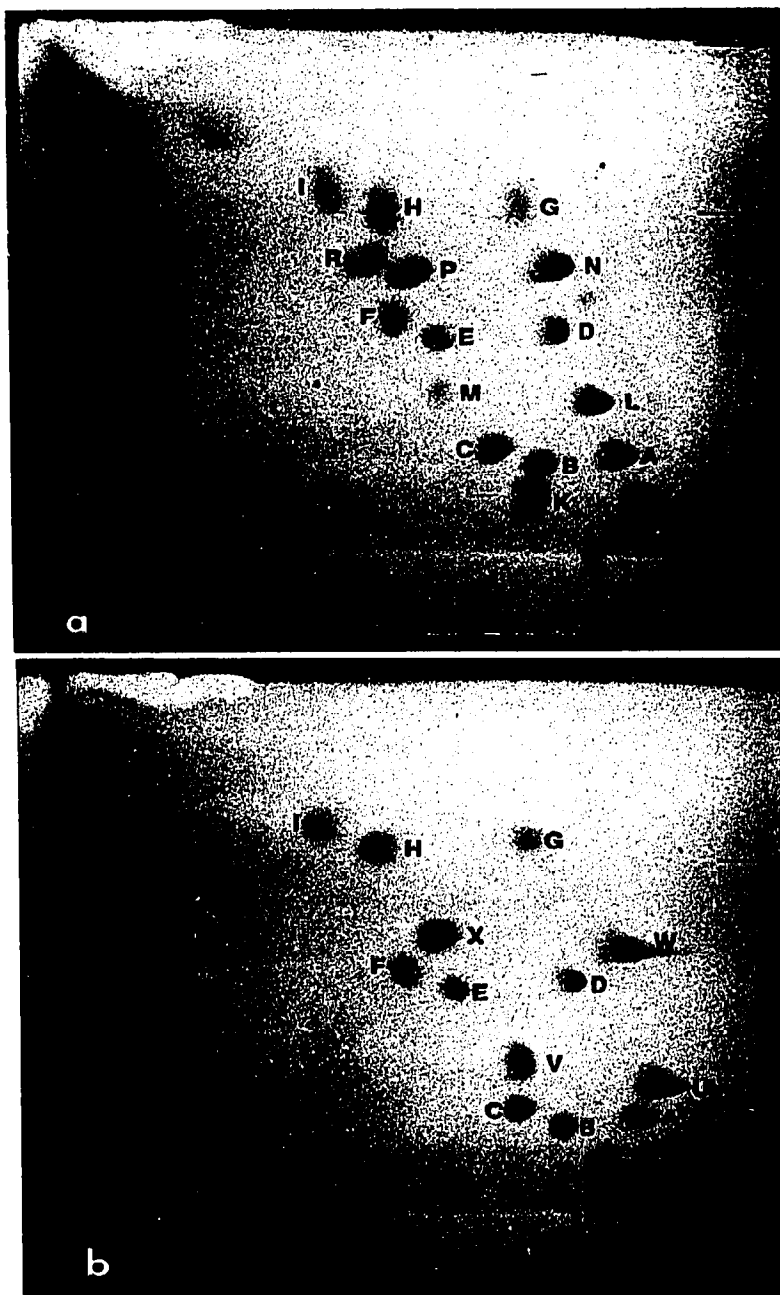


Fig. 1. TLC separation of adenine and hypoxanthine 5'-nucleotides (a) and adenine and guanine 5'-nucleotides (b) on PEI-cellulose plates. Two-dimensional development was performed as described in the text. The solvent fronts after each of the stepwise developments in the first dimension are indicated by the arrows to the right. Photography was under UV light (254 nm). O = Origin; A = ATP; B = ara-ATP; C = dATP; D = ADP; E = ara-ADP; F = dADP; G = AMP; H = ara-AMP; I = dAMP; J = ITP; K = dITP; L = IDP; M = dIDP; N = IMP; P = ara-HMP; R = dIMP; S = GTP; T = dGTP; U = GDP; V = dGDP; W = GMP; X = dGMP; Z = purines and purine nucleosides.

plex mixture of all three sets of nucleotides listed in the legend to Fig. 1 could be resolved on a single chromatogram.

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