

The Separation of Oligonucleotides of Baker's-Yeast Valine Transfer Ribonucleic Acid 2b by High-Voltage Electrophoresis on DEAE-Paper and by Thin-Layer Chromatography

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A modified procedure for the separation of oligoribonucleotides is described that is based on the combination of t.l.c. on cellulose and electrophoresis on DEAE-paper at 4000 V on a cooling plate. The technique is relatively rapid and allows the analysis of larger quantities than is possible by electrophoresis on cellulose acetate.

Our studies of the primary structure of baker's-yeast (*Saccharomyces cerevisiae*) tRNA_{2b}^{Val} necessitated the rapid separation of oligonucleotides. Previously we had used a combination of some column and t.l.c. methods (Isaenko & Axel'rod, 1976; Kryukov *et al.*, 1976), but this technique was tedious and time-consuming.

The two-dimensional electrophoresis of Sanger *et al.* (1965) is commonly used to separate oligonucleotides. This method has high resolution and has been used successfully in the study of the primary structure of nucleic acids and their fragments. However, because of the low capacity of cellulose acetate and the use of u.v.-absorbing buffer, this method may be applied only to the analysis of a labelled material. Murray (1970), developing the Sanger *et al.* (1965) method, suggested the two-dimensional electrophoresis of deoxyoligonucleotides on DEAE-paper. In the first dimension he used 5% (v/v) triethylamine carbonate buffer, pH 9.7, followed by formic acid/acetic acid buffer, pH 1.9, in the second dimension. This combination was found to be superior to the Sanger *et al.* (1965) system both in resolution (mainly for the short oligomers) and in capacity. However, the high pH of the first dimension makes this method unapplicable to separation of oligonucleotides of tRNA, on account of the presence of minor components which are sensitive to alkaline conditions (1-methyladenosine, 7-methylguanosine, for example).

An attempt was made to overcome the low capacity of cellulose acetate by Gangloff *et al.* (1972). These authors combined the column-separation method of Tomlinson & Tener (1962) in 7M-urea with electrophoresis on DEAE-paper in 7% (v/v) formic acid.

This approach gives good results, but it is inconvenient, since it involves column chromatography followed by desalting.

Another limitation of the Sanger *et al.* (1965) method is that the cooling system in a tank with Varsol limits the voltage to 1500 V on DEAE-paper, and this causes an increase in the time required.

Materials and Methods

tRNA_{2b}^{Val} was purified by consecutive column chromatography on BD-cellulose (benzoylated DEAE-cellulose) and DEAE-Sephadex, as described for tRNA_{2b}^{Val} (Kryukov *et al.*, 1972), and then on hydroxyapatite by the method of Pearson & Kelmers (1966) and on RPC-5 system as described by Pearson *et al.* (1970). The tRNA_{2b}^{Val} finally obtained was about 90% pure.

Guanyloribonuclease from *Actinomyces aureoverticillatus* (EC 3.1.4.8) was prepared as described by Tatarskaya *et al.* (1966). Ribonuclease I from bovine pancreas (EC 3.1.4.22) was kindly provided by Dr. Y. Lebedev (Institute of Molecular Biology, Moscow, U.S.S.R.), and was homogeneous by chromatography on Bio-Rex 70.

Cellulose FND for t.l.c. was obtained from Filtrak, 9303 Niederschlag/Erzg., German Democratic Republic. Ion-exchange paper was Whatman Chromedia DE81.

All the solvents used were freshly distilled.

Results and Discussion

In the present study we used electrophoresis on DEAE-paper in formic acid, pH 1.55, at 4000 V on a cooling plate equipped with a paper-pressure device [a rubber pillow at a pressure of 5 kPa (0.5 kg/cm²)]. This apparatus, designed at the Special Design

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Bureau of Biophysical Equipment (Moscow, U.S.S.R.), allowed us to maintain the temperature of DEAE-paper during the experiment at a constant value (in our case +4°C) at 4000V. To obtain a complete separation of oligonucleotides, we used t.l.c. on cellulose plates (4cm×18cm) in isobutyric acid/0.5M-NH₃ (5:3, v/v), pH3.7, as the first dimension. The sample was applied to the chromatogram as a 10–15mm-long band. After two developments the plate was washed with water-saturated butan-1-ol and then the elution, perpendicular to the development direction, was performed with formic acid, pH1.55. In this way the elution front displaced all the components to the edge of the plate. Then the plate (without drying) was placed face downwards on a sheet of Whatman DE81 DEAE-paper (18cm×57cm) wetted with formic acid, pH1.55, and the electrophoresis was carried out at 4000V in the second dimension for 2h (for a pancreatic ribonuclease digest) and 3h (for a guanyloribonuclease digest) (see Plates 1a and 1b).

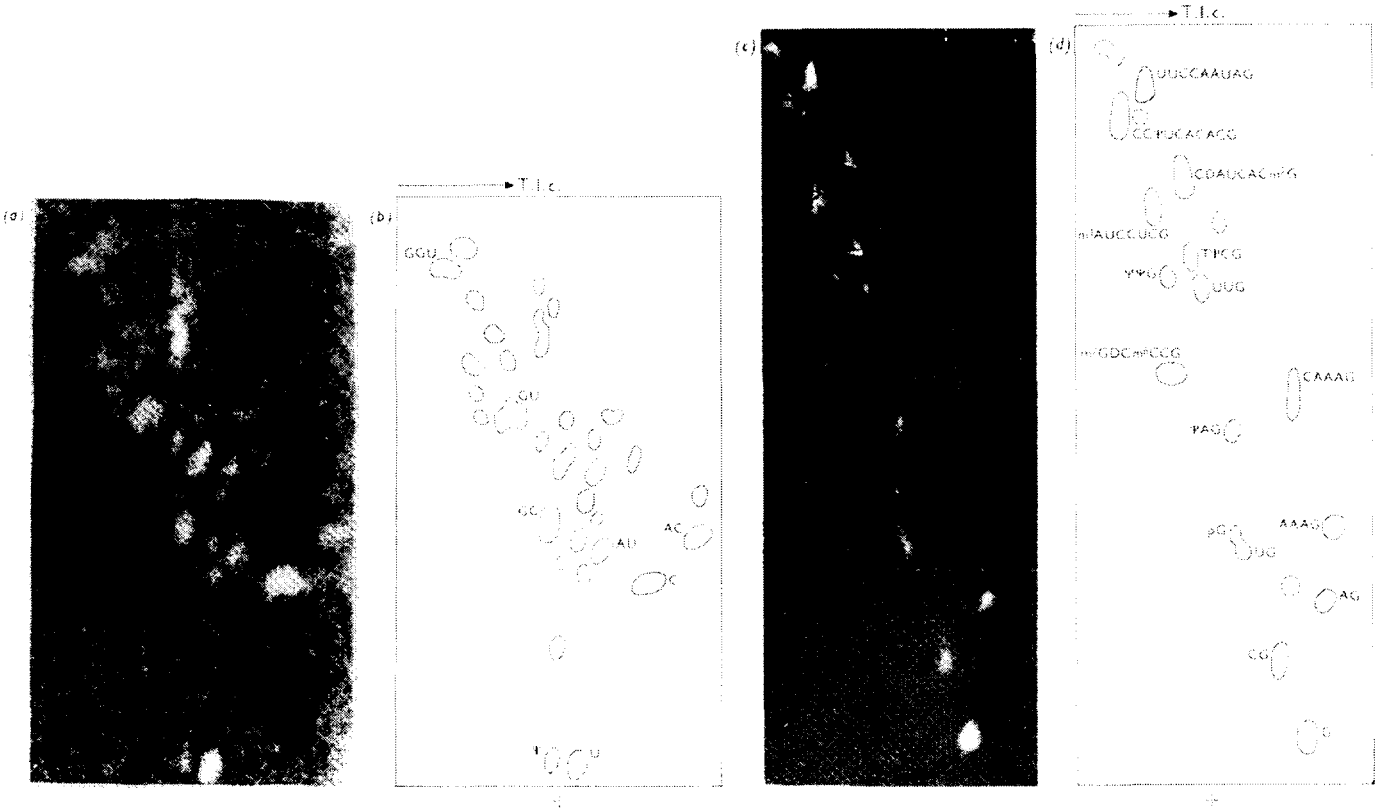
By using such a combination of t.l.c. and electrophoresis, we were able to separate a large amount of the material during the first stage, with the result that the capacity of DEAE-paper on the second stage became the limiting factor. It was found that in handling large quantities (in excess of 300µg of the digest of the individual tRNA) there was overloading in the starting-points on DEAE-paper, resulting in deterioration of the electrophoresis. The results of the

electrophoresis at +4°C were much better than at +20°C.

We have successfully used this developed technique to separate the guanyloribonuclease digest in the study of the primary structure of baker's-yeast tRNA₂₅^{Val}. Intact tRNA₂₅^{Val} was digested by this enzyme (360 units of activity/270µg of tRNA for 18h at 37°C) (Tatarskaya *et al.*, 1966) and subjected to the separation procedure (Plates 1c and 1d).

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EXPLANATION OF PLATE I

Electrophoretic patterns of the separation of oligonucleotides

(a) 'Fingerprint' of the pancreatic-ribonuclease digest of the total tRNA from baker's yeast. The loading was 400 μ g. T.l.c. was in isobutyric acid/0.05M-NH₃ (5:3, v/v), pH 3.7; electrophoresis was in formic acid, pH 1.55 (4000 V, 2 h, +4°C). (b) Line drawing of u.v. spots from (a). (c) 'Fingerprint' of the guanyloribonuclease digest of tRNA^{Phe} from baker's yeast. The loading was 270 μ g. All conditions were as in (a), except for the periods of electrophoresis (3 h). (d) Line drawing of u.v. spots, showing the structure of oligonucleotides. Abbreviations for nucleotide substituents: m, methyl; p, phosphoric residue.

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(Facing p. 410)