CHROM. 3749

Polyamide layer chromatography and electrophoresis of nucleotides

Recently, polyamide-layer chromatography was successfully used for the separation of nucleic acid bases and nucleosides[1]. The sensitivity was high and resolution was good. Its performance was comparable to other thin-layer media, such as ion-exchange cellulose[2]. We would like to describe our further efforts on the separation of nucleotides.

Samples of 1 mg were dissolved in 0.5 ml of distilled water and transferred to a polyamide layer (supplier; Chenchin Trading Co., No. 75, section 1, Hankow St., Taipei, Taiwan). 1 μl of the sample solution was applied for each spot, which is equivalent to 2 μg of sample. The chromatographic method of the previous publication was followed[3] and detection was effected by irradiation of chromatograms with a short-wavelength ultraviolet lamp (2375 Å), spots appearing dark blue on a slightly fluorescent background. The quenched spots were marked by a ball pen, then, the chromatograms were contact-photographed as described before[3].

Table I tabulates the R_F values of 10 nucleotides, in 3 solvent systems. Figs. 1, 2, and 3 are contact photographs of marked chromatograms.

Solvent system II required a 3-h developing time; the spots were circular without any tailing. In Solvent systems I and III, the spots appeared oval.

As Fig. 3 shows, 2'-, and 3'-AMP could be separated by Solvent III. We could not establish which one was the 2' or the 3' nucleotide but the separation is apparent-

Since we could separate 10 common nucleotides, we tried to resolve the hydro.

TABLE I

R_F VALUES OF NUCLEOTIDES ON POLYAMIDE LAYERS

Solvent I: water–glacial acetic acid (20:1, v/v); Solvent II: isopropanol–water–glacial acetic acid (2:2:1, v/v/v); Solvent III: acetone–water–glacial acetic acid (2:2:1, v/v/v). Abbreviations: 5'-ATP, adenosine 5'-triphosphate; 3'(2')-UMP, uridine 3'(2')-phosphoric acid; 3'(2')AMP, adenosine 3'(2')-phosphoric acid; 3'(2')-CMP, cytidine 3'(2')-phosphoric acid; 5'-CMP, cytidine 5'-monophosphoric acid; 5'-GMP, guanosine 5'-monophosphoric acid; 5'-deoxyGMP, deoxyguanosine 5'-phosphoric acid; 5'-deoxyGMP, deoxycytidine 5-monophosphoric acid; 5'-AMP, adenosine 5'-monophosphoric acid.

Sample	*Solvent I*	*Solvent II*	*Solvent III*
3'(2')-UMP**	0.41	0.48	0.36
3'(2')-AMP**	0.82	0.82	0.72
3'(2')-GMP**	0.30	0.51	0.28
3'(2')-CMP**	front	front	front
5'-CMP*	0.97	0.94	front
5'-GMP*	0.50	0.66	0.28
5'-deoxyGMP*	0.51	0.68	0.56
5'-deoxyCMP*	0.97	0.94	front
5'-AMP*	0.50	0.56	0.81
5'-ATP*	0.08	0.1	0.05

* Sigma Chemical Co., St. Louis, Mo., U.S.A.

** Courtesy of Dr. A. T. Tu, Department of Biochemistry, Colorado State University, Fort Collins, Colo., U.S.A.

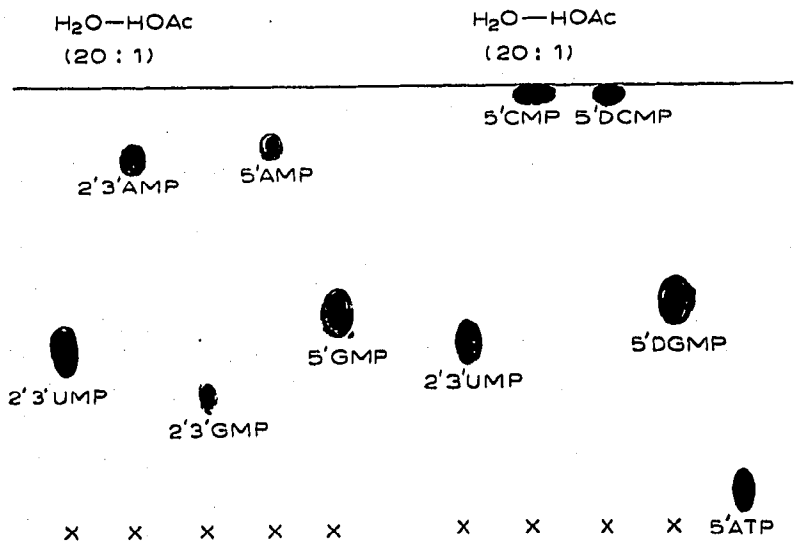

Fig. 1. One-dimensional chromatogram. Solvent: water–glacial acetic acid (20:1, v/v), 50 min for 10-cm development. Crosses show the location of the samples.

lyzate of yeast ribonucleic acid* which was prepared according to JONES AND GERMANN[4]. The result was the same as reported by HIBY AND KROGER[5].

Electrophoresis using polyamide layers exhibits several advantages over other media[6]. We eluted 7 nucleotides at pH 3.4 and 9.3 in a 1-h run with 300 V loaded (20 V/cm) using a home-made electrophoresis cell[7]. The results are fairly good under these conditions (Fig. 4).

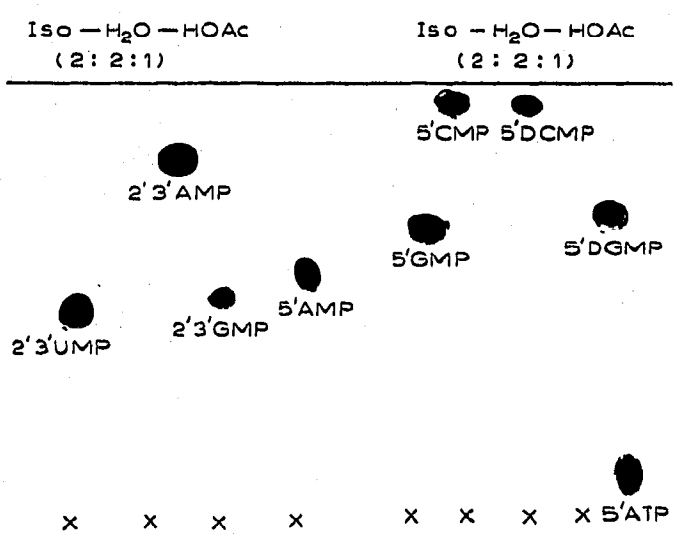

Fig. 2. One-dimensional chromatogram. Solvent: isopropanol–water–glacial acetic acid (2:2:1, v/v/v), 3 h, 10 cm. Spots were complete circles, but 5'-ATP was oval shaped.

* Courtesy of Dr. Y. C. Su, Department of Agricultural Chemistry, National Taiwan University.

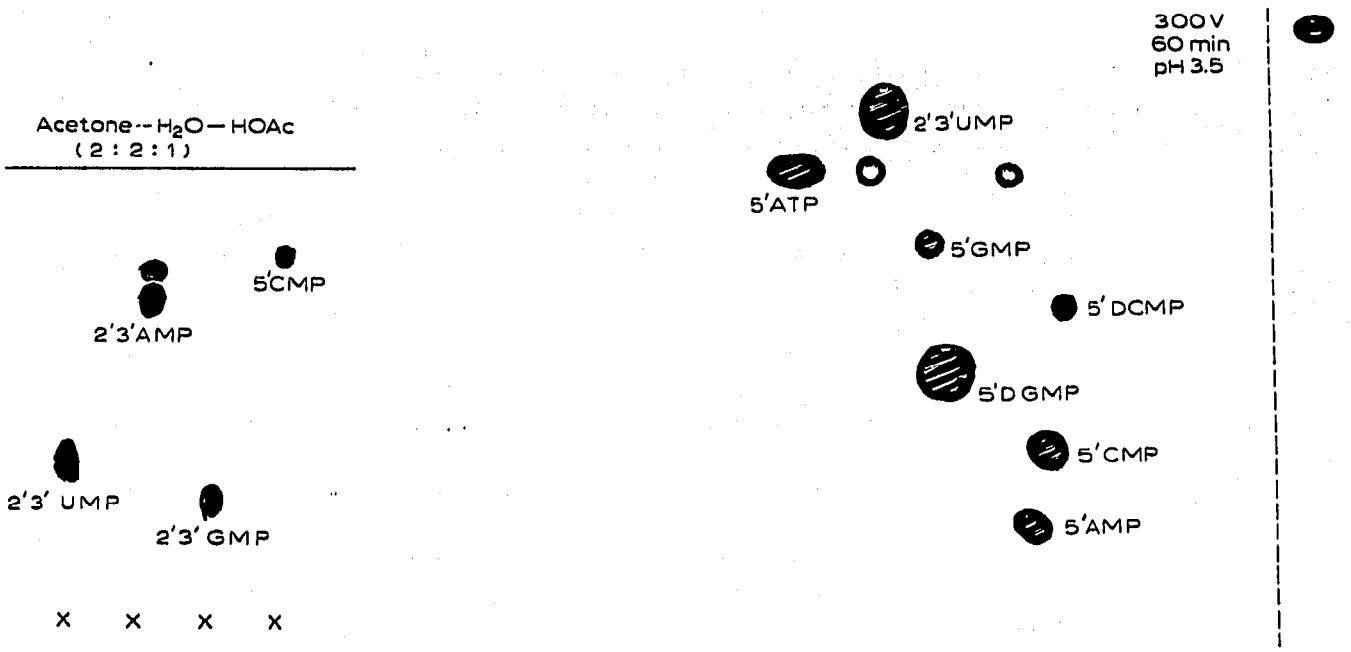

Fig. 3. One-dimensional chromatogram. Solvent: acetone–water–glacial acetic acid (2:2:1, v/v/v), 50 min, 10 cm. In this solvent system, we could separate 3'(2')-AMP which appeared as 2 spots.

Fig. 4. Broken line indicated the location of applied samples. The electrophoresis was run with 300 V loaded, 60 min, with pH 3.5 buffer[8], 7 authentic samples of nucleotides and deoxynucleotides could be separated.

Finally, we would like to point out that the separation pattern of nucleotides by polyamide-layer chromatography was similar to PEI-cellulose thin-layer chromatography of RANDERATH[2]. Further studies on more nucleotides are in progress.

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