THE FRACTIONATION OF DEOXYRIBONUCLEIC ACIDS ON ECTEOLA

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INTRODUCTION

The column-chromatographic fractionation of nucleic acids on the cellulose-anion exchanger ECTEOLA developed by BENDICH *et al.*[1,2] is now one of the major techniques used for the fractionation of these compounds.

During a study concerning the column-chromatographic fractionation of DNA* preparations, isolated from different sources, results were obtained which show that the fractionation patterns of DNA preparations isolated from the same source are not identical. A number of experiments were then carried out to study the reproducibility of this column-chromatographic technique. The results of these experiments will be reported and discussed.

METHODS AND MATERIALS

ECTEOLA was prepared according to the method of PETERSON AND SOBER[3]. Calf thymus DNA was isolated by the detergent method, using sodium lauryl sulphate as detergent[4].

Excessive drying of DNA was avoided. After precipitation of DNA in 50% ethanol, the DNA was lifted out of the solution, carefully pressed out between filter paper and dissolved in 0.14 *M* NaCl–0.001 *M* citrate, pH 6.8 or in 0.01 *M* phosphate buffer, pH 6.8. Traces of ethanol were removed by dialysis. The isolations of DNA were carried out at 2–4°. DNA determinations were performed according to the method of DISCHE[5], RNA was determined by the orcinol reaction[5], protein by the biuret assay[6]. Nitrogen was determined by the Kjeldahl method and inorganic phosphate by the method of FISKE-SUBBAROW[7] or by the method described by MICHELSON[8].

Column chromatography

ECTEOLA was washed thoroughly with 1 *N* NaOH and 0.01 *M* phosphate buffer, pH 7, until no U.V.-absorbing material could be detected. The columns were then prepared in the following way: 0.5 g ECTEOLA (200–300 mesh) was suspended in

* Abbreviations: DNA = deoxyribonucleic acid; RNA = ribonucleic acid.

50 ml 0.01 *M* phosphate buffer, pH 7, and deaerated *in vacuo* for 15 min. The slurry was added to the column and allowed to settle under gravity. The columns were washed with 0.01 *M* phosphate buffer, pH 7, for 24 h. The size of the ECTEOLA columns was 5 × 0.8 cm. Solutions of DNA in 0.01 *M* phoshate buffer, pH 7, with concentrations of about 300 μg/ml were added to the column and allowed to percolate by gravity. The columns were washed with 0.01 *M* phosphate buffer until the optical density of the effluent at 260 mμ was lower than 0.050. The DNA that passed into the effluent through the columns during the absorption was measured by the method of DISCHE[5] or by means of the optical density at 260 mμ. The absorption capacity of the amount of ECTEOLA used in a column is sufficient to absorb 2–3 mg DNA, so that usually no DNA was detected in the effluent.

The fractionation of DNA was performed by gradient elution with a single mixing chamber of 500 ml[1]. The flow rate was kept at 5–6 ml/h by using a pressure of 20–25 cm Hg. After reaching pH 10.5 the resistance of the column decreased and a lower pressure, namely 5–10 cm Hg, was sufficient to maintain the flow rate at 5.0 ml/h. The connections between the supply chamber, the mixing chamber and the column were glass–polyethylene–glass connections. The polyethylene tube was treated thoroughly with 2 *N* HCl and 2 *N* NaOH several times until no U.V.-absorbing material was released, and subsequently washed for 48 h in 0.1 *M* phosphate buffer,

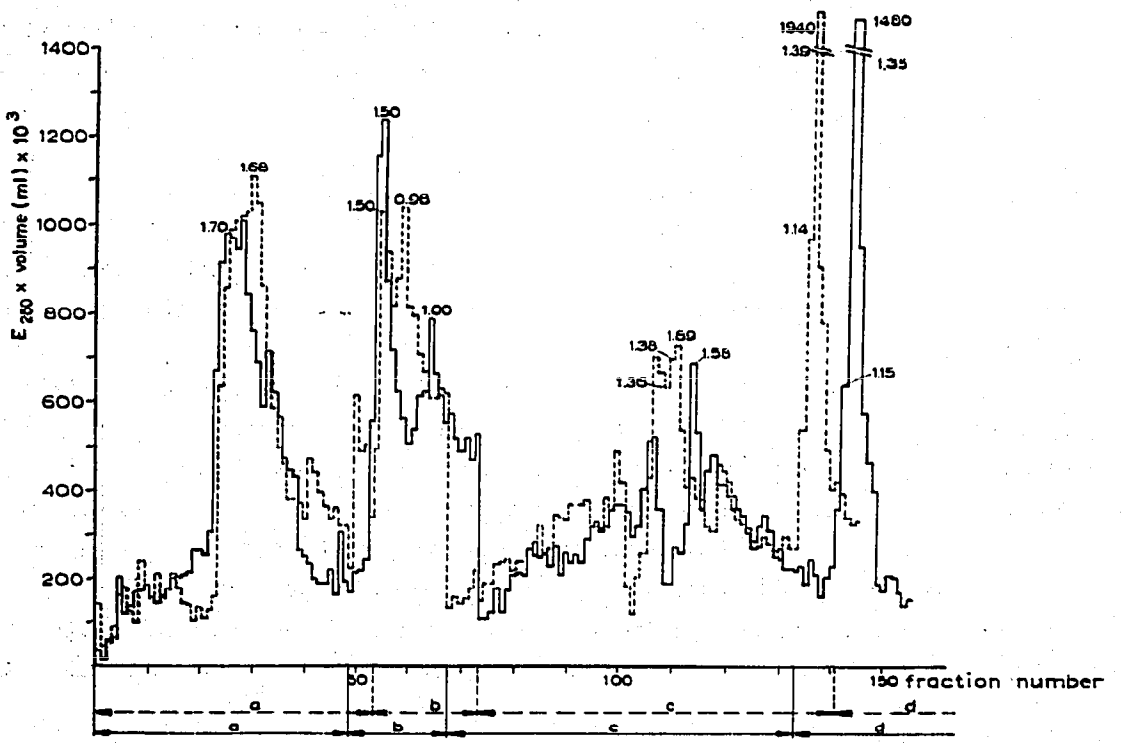

Fig. 1. Chromatography of 2.9 mg of calf thymus DNA (DNA I) on a column of ECTEOLA-SF (6 × 0.8 cm; 0.5 g; 0.29% N; flow rate 5–6 ml/h). Duplicate experiments; analytical characteristics of the DNA preparation: 3% RNA; 3% protein; E_{260}/E_{280} = 1.81; N/P = 3.8. The E_{260}/E_{280} ratios of several fractions and the optical densities at 260 mμ of the fractions eluted with 0.5 *N* NaOH are marked in the figure. Elution fluids: (a) gradient 0 to 0.5 *M* NaCl; (b) 0.1 *M* ammonia + 2.0 *M* NaCl; (c) 2.0 *M* NaCl and gradient from 0.1 to 1.0 *M* ammonia; (d) 0.5 *M* NaOH. a, b and c were made up in 0.01 *M* phosphate buffer, pH 7.0.

pH 7.0. The fractions were collected in 4.5 ml amounts in an L.K.B. Fraction-Collector. All operations were carried out in the cold room.

RESULTS

Reproducibility of the fractionation

Two thymus DNA preparations (DNA I and DNA II) were fractionated in duplicate experiments. Two columns were run with the same amounts of DNA and under almost identical conditions of elution (Figs. 1–4). The two curves in each figure rep-

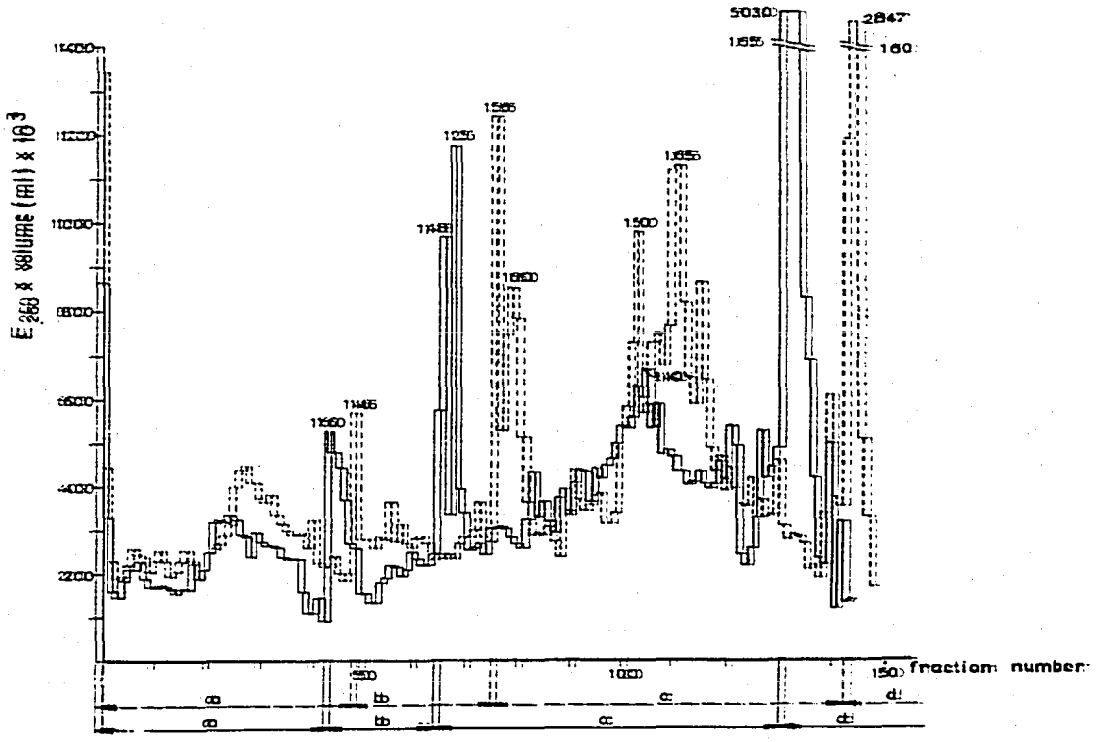

Fig. 2. Chromatography of 22.9 mg of calf thymus DNA (DNA I) on a column of ECTEOLA-SF (6 × 0.8 cm; 0.5 g; 0.39% N; flow rate 5–6 ml/h). Duplicate experiments; for the analytical characteristics of the DNA preparations and further details see Fig. 1.

resenting duplicate experiments are very similar. The experiments illustrated in Figs. 1 and 3 were repeated two weeks later under the same conditions and the results are presented in Figs. 2 and 4 respectively. During that period the thymus DNA was stored in the refrigerator in 0.14 *M* NaCl. Comparison of the figures shows that this storage of two weeks had brought about changes in the fractionation patterns. In the figures the ratios of the optical densities measured at 260 and 280 mμ are shown for different peaks. These ratios vary from 1.40–1.80.

Fractionations with two different ECTEOLA preparations

The fractionation of thymus DNA on two different ECTEOLA preparations was studied next. The ECTEOLA preparations were made under the same conditions. Their nitrogen contents show a small difference (Fig. 5). The results of these fractionations are presented in Fig. 5.

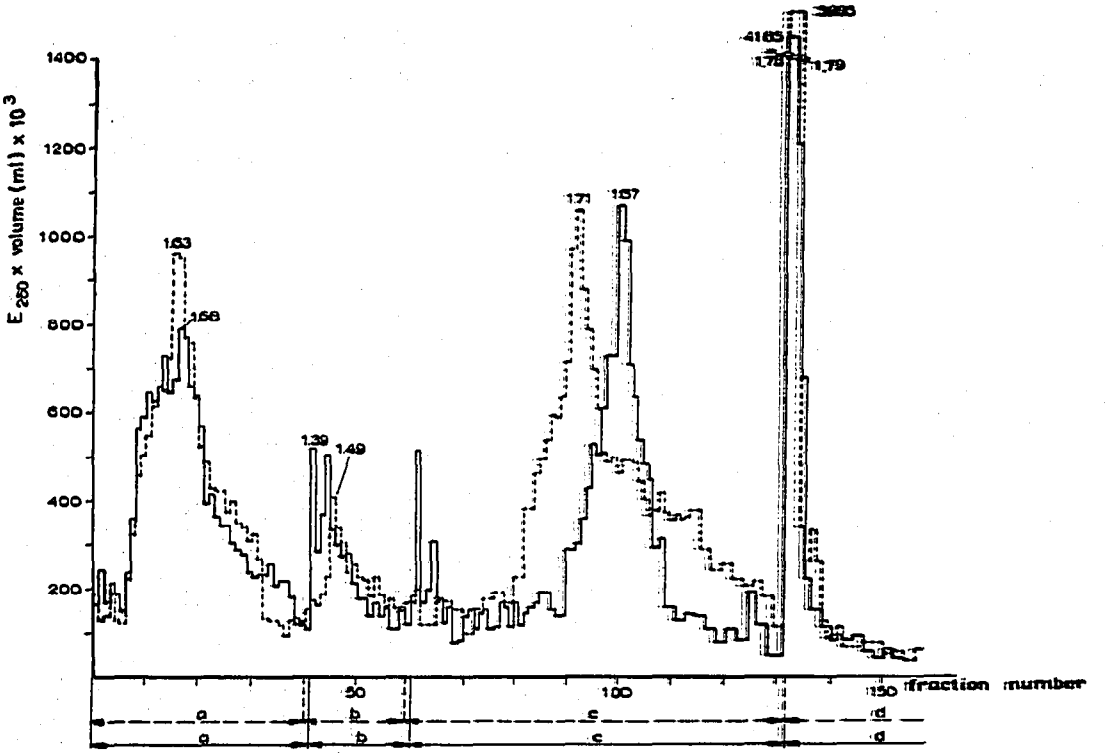

Fig. 3. Chromatography of 3.1 mg of calf thymus DNA (DNA II) on a column of ECTEOLA-SF (5 × 0.8 cm; 0.5 g; 0.29% N; flow rate 5–6 ml/h). Duplicate experiments; analytical characteristics of the DNA preparation: 1.5% RNA; 1% protein; $E_{260}/E_{260} = 1.80$; N/P = 3.7. For further details see Fig. 1.

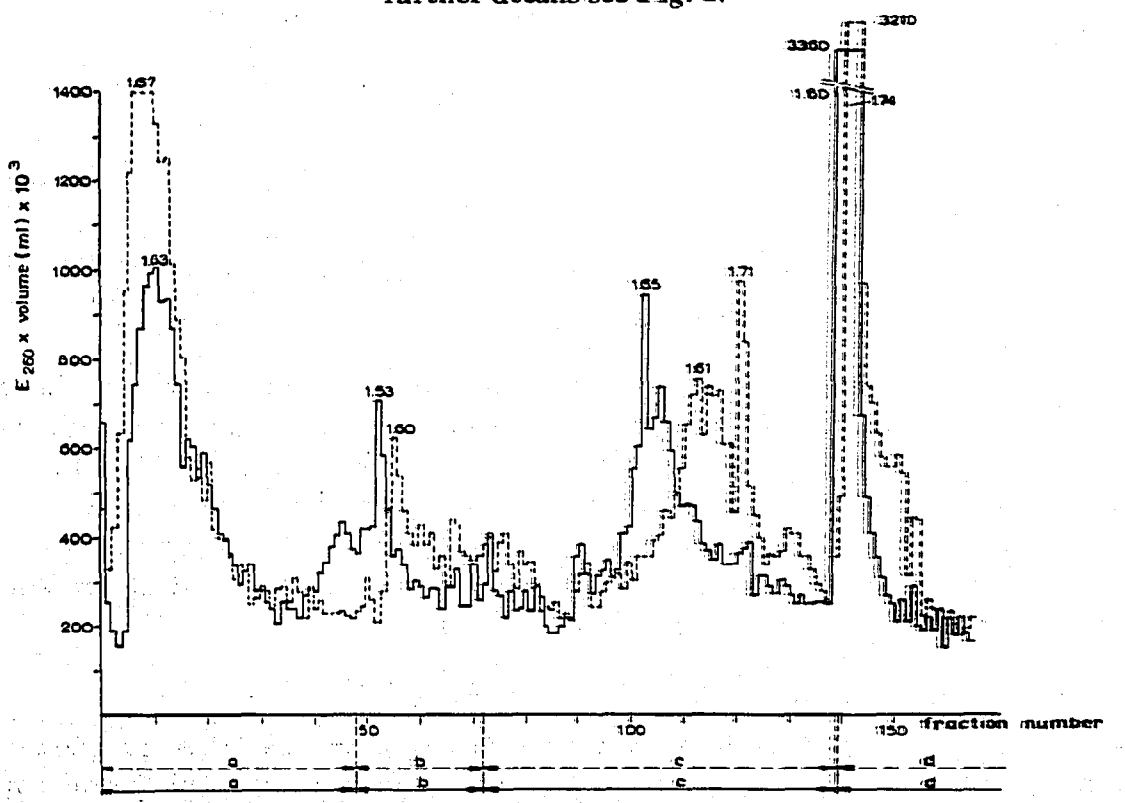

Fig. 4. Chromatography of 3.6 mg of calf thymus DNA (DNA II) on a column of ECTEOLA-SF (5 × 0.8 cm; 0.5 g; 0.29% N; flow rate 5–6 ml/h). Duplicate experiments; for the analytical characteristics of the DNA preparation see Fig. 3; for further details see Fig. 1.

DISCUSSION

The results (Figs. 1–5) show that it is possible to use ECTEOLA as anion-exchanger to separate DNA into a number of distinct fractions. These fractions show differences in the ratio of optical densities measured at 260 and 280 mμ (Figs. 1–5) and in base composition[2]. The variations in the ratio of the optical densities are sometimes very

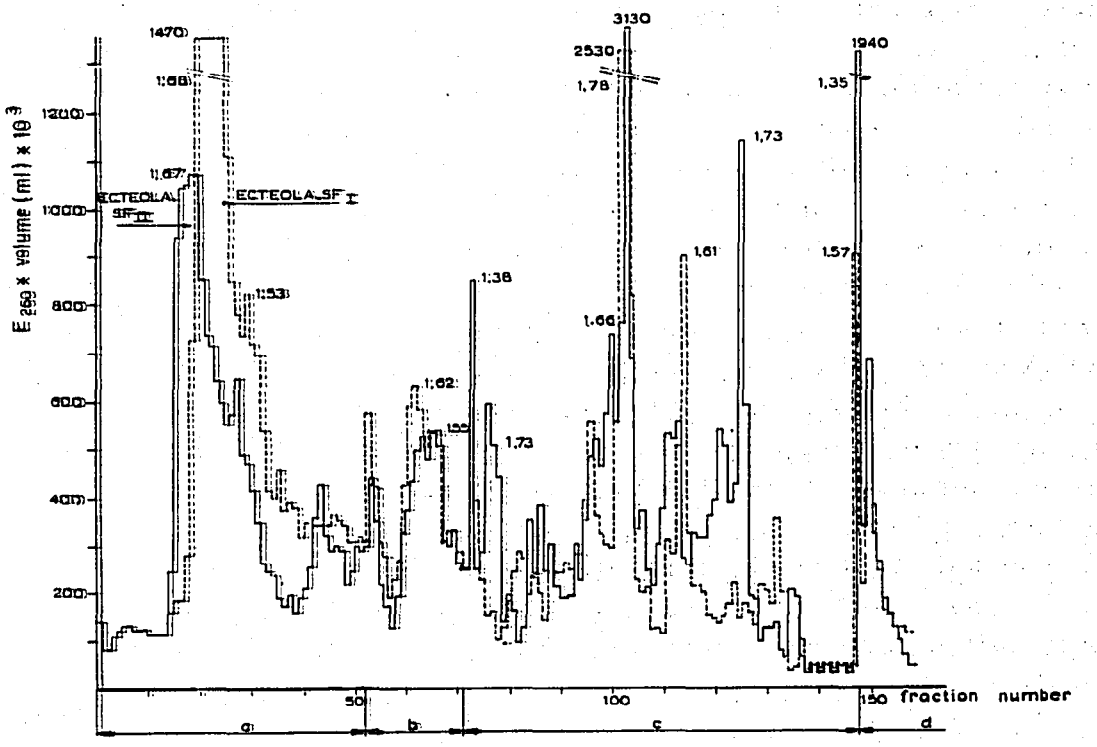

Fig. 5. Chromatography of two samples of 2.2 mg calf thymus DNA each on two columns of different ECTEOLA-SF preparations. ECTEOLA-SF I column: (6 × 0.7 cm; 0.5 g; 0.29% N; flow rate 5–6 ml/h). ECTEOLA-SF II column: (6 × 0.7 cm; 0.5 g; 0.32% N; flow rate 5–6 ml/h). Analytical characteristics of the DNA preparation: 3% RNA, 1% protein, $E_{260}/E_{280} = 1.78$; N/P = 3.7. For further details see Fig. 1.

pronounced. They were found in experiments with DNA isolated from calf thymus, rat liver and haemophilus DNA and cannot be explained as a result of the increase of the pH or the NaCl concentration during the fractionation. It has been reported that the fractions obtained after chromatography of DNA on ECTEOLA show also differences in physicochemical properties[9,10] and, in the case of biologically active DNA, in biological activity[2]. Our experiments demonstrate that fractionations in duplicate of a DNA preparation produce almost identical patterns. The small divergence in pattern of the duplicates of Figs. 3 and 4 might be due to slight differences in elution rate. Our results show further that alterations of the fractionation patterns occur when DNA preparations are aged for 2–3 weeks. During this period the DNA preparations were stored in 0.14 *M* NaCl–0.001 *M* citrate solutions at pH 6.8. It remains uncertain whether it will be possible to get fractionation patterns which are highly typical for DNA preparations of a given source. It has been suggested by ROSOFF

et al.[10] that the fractionation of DNA over ECTEOLA is determined by the size and shape of the molecules, and it was demonstrated that DNA with relatively low molecular weight is eluted prior to DNA with relatively high molecular weight[9]. In physicochemical studies on DNA HERMANS[12] found differences in molecular weight, gyration radius and viscosity between preparations isolated from the same source under identical conditions. Preliminary experiments carried out by VAN DE VEN[11] have confirmed the results of HERMANS[12] and showed further that the physicochemical properties mentioned change during storage of DNA solutions at 0–3°. Obviously variations in size and shape exist between DNA molecules of a particular sample and DNA molecules of different samples and it is likely that these molecular parameters undergo alterations very easily. The evidence from column-chromatographic fractionations of DNA on ECTEOLA and from physicochemical studies of other authors[11,12] is consistent with the hypothesis of ROSOFF *et al.*[10] that fractionation on ECTEOLA is governed by the size and shape of the DNA molecules. Slight variations in chromatographic patterns also occur when different samples of ECTEOLA are used (Fig. 5). It is further necessary to emphasize that variations in RNA and protein impurities in different DNA preparations may occur, even when the isolations are performed under completely identical conditions. It has been established that protein impurities of the DNA preparations influence the physical properties of the DNA molecules[12].

We conclude that DNA preparations even when isolated from the same source will yield variable patterns when separated on ECTEOLA columns. Furthermore, the fractionation patterns change as a result of storage of DNA solutions in 0.14 *M* NaCl in the refrigerator.

SUMMARY

DNA preparations were fractionated on ECTEOLA-SF columns. Variations in column-chromatographic pattern were observed amongst DNA preparations isolated from the same source and amongst the chromatographic patterns of the same preparation fractionated at different times.

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