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Note

Ion-exchange and ion-exclusion chromatographic separation of nucleosides on Dowex 50 type resin-coated chromatoplates

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The separation of complex mixtures of nucleosides into components is a fundamental problem in nucleic acid analysis. Column and thin-layer chromatographic (TLC) procedures have been developed for this purpose. Column chromatographic separations on strong ion-exchange resins have acquired the greatest importance[1–8], and ion-exclusion chromatography has also been introduced recently[9–11]. On the other hand, unsubstituted cellulose[12,13] and cellulose ion exchangers[14–17] have been used for TLC resolutions. Only a few reports have suggested the use of layers or papers coated with ion-exchange resins for this purpose[18,19].

The use of commercial chromatoplates coated with Dowex 50 type resin (Ionex-25 SA or Fixion 50-X8) has recently been reported for the separation of nucleic acid bases and nucleotides[20,21]. The behaviour of the principal ribo- and deoxyribonucleosides has been investigated on this type of plate and the aim of the present paper is to summarize the main results.

EXPERIMENTAL

Experiments were performed on Polygram Ionex-25 SA chromatoplates (Macherey, Nagel & Co., Düren, G.F.R.). Identical results were obtained by the use of Fixion 50-X8 chromatosheets (Chinoin, Nagytétény, Hungary). Layers were employed in the acidic, ammonium and sodium forms. The commercially available chromatoplates were in the sodium form and were pre-treated by continuous development[22] with 1.0 *M* sodium chloride solution and then with de-ionized water for 16 h with both solvents. Layers in the acidic and ammonium forms were obtained in the same way, but 1.0 *N* hydrochloric acid or 1.0 *M* ammonium carbonate solution was used instead of 1.0 *M* sodium chloride solution. In general, chromatoplates were equilibrated for 16 h in buffers which had the same pHs but were one order of magnitude more diluted than the developing buffers.

Nucleosides were commercial products (Sigma, St. Louis, Mo., U.S.A.) and their purity was checked by paper chromatography[23] and UV absorption[24]. Stock solutions of nucleosides were prepared in de-ionized water (10 mg/ml) and volumes of 1 μl of these solutions were applied for each spot.

The spots were detected under a short-wave (254 nm) UV lamp (Desaga Uvis).

RESULTS AND DISCUSSION*

The applicability of Dowex 50 type resin-coated chromatoplates in the salt and acidic forms was studied for the separation of nucleosides. Dilute (0.1–1.0 *M*) aqueous buffers were employed as developing solutions. Adequate separation could be achieved under different conditions. It was found that the separation depended primarily on the pH of the developing solution and changes in the concentration of eluting ions could affect it to a much lesser extent.

TABLE I

R_F VALUES OF NUCLEOSIDES ON IONEX-25 SA (NH_4^+) CHROMATOPLATES IN AMMONIUM ACETATE, [NH_4^+] = 0.4, AND IN 0.1 *M* AMMONIUM CARBONATE BUFFERS AT DIFFERENT pH VALUES

A period of 60 min was necessary for 10 cm development.

Nucleoside	*Acetate pH*		*Carbonate pH*			
	3.5	*6.5*	*9.0*	*9.5*	*10.0*	*10.5*
Ado	0.39	0.41	0.54	0.55	0.54	0.56
dAdo	0.37	0.35	—	—	0.48	—
Cyd	0.27	0.51	0.63	0.63	0.64	0.67
dCyd	0.26	0.41	—	—	0.60	—
Guo	0.65	0.38	0.51	0.63	0.79	>0.90
dGuo	0.62	0.31	—	—	0.70	—
Urd	0.80	0.66	0.85	0.90	0.90	>0.90
dThd	0.81	0.58	—	—	0.82	—

As shown in Table I, ion exchange governed the separation on plates in the ammonium form and in ammonium acetate buffers at about pH 3.5, as the resolution of nucleosides occurred in the order of decreasing basicity. Because of the close similarity of their basicities, the riboside and the 2'-deoxyriboside of the same base were indistinguishable under these conditions. A mixture of some deoxyribonucleosides was separated in a similar pattern on a Dowex 50 (NH_4^+) column with 0.1 *M* ammonium acetate solution (pH 3.9) as eluent, but this mixture did not contain dAdo[25]. We found that the pattern of substances did not alter up to about pH 4.5, but the efficacy of resolution decreased as the pH increased. Beyond pH 4.5, and particularly at about pH 7, compounds were separated primarily according to differences in their polarities. Two types of separation were obtained under these conditions: (a) the separation of pyrimidine nucleosides from each other and from the mixture of the two purine nucleosides within one series; and (b) the separation of a given ribonucleoside from its 2'-deoxy derivative.

Ion exclusion seemed to play the decisive role on layers in the ammonium form and in ammonium carbonate buffers between pH 9.0 and 10.5. A successful separation was attained at pH 10.0, but only within one series of compounds (Table I). As far as we know, ion-exclusion TLC has not yet been employed for the separation of nucleic

* The abbreviations used are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.*, 241 (1966) 527).

acid constituents. In every instance, the ribo derivative had the higher R_F value, but the differences were insufficient to obtain complete resolution of all eight compounds. As shown in Table 1, the R_F value of Guo became identical with that of Cyd when the pH of the developing solution was decreased from 10.0 to 9.5. Guo and Ado were indistinguishable at pH 9.0. Guo and Urd approached the solvent front as the pH of the eluent was increased from 10.0 to 10.5. The extreme sensitivity of the mobility of Guo to the pH of the eluent agreed well with the literature data obtained from column experiments[10].

As shown in Fig. 1, complete resolution of the eight principal ribo- and deoxyribonucleosides was achieved by the two-dimensional combination of acetate buffers of pH 3.5 and 6.5.

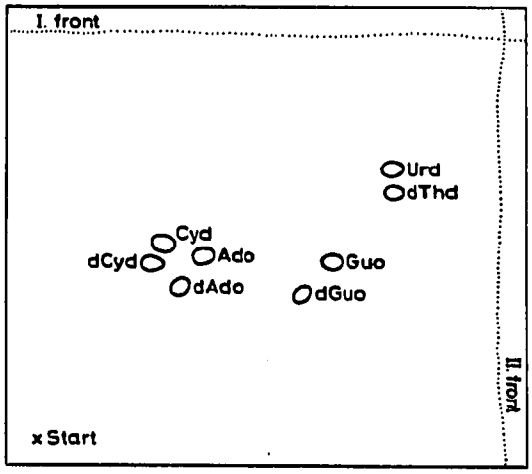

Fig. 1. Two-dimensional separation of nucleosides on Ionex-25 SA (NH_4^+) chromatoplates in ammonium acetate buffers, $[NH_4^+] = 0.4$. First dimension (I): pH 6.5, 195 min for 16 cm; second dimension (II): pH 3.5, 190 min for 15 cm.

It should be pointed out that Ado and Cyd remained unresolved when formate buffer was used instead of acetate in the pH range 2.4–5.0. This result was contrary to the literature data obtained from column experiments, as most of these separations were carried out with formate buffers of about pH 4.5.

Layers in the sodium form gave analogous results when the appropriate sodium acetate buffers were used. Nevertheless, this form was found to be less suitable because of the formation of relatively wide UV-absorbing solvent fronts, which interfered in the separation of compounds of high R_F values.

Chromatoplates in the acidic form proved to be unsuitable. All compounds remained at the start, with the exception of Urd and dThd, in de-ionized water as developing solvent. Cyd and Guo migrated with identical R_F values in dilute aqueous mineral acids, whereas dAdo, dCyd and dGuo were hydrolyzed quantitatively to the parent base by the acidity of the layer. Our observations agreed with earlier TLC data[18], but were inconsistent with the results of the classical Cohn experiments on columns[26]. This difference may be ascribed to the presence of microcrystalline cellulose[18] or, as in our case, silica gel in the layers.

Our method offers an alternative possibility for the TLC separation of the eight major nucleosides. This resolution was achieved earlier by the use of two-dimensional partition chromatography on purified cellulose layers[27]. In addition, it may provide simple means of distinguishing between the riboside and the 2'-deoxyriboside of the same base, which was hitherto achieved by using the complexing ability of ribonucleosides with borate ions[17,23].

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