A new method for the determination of base ratios in ribonucleic acids

The determination of the base composition of RNA is usually carried out in such a manner that after hydrolysis of the RNA the resulting mononucleotides are separated by paper chromatography[1], paper electrophoresis[2] or ion-exchange chromatography[3]. The amount of each one of the nucleotides is estimated by means of U.V. absorption. A rather new procedure to solve separation problems has been found in chromatography or electrophoresis on thin layers[4-7].

The method here described is a combination of electrophoresis and chromatography on thin layers of phosphorus-free cellulose. The 2′,3′-nucleoside monophosphates, derived from alkaline hydrolysis of yeast RNA, can be separated by this procedure. The amount of each nucleotide is easily determined by its phosphorus content.

Methods

Preparation of thin cellulose layers. 15 g of MN cellulose powder 300 HR and 75 ml of deionized water were mixed with an Ultra-Turrax (Janke & Kunkel, Staufen i.Br., Germany) for one minute. The suspension was spread out on carefully cleaned glass plates (20 × 20 cm) with a Camag-applicator (Camag, Muttenz, Switzerland), the slit-width of which we adjusted to 0.3 mm. The plates should then dry overnight at room temperature. It is worth mentioning that the back and the rims of the plates should be cleaned thoroughly, in order to guarantee a perfect contact during the electrophoresis.

Before the application of the nucleotide mixtures we prepared the plates according to Fig. 1. The three shaded streaks show where the cellulose powder was carefully scratched off. The lines a–a and c–c were marked with a soft pencil pre-

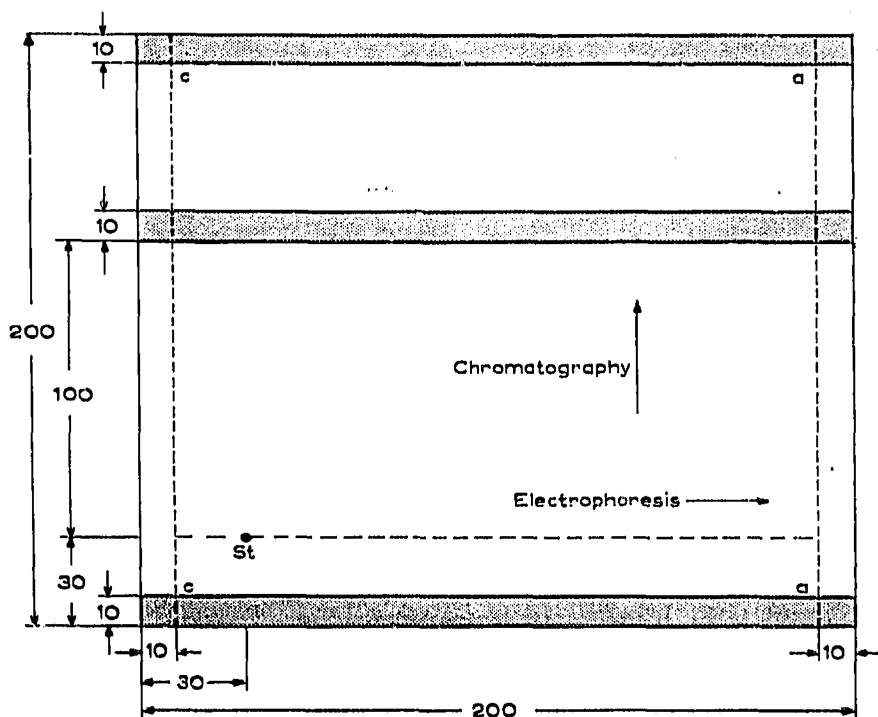

Fig. 1. Plate with thin cellulose layer. For preparation see Methods. (Dimensions in mm).

venting any damage of the layer. St stands for starting point, where the mixture in question is spotted.

High-voltage electrophoresis. 0.02–0.05 ml (1.5–2.5 OD) of the nucleotide solution (preparation see Table III) were applied dropwise to the starting point, the diameter of which should not exceed 2–4 mm. The buffer for the separation (0.05 *M* sodium formate; pH 3.4) was evenly sprayed on the cellulose layer directly before the electrophoresis. We used the high-voltage electrophoresis apparatus designed by WIELAND AND PFLEIDERER (Hormuth & Vetter, Heidelberg, Germany). Application of 1500 V and 45 mA at —4° yielded a field intensity of approximately 100 V per cm. This condition brought about a sufficient separation of the 2',3'-nucleoside monophosphates in 50 min. After the electrophoresis the plates were quickly dried with a fan.

Chromatography. Following the high-voltage electrophoresis the plates with the thin cellulose layer were developed by ascending chromatography. The development was carried out at room temperature in a closed rectangular glass jar. It contained the solvent on the bottom (1–2 cm) and in addition filter paper moistened with solvent on three sides. The solvent, saturated ammonia sulfate–1 *N* sodium acetate–isopropanol (80:18:2, v/v)[1], was allowed to rise a distance of 10 cm. This was usually achieved in 60 min. After the development the plates were again dried with a fan.

Quantitative determination of the nucleotides. The positions of the spots corresponding to nucleoside monophosphates were determined by their U.V. absorption and carefully scraped off the plate, cellulose areas of the same size serving as blanks. The phosphorus content of the samples was determined by the method of GERLACH AND DEUTICKE[8].

Material. Yeast RNA was obtained from Schwarz Bioresearch (Orangeburg, N.Y., U.S.A.), the 2',3'-ribonucleoside monophosphates from Calbiochem (Los Angeles, Calif., U.S.A.) and MN cellulose powder 300 HR from Machery and Nagel (Dueren, Germany).

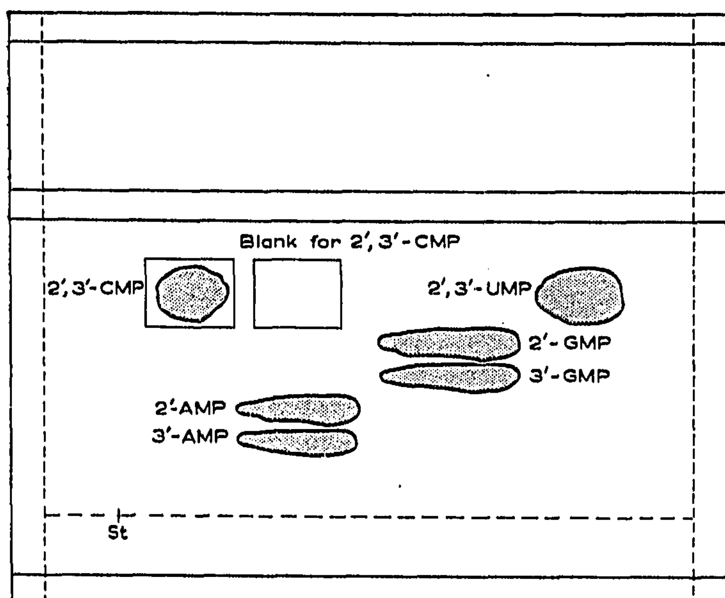

Fig. 2. Separation of a nucleotide solution resulting from alkaline hydrolysis of yeast RNA by electrophoresis and chromatography on thin cellulose layer.

Results

As can be seen in Fig. 2 the combination of electrophoresis and chromatography on a thin cellulose layer leads in fact to a perfect separation of a mixture of 2',3'-nucleoside monophosphates. In the case of AMP and GMP a single spot appears not only for the 2'- but also for the 3'-nucleotide. The R_F values are listed in Table I. The recovery of the substances is quite good (Table II). We could recover an average of 95.7 % of each nucleotide from artificial mixtures. Since we related the phosphorus content of each nucleotide spot to the amount of phosphorus in the mixture applied

TABLE I

R_F VALUES OF 2',3'-RIBONUCLEOSIDE MONOPHOSPHATES AFTER ELECTROPHORESIS AND CHROMATOGRAPHY ON THIN CELLULOSE LAYERS

Nucleotide	R_F
2',3'-CMP	0.73 ± 0.04
2'-AMP	0.35 ± 0.03
3'-AMP	0.28 ± 0.06
2'-GMP	0.58 ± 0.06
3'-GMP	0.49 ± 0.05
2',3'-UMP	0.73 ± 0.07

TABLE II

RECOVERY OF 2',3'-RIBONUCLEOSIDE MONOPHOSPHATES AFTER ELECTROPHORESIS AND CHROMATOGRAPHY ON THIN CELLULOSE LAYERS

Nucleotide	*μmole nucleotide*		*% recovery*	*Number of determinations*
	Spotted	*Recovered*		
2',3'-CMP	0.0399	0.0394 ± 0.0017	94.5–100.5	5
2',3'-AMP	0.0150	0.0144 ± 0.0005	92.0– 99.4	5
2',3'-GMP	0.0151	0.0142 ± 0.0007	89.4– 98.7	5
2',3'-UMP	0.0365	0.0352 ± 0.0019	90.9–101.6	5

TABLE III

COMPOSITION OF NUCLEOTIDE SOLUTIONS RESULTING FROM ALKALINE HYDROLYSIS OF YEAST RNA CALCULATED IN PER CENT OF TOTAL PHOSPHORUS CONTENT

Commercial ribonucleic acid from yeast was hydrolyzed with 0.3 N KOH at 30° for 18 h. After adjusting the hydrolysate to pH 1 with 1 N $HClO_4$, it was kept in ice for one hour. The $KClO_4$ was removed by centrifugation, and the supernatant was adjusted to pH 6 with 1 N KOH. After another hour in ice the salt was again centrifuged down. The resulting nucleotide solution had an optical density at 260 mμ of approximately 75 per ml. This mixture was applied to the plates.

Nucleotide	*% of total phosphorus*
2',3'-CMP	21.25 ± 1.25
2',3'-AMP	25.89 ± 1.43
2',3'-GMP	26.20 ± 1.14
2',3'-UMP	26.66 ± 1.62

to the plate, we knew how much of the single nucleotide the mixture had contained (Table III). These values served us for the calculation of the base ratios. In the case of yeast RNA we found $A+C/G+U = 0.89$ and $A+G/C+U = 1.09$. The values correspond well with those in the literature[9]. More recently we used the new method to determine the base ratios of different RNA fractions from rat liver. As expected we found the values were also the same as previously published[10].

Finally it should be noted that the application of the method described here allows the estimation of the base ratios of extremely small amounts of RNA.

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