A CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF NUCLEIC ACIDS IN MICROGRAM AMOUNTS

by

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Methods of analyzing nucleic acids by paper chromatography on their constituents absorbing ultraviolet light have been worked out during the last years[1-5]. They are based on the photometrical quantitative determination of solutions of the bases and nucleotides after their elution from paper strips. The lowest amount which can be analyzed with sufficient accuracy lies around 100 μg for yeast nucleic acid. It might be of interest to be able to estimate nucleic acid components in smaller amounts, *e.g.* at quantitative determinations within the nervous system. A method has been worked out which allows less than 2 μg of ribose nucleic acid to be determined accurately with reference to purine bases and pyrimidine nucleotides.

PROCEDURE

1.5–5 μg of hydrolyzed yeast nucleic acid was put on a narrow (0.5–1 mm) filter paper strip. Separation was performed according to the methods adopted for ordinary paper chromatography. The strip was then dried and contact-photographed at 257 mμ and 275 mμ together with a calibration system. The densities of the calibration system and the prints of the strips were then measured photometrically on the photographic plate. From the extinction values the amounts of the individual bases and nucleotides could be computed.

METHODS

Paper strips. Filter paper (Munktell OB) was cut in strips, 0.5–1 mm broad, with a Schneider paper-cutting machine (Schneiderwerk, Lübeck). The breadth of each strip was determined and controlled microscopically.

Capillary pipette. A thermometer capillary with an inner diameter of 75 μ was used. It allows amounts from 0.5 μl to be put on the strip with a dispersion of about $\pm$ 1%.

Chromatography. A glass cylinder (diameter 8 cm, height 40 cm) was used and the solvent was placed in a 10 ml glass trough. (Equipment for ordinary one-dimensional chromatography is quite satisfactory.)

Two solvents were used, No. I: 8,5 *M iso*propanol, 2 *N* HCl in water[6], No. II: *n*-butanol (3), ethanol (2), 5 *M* HCl (2), v/v/v. No. II separates the purines, the pyrimidine nucleotides and nucleosides in a short length of flow, taking 4–6 hours.

R_F-values:	guanine	0.24
	adenine	0.35
	cytidine	0.43
	cytidylic acid	0.54
	uridine	0.63
	uridylic acid	0.78

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After the chromatography the strips were dried for 18 hours at 20° C.

Materials. Commercial yeast nucleic acid was purified according to a modification of the procedure of FLETCHER *et al.*[1]. Commercial purines, pyrimidine nucleosides and nucleotides were employed.

Hydrolysis. The nucleic acid was hydrolyzed for 1 hour at 100° C with 1 *N* HCl[4].

Optical method. The dried strips were cut in two or three pieces and put against a photographic plate. They were held in place by a quartz window with low light-absorption down to 230 mμ. Another quartz window on the same plate was completely covered by a piece of untreated filter paper. During the exposure a rotating sector, logarithmically cut, was placed in front of it. See Fig. 1. Contact prints were taken at 257 mμ and at 275 mμ. The light was generated between rotating cadmium electrodes of an ultraviolet microscope constructed according to Köhler. The KÖHLER microscope has been used earlier for similar purposes by the author[7]. The illumination was adjusted according to KÖHLER's rule. Optical equipment: a quartz condenser of numerical aperture 0.3 and a 6 mm monochromator of numerical aperture 0.35, corrected for 257 mμ (Cooke, Troughton and Simms).

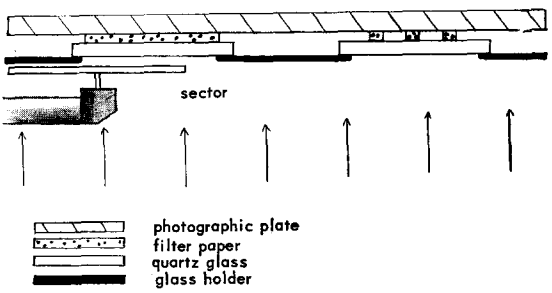

Fig. 1. Arrangement for contact photography of filter-paper strips.

Photographical: Kodak Process plates were used. Calibration errors owing to the intermittency effect and interference with the periods of the spark do not exist at 1400 r.p.m. and cut zones of 2·90°, 2·45°, 2·22.5° and 2·11.25°. See THORELL[8]. The homogeneity of the light-field was controlled by photometry of exposed empty plates. The exposure was chosen so that the blackening of the prints of the strips with their spots would lie between extinction values from 0.3 to 2, *i.e.* within the straight part of the photographic blackening curve.

The calibration system constitutes an extinction scale with a difference of log 2 between each step.

Photometry. The plates were investigated by photometry with a "Moll recording microphotometer" model A[1] (P. J. KIPP en Zonen). The strips were measured continuously in their whole length. The total extinction of every spot was computed and compared with standards, empirically found, for the different substances, treated in the same way. For the quantitative estimation of adenine, guanine and uridylic acid, where solvent I or II was used, the plate at 257 mμ was taken. For cytidylic acid, where only solvent II was used because of interference with uridine at solvent I, the plate at 275 mμ was taken.

RESULTS

Fig. 2 shows a photography at 275 mμ of the calibration system and a 0.7 mm broad strip where 1.75 μg hydrolyzed yeast nucleic acid is separated. g, a, c, u and f refer to adenine, guanine, cytidylic acid, uridylic acid and the front, respectively. Solvent I was used. As can be seen there is no impairment of the resolution compared with ordinary paper chromatography. Fig. 3 shows the curves obtained by photometry of the plates at 257 mμ and 275 mμ of the same strip as in Fig. 2. (Guanine is excluded for practical reasons.) Note the differences of the extinction curves

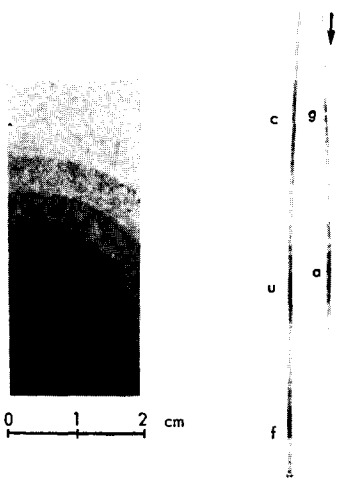

Fig. 2. Contact print at 275 mμ of a strip chromatogram with calibration system. 1.75 μg of hydrolyzed yeast nucleic acid is subjected to separation. Solvent I. g, a, c, u and f refer to guanine, adenine, cytidylic acid, uridylic acid and the front, respectively.

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of each substance at the two wave-lengths. For the quantitative determination of guanine, adenine and uridylic acid the plate at 257 mμ was used, this wave-length being ideal for these substances since it lies in the immediate neighbourhood of the maxima of their extinction curves. For the same reason 275 mμ is ideal for cytidylic acid.

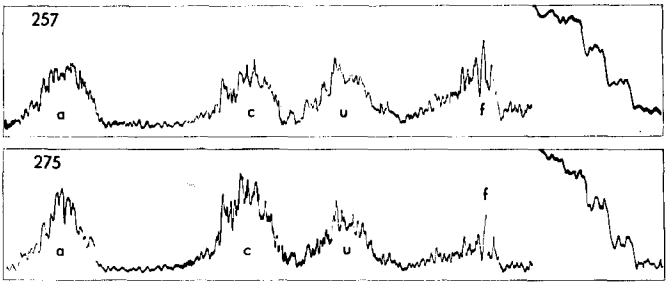

Fig. 3. Extinction curves of the prints at 257 mμ and 275 mμ of a strip chromatogram where 1.75 μg of hydrolyzed yeast nucleic acid is separated. Symbols as in Fig. 2.

A detailed report with analyses of biological material and a discussion of the accuracy of the method will be given later.

SUMMARY

A chromatographic method for the determination of nucleic acid components, fifty times more sensitive than the older ones, is described.

RÉSUMÉ

L'auteur décrit une méthode chromatographique pour la détermination de composantes d'acides nucléiques; cette méthode est cinquante fois plus sensible que les méthodes plus anciennes.

ZUSAMMENFASSUNG

Eine chromatographische Methode zur Bestimmung von Nukleinsäure-Bestandteilen, welche fünfzigmal empfindlicher ist als die älteren Methoden, wird beschrieben.

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