Separation of Ribonucleosides and Ribonucleotides by a One-Dimensional Paper Chromatographic System

During the course of studies on the products resulting from the enzymic digestion of RNA, a method was developed for the complete separation on paper of the four ribonucleosides and ribonucleotides in a single step. The samples were chromatographed using a modification of the isopropanol/ammonia solvent system of Markham and Smith (1) on paper pretreated with 0.2 *M* sodium phosphate pH 7.5.

Whatman No. 3MM papers 57 cm in length were completely saturated with 0.2 *M* sodium phosphate, pH 7.5, by spraying or dipping the papers through an excess of the salt solution and allowing them to drain and air-dry in a hood. This salt concentration was required to resolve the four ribonucleotides, although 0.01 *M* sodium phosphate was sufficient to separate the ribonucleosides. A mixture of 2′(3′)-ribonucleotides and ribonucleosides was applied as a 1 cm streak 10 cm from one end of the paper. The far end of the paper was left unserrated to delay the elution of nucleosides from the end of the paper. Development was by descending chromatography at 23°C in the solvent isopropanol/concentrated ammonium hydroxide/water (7/1/2 v/v). Forty-eight hours was required for full separation of the nucleotides. The ultraviolet-absorbing spots were detected with a Mineralight and contact prints made according to the procedure of Markham and Smith (2). A contact print of a typical chromatogram is shown in Figure 1. Where further separation of the nucleotides was desired, the paper could be removed from the tank after 48 hr, dried, and rechromatographed for an additional period of time after clipping off the nucleoside region.

The nucleotides and nucleosides could be recovered quantitatively from the paper by cutting out each ultraviolet-absorbing spot and eluting with 0.1 *N* HCl.

Similar results were obtained with Whatman No. 1 and No. 4 papers. This method did not resolve the corresponding deoxy compounds. Thymidylic acid was only partially separated from deoxycytidylic acid and thymidine and deoxyadenosine moved as a single spot. The mobilities of these and other compounds relative to that of adenosine are given in Table 1.

To the best of our knowledge this is the first one-dimensional paper chromatographic system that has been described for separating all of the common ribonucleosides and ribonucleotides. The principal advantages of this method are its simplicity and insensitivity to contaminating salt and protein. (Solutions containing up to 1 mg protein or 0.1 mmole of sodium phosphate have been chromatographed with no apparent loss of resolution.)

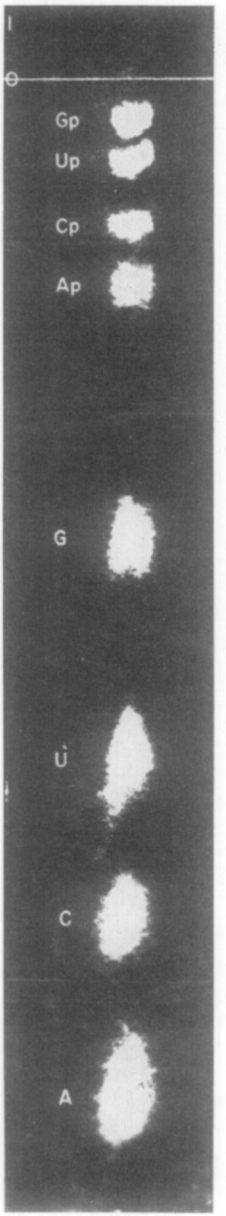

FIG. 1. Contact print photograph of 2′(3′)-ribonucleotides and ribonucleosides chromatographed 48 hr in isopropanol/NH_3/H_2O (7/1/2 v/v) on Whatman No. 3MM paper pretreated with 0.2 *M* sodium phosphate, pH 7.5. Abbreviations: Gp = Guanylic acid, Up = Uridylic acid, Cp = Cytidylic acid, Ap = Adenylic acid, G = Guanosine, U = Uridine, C = Cytidine, A = Adenosine.

TABLE 1
Mobilities of Nucleosides and Nucleotides[a] Relative to Adenosine (R_A)

Compound	R_A	Compound	R_A
Guanylic acid	0.05	Deoxyguanylic acid	0.05
Uridylic acid	0.08	Deoxycytidylic acid	0.16
Cytidylic acid	0.16	Thymidylic acid	0.19
Adenylic acid	0.22	Deoxyadenylic acid	0.22
Guanosine	0.45	Deoxyguanosine	0.66
Uridine	0.66	Deoxycytidine	1.09
Cytidine	0.81	Thymidine	1.20
Adenosine	1.00	Deoxyadenosine	1.20

[a] The mobilities of the 5′-ribo- or deoxyribonucleotides are approximately the same as those of their 2′- or 3′-analogs.

Thus, enzyme digests can be directly applied to the paper for subsequent identification and analysis of the nucleoside and nucleotide products.

This procedure is easily adaptable to thin-layer chromatography. Powdered cellulose (Macherey, Nagel & Co. No. 300) is suspended to 18% in 0.2 *M* sodium phosphate, pH 7.5, and spread on glass plates 20 × 20 cm in a 0.5 mm layer. The plates are dried and heated at 100°C for 1 hr. A mixture of 10 μg nucleotides and nucleosides is spotted 2.5 cm from the bottom of the plate and developed in the isopropanol/ammonium hydroxide/water solvent at room temperature until the solvent front reaches the top of the plate (about 4 hr). Under these conditions, the relative mobilities of the nucleosides and nucleotides are essentially the same as in Figure 1.

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