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Note

Separation of nucleic acid bases, nucleosides and nucleotides on Dowex 50 type resin-coated chromatoplates

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Ion exchangers of different types are widely used for the thin-layer chromatographic (TLC) separation of nucleic acid derivatives. Usually, substituted cellulose-type sorbents, *e.g.*, PEI-cellulose, are used [1,2] and only a few papers have described layers or papers coated with ion-exchange resins[3–5]. Much work has been done on anion exchangers[1,2], while cation exchangers have been applied far less frequently[5,6]. Therefore, a systematic investigation study was started of the applicability of Dowex 50 type resin-coated chromatoplates[7] for the TLC separation of nucleic acid bases, nucleosides, nucleotides and related compounds and for the base analysis of DNAs. This type of plate has recently been used for the resolution of complex mixtures of amino acids[8].

EXPERIMENTAL

Most experiments were performed on Fixion 50-X8 (H^+) chromatoplates (Chinoin, Nagytétény, Hungary). Identical results were obtained using Ionex 25 SA (H^+) chromatosheets (Macherey, Nagel & Co., Düren, G.F.R.). The chromatoplates consisted of about one third of Dowex 50 type resin and two thirds of silica gel[7]. The commercially available plates were in the sodium form. Layers in the acid form were obtained by continuous development[8] with 1.0 *N* HCl followed by treatment with de-ionized water for 16 h with both solvents.

The compounds investigated were commercial products (P-L Biochemicals, Milwaukee, Wisc., U.S.A.) and their purity was checked by paper chromatography[9] and UV absorption[10].

Stock solutions were prepared in de-ionized water (10 mg/ml of nucleoside or nucleotide) or in 1.0 *N* HCl (5 mg/ml of base). Volumes of 1 μl from the stock solutions were applied for each spot, this volume being equivalent to 5 μg of base and 10 μg of nucleoside and nucleotide, respectively.

The spots were rendered visible under a short-wave (254 nm) UV lamp (Desaga Uvis).

DNA from chicken erythrocytes was a commercial product (Reanal, Budapest, Hungary). DNAs of *Rhizobium meliloti* phage 16–3 temperature inducible (ti.) double mutant and of *Salmonella typhimurium* LT-2 were prepared by Dr. T. Sik[11] (Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged) and by Dr. A. Udvardy[12] of this Institute.

The DNA samples were hydrolyzed in the usual way (1 mg of DNA in 0.5 ml of 88% formic acid at 175° for 30 min)[9,13]. The colourless or pale yellow hydrolyzate was evaporated under reduced pressure (Rotavapor, W. Büchi, Flawil, Switzerland) and the residue was dissolved in 50 μl of 1.0 *N* HCl. The solution was applied to the layers in the form of two approximately equivalent spots. After two developments with 1.0 *N* HCl, the spots were cut out and were eluted with 5 ml of 10% NaCl solution in 0.1 *N* HCl by continuous shaking at 85° for 15 min using a thermostatted gyrotory shaker (Vibroterm, Labor-Mim, Esztergom, Hungary). Equivalent areas of the chromatoplates that contained no samples were treated simultaneously under the same conditions and served as blanks. After cooling and centrifugation, the absorbances of the clear supernatant liquids were read against the appropriate blanks in an Opton PM QII spectrophotometer at two different wavelengths according to the differential extinction technique[14].

In order to determine the recoveries, 5-μl aliquots of the stock solutions (equivalent to 25μg of base) were applied to the chromatoplates as a single mixed spot and the same aliquots were transferred separately by pipette into 5 ml of 10% NaCl in 0.1 *N* HCl (reference solutions) using an Eppendorf micropipette in both instances. The artificial mixtures of bases on the chromatoplates were separated and eluted and the absorbances of the individual compounds were compared with those of the appropriate reference solutions.

Fig. 1. Separation of 5'-ribonucleotides on Fixion 50-X8 (H^+) chromatoplates in de-ionized water. Development time 60 min for 10 cm. Points show the location of samples. 1 = AMP; 2 = CMP; 3 = GMP; 4 = UMP; 5 = 1 + 2 + 3 + 4.

RESULTS AND DISCUSSION

As shown in Fig. 1, the 5′-ribonucleotides could be separated on Fixion 50-X8 (H^+) chromatoplates with de-ionized water as eluting agent. To our knowledge, the only known example of the separation of nucleotides on cation-exchange resins is the resolution of a mixture of 5′-deoxyribonucleotides on a Dowex 50-X2 column at pH 3.25 using sodium citrate and sodium chloride as the eluting solution[15].

The isomeric ribonucleotides were indistinguishable by our method, although partial resolution of 2′- and 3′-purine ribonucleotides was achieved. The overlap between the double spot of 2′(3′)-AMP and 2′(3′)-CMP did not make possible the fractionation of 2′(3′)-ribonucleotides. However, the system was suitable for the separation of ATP (R_F=0.91), ADP (R_F=0.80), 3′,5′-cyclic AMP (R_F=0.18), AMP (R_F=0.08) and adenosine (R_F=0.00).

In dilute aqueous NaCl or HCl solution, cytidine and guanosine phosphates migrated with identical R_F values, but the 5′-ribonucleotides were separated from the parent bases and ribonucleosides in 1.0 *N* HCl (Fig. 2).

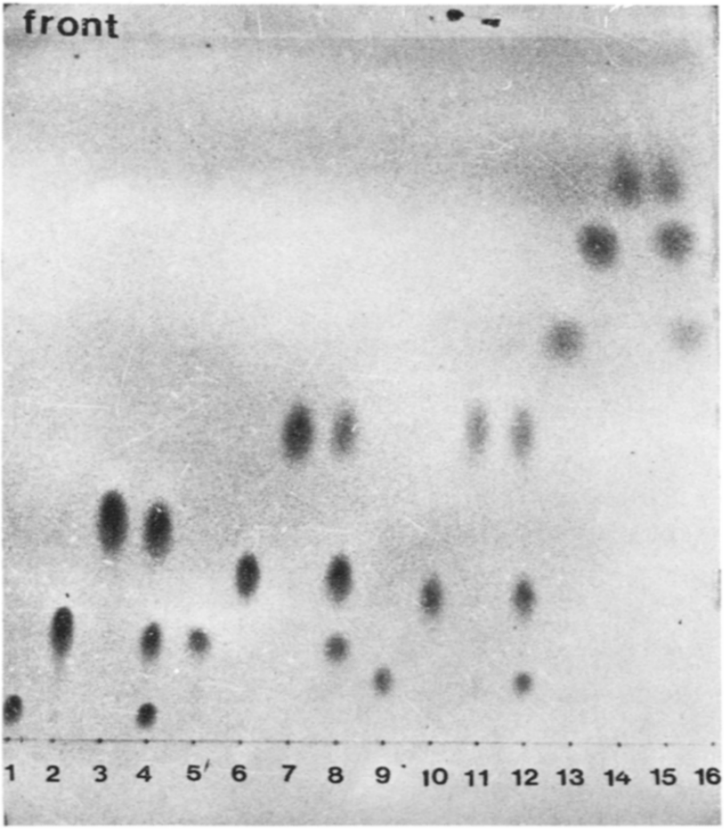

Fig. 2. Separation of bases, ribonucleosides and 5′-ribonucleotides on Fixion 50-X8 (H^+) chromatoplates in 1.0 *N* HCl. Development time 70 min for 10 cm. 1=Adenine; 2=adenosine; 3=AMP; 4=1+2+3; 5=cytosine; 6=cytidine; 7=CMP; 8=5+6+7; 9=guanine; 10=guanosine; 11=GMP; 12=9+10+11; 13=uracil; 14=uridine; 15=UMP; 16=13+14+15.

The observed R_F values of the bases and nucleosides were in good agreement with those reported by Lepri *et al.*[5], who used a different TLC system.

A very convenient separation of the principal DNA bases and their deaminated

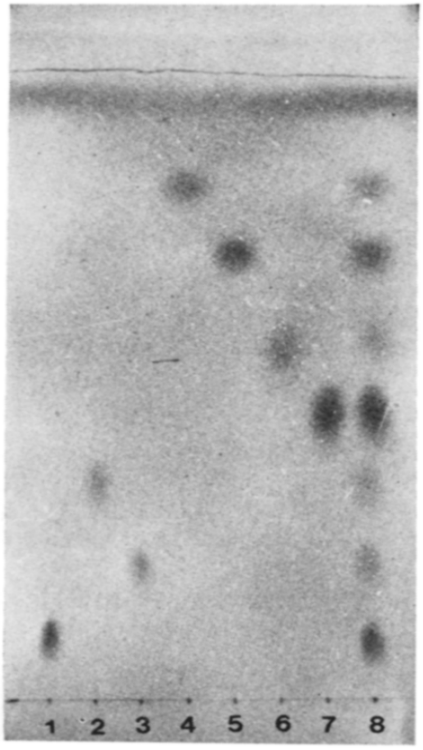

Fig. 3. Separation of purine and pyrimidine bases on Fixion 50-X8 (H^+) chromatoplates after two successive developments in 1.0 *N* HCl. First run: 90 min, 15 cm. Second run: 90 min, 16 cm. 1 = Adenine; 2 = cytosine; 3 = guanine; 4 = uracil; 5 = thymine; 6 = xanthine; 7 = hypoxanthine; 8 = 1 + 2 + 3 + 4 + 5 + 6 + 7.

derivatives could be achieved by two successive developments in 1.0 *N* HCl (Fig. 3). This system could be applied to the quantitative determination of the base composition of DNAs from different sources, using hydrolysis in formic acid[9,14] and the spectrophotometric technique after elution[14].

The usual elution technique (0.1 *N* HCl, room temperature[13,14] or 100° (ref. 16)) proved to be inapplicable owing to the ion-exchange properties of the layers. The observed approximate recoveries in this instance were as follows: 20–25% for adenine, 45–50% for guanine, 65–70% for cytosine and 95–100% for thymine. Adequate recoveries, irrespective of the individual bases, were attained by carrying out the elution with 10% NaCl solution in 0.1 *N* HCl at 85° for 15 min (Table I). Blank values were low (<0.050) and with hydrolyzed samples absorbances of not more than 0.020 were observed at the starting points. The absorbances of bases in the eluting solution were identical with those of their solution of the same concentration in 0.1 *N* HCl[14] within the limits of experimental error, and remained unaltered after treatment at 85° for 15 min.

TABLE I

RECOVERIES OF DNA BASES FROM FIXION 50-X8 (H^+) CHROMATOPLATES

Base	*Amount applied (μmole)*	*Amount recovered (μmole)*
Adenine	0.185	0.177, 0.181, 0.190
Cytosine	0.225	0.219, 0.222, 0.222
Guanine	0.165	0.155, 0.159, 0.162
Thymine	0.198	0.191, 0.193, 0.202

In consequence of the high UV absorption of the resin matrix, the sensitivity of the UV detection was about one order of magnitude lower than that obtained with cellulose layers[2] ($3 \cdot 10^{-2}$ μmol *vs.* 10^{-3} μmol). On the other hand, round spots were formed and relatively large amounts (up to 100 μg from each base) could be separated.

The alternative method of hydrolysis in 7.5 *N* perchloric acid[9,13] could not be used because of the corrosive action of this acid on the layers.

The base compositions of the DNA samples, compared with the literature data, are summarized in Table II. Each figure is the average of three determinations. For the *Rhizobium meliloti* phage 16–3 ti. double mutant, only the base composition of the wild type is known.

It seems probable that the separation obtained is due primarily to charge differences between the compounds fractionated. The resin phase of a strong cation exchanger of the sulphonic acid type is comparable with moderately concentrated (3–7 *N*) aqueous sulphuric acid solutions[21]. On the basis of UV spectral data, it seems that under the conditions of separation the second protonation of the adenine and guanine ring occurs to some extent[10,22] (the pK_a values for the first protonation of adenine and guanine are 4.1 and 3.2, respectively[23], while those for the second protonation have not been described in the literature). For this reason, they must

TABLE II

BASE COMPOSITION OF DIFFERENT DNAs

Literature values are given in parentheses.

DNA	*Composition (%)*				*Reference*
	Adenine	*Thymine*	*Cytosine*	*Guanine*	
Chicken erythrocytes	27.8 (27.9)	29.8 (29.4)	21.6 (21.5)	20.8 (21.2)	17
Rhizobium meliloti phage 16–3 ti. double mutant	20.2 (20.6)	20.2 (23.2)	29.8 (27.3)	29.8 (28.8)	18*
Salmonella typhimurium LT-2	22.9 (22.6) (24.0)	23.8 (22.6) (23.7)	26.0 (28.4) (25.9)	27.3 (26.4) (26.4)	19 20

*Base composition of the wild type.

have an additional fraction of positive charge, adenine having a larger one than guanine[22]. On the other hand, in moderately concentrated aqueous solutions of strong acids, the first anionic dissociation of nucleotides ($pK_n = 0.5–1.0$)[23] is markedly repressed, and these compounds are therefore, with the exception of UMP, also cations with different net positive charges.

The further applicability of the strong cation-exchange resin-coated chromatoplates in this field (base analysis of RNAs, determination of adenylcyclase activity, etc.), as well as the mechanism of separation, are under investigation and the results will be published later.

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