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Resolution of RNA hydrolysis products by two-dimensional anion-exchange thin-layer chromatography*

Separations of nucleic acid hydrolysates and complex nucleotide mixtures have been reported[1–4]. RAAEN AND KRAUS[5] recently presented a technique capable of group separation of the four major nucleotides of alkali-hydrolyzed RNA and the separation of the 2'- and 3'-isomers of adenylic acid monophosphate. LEECH *et al.*[6] have presented a method for the determination of base ratios of acid-hydrolyzed ribonucleic acid. CASHEL *et al.*[7] have demonstrated the effect of pH on nucleotide migration in phosphate solvents. The technique presented here is capable of resolving the four major nucleotides found in RNA and both the 2'- and 3'-isomers of each of them. Preliminary application of this technique to maize tRNA hydrolysates demonstrates an additional component, probably a substituted guanylic acid. The advantages of this system reside in (1) the high degree of resolution and (2) its adaptability to radioisotope studies involving quantitation in the $\mu\mu$mole range.

Materials and methods

Poly(ethyleneimine) (Chemicat P-145) was generously supplied by Chemirad Corporation, Baltimore, Md., U.S.A.; MN Cellulose 300, manufactured by Macherey, Nagel and Co., Duren, G.F.R., and a Desaga applicator, manufactured by Desaga, Heidelberg, G.F.R., were obtained from Brinkmann Instruments Inc., Westbury, N.Y., U.S.A. Bakelite ® Rigid Vinyl Sheets (21 × 50 in.) type VSA 3310 Clear 31 Matte 06, 0.010 in., manufactured by Union Carbide Corporation, Cincinnati, Ohio, U.S.A., were obtained from Commercial Plastics and Supply Corporation, New York, N.Y., U.S.A. RNA (Torula Yeast, Grade VI) was obtained from Sigma Chemical Company, Company, St. Louis, Mo., U.S.A. Nucleotide monophosphate standards, *i.e.*, adenylic acid, 2'(3') mixed isomers (2',3'-AMP); cytidylic acid, 2'(3') mixed isomers (2',3'-CMP); guanylic acid, 2'(3') mixed isomers (2',3'-GMP), and uridylic acid, 2'(3') mixed isomers (2',3'-UMP), were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

Preparation of the PEI-cellulose thin layers has been reported elsewhere[2].

Effect of sample pH on migration and resolution. Three grams of commercially prepared RNA were dissolved in 250 ml of 0.3 *N* KOH and incubated in a water bath at 37° for 12–15 h. Any insoluble material was then centrifuged away. 10-ml aliquots were diluted with 20 ml of doubly distilled water and adjusted to the various pH values, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 7.0, 9.0, 11.0, and 13.0 (the pH of the alkaline hydrolysate). The samples were applied 2 cm above the bottom of the plate, 2–3 cm apart. The sample contained approximately 3×10^{-6} g of the original 3-g sample. The plate was then run 10 cm from the sample origin in a closed rectangular chromatography chamber. Various solvents were used, including 1.0 *N* formic acid pH 2.0, 0.1 *N* formic acid pH 3.6, 1.0 *N* formic acid pH 3.6, 1.0 *M* LiCl pH 7.0, 0.5 *M* formic acid–sodium formate pH 3.6, and 2.0 *M* formic acid–sodium formate, pH 3.6.

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The standard nucleotide monophosphates were run in the various solvents for the purpose of identification of the hydrolyzed RNA components.

Two-dimensional chromatography. The solvent for the first dimension was either 1.0 N formic acid pH 3.6 or 0.1 N formic acid pH 3.6. The sample, with a pH of 3.0 ± 0.1 (3×10^{-6} g in 2 μl), was spotted 2 cm from the bottom of the plate and approximately 3 cm from either side. The solvent was allowed to run a distance of 10–15 cm from the base of the plate, after which the plate was dried in a current of air and the R_F values were determined. The plate was next trimmed below the front area to eliminate impurities that migrated with the front. The solvent for the second dimension (1 M LiCl, pH 7.0) was then run a distance of approximately 8 cm on the plate, dried in a current of air and the R_F values were determined.

The chromatographic plates were illuminated with a Mineralight UVS-11 short-wave UV light source and photographed with Agfa 500 color film. The 35-mm slide was then used to draw Figs. 1 and 2 by projection through a Beseler enlarger.

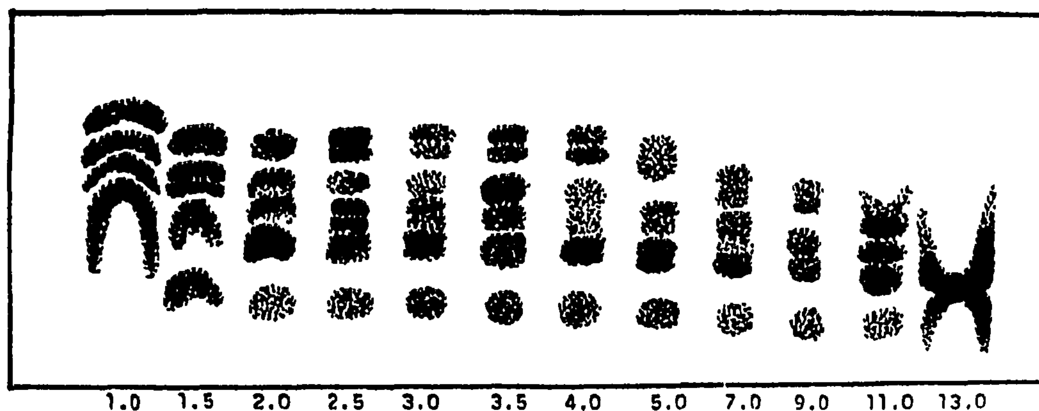

Fig. 1. One-dimensional chromatogram of alkaline hydrolysate of yeast RNA. The effect of sample pH on separation and resolution is shown, pH values are given for each sample from left to right. Solvent: 0.1 N formic acid, pH 3.6. The lower border represents the origin for all samples.

Results and discussion

Effect of sample pH. Sample pH was found to have a marked effect on component separation and resolution in the unbuffered system (Fig. 1). In the buffered system, the R_F values were similar regardless of sample pH. (R_F values not given.) Optimum

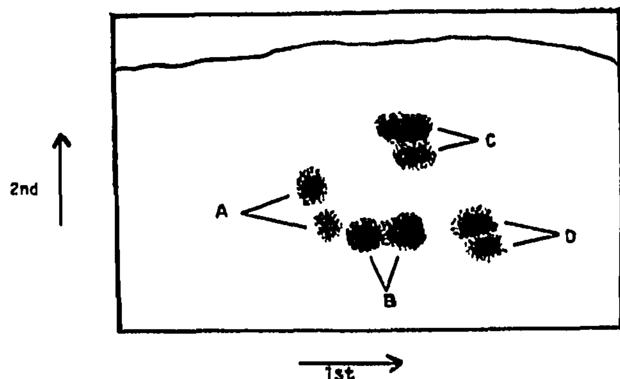

Fig. 2. Two-dimensional chromatogram of alkaline hydrolysate of yeast RNA. A = 2'- and 3'-GMP, B = 2'- and 3'-AMP; C = 2'- and 3'-UMP, D = 2'- and 3'-CMP. Pure 2' and 3' forms were not available for reference.

separation and resolution were found to occur at pH 3.5 ± 0.1; all samples subsequently tested were first brought to this pH.

Two-dimensional chromatography. It was of primary importance in a two-dimensional elution scheme to avoid any effect by the solvent system for the first dimension upon migration in the second dimension. RANDERATH *et al.*[1] thoroughly discussed the effects of the presence of salt on migration and separation of nucleotides. They proposed a methanol wash after the first dimension, but this involved some loss of sample. To avoid this we used a volatile solvent. The ideal solvent for the first dimension varied with the aims of the experimenter. 1.0 *N* formic acid and 3.0 *N* formic acid, pH 2.0, gave sharp group separation. In these systems, adenylic and cytidylic nucleotides migrated close to the solvent front and far ahead of the guanylic and uridylic nucleotides. Maximum resolution of nucleotides was obtained with 0.1 *N* formic acid, pH 3.6. This solvent separated the 2'- and 3'-isomers of adenylic and guanylic acid monophosphates in addition to the group separation of uridylic and cytidylic acid monophosphates (Fig. 2).

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