THIN-LAYER CHROMATOGRAPHY OF DEOXYRIBONUCLEIC ACID ON ECTEOLA CELLULOSE

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INTRODUCTION

Thin-layer chromatography has found wide application in the past few years, with notable success in the separation and detection of nucleic acid derivatives including deoxyribooligonucleotides[1]. Recently, JOSEFSSON[2] reported a semi-quantitative microdetermination of nucleic acid derivatives on cellulose powder using thin-layer adsorbent techniques. However, studies have been lacking in the application of this technique to the nucleic acids themselves. Since ECTEOLA cellulose has been found to be a good support for the column chromatography of deoxyribonucleic acid (DNA)[3], this seemed to be the support of choice for thin-layer chromatography. ROSENKRANZ AND BENDICH[4] showed a correlation between the chromatographic migration using ECTEOLA as a column support and the sedimentation coefficient of DNA. This technique has shown wide application in ascertaining the molecular state of the nucleic acid. Also, these workers have reported a correlation between the migration of DNA on paper and the sedimentation coefficient[5].

This study shows how ECTEOLA-cellulose may be used as a support for the convenient thin-layer chromatography of DNA. A correlation between the thin-layer and column techniques using the same chromatographic support is made here. The results of these chromatographic techniques can also be related to the sedimentation coefficients as obtained by ultracentrifugation.

EXPERIMENTAL

Calf thymus DNA (sodium salt), as obtained from Worthington Biochemical Corporation, Freehold, New Jersey, was used in this study.

Heat treatment

A solution of DNA (2 mg/ml of distilled water) was heated for varying amounts of time ranging from five minutes to one hour and then rapidly cooled in an ice bath. At all conditions other than heat treatment the DNA was dissolved in 0.01 *M* phosphate buffer, pH 7.

Column chromatography

Fractionation of unheated and heated DNA was carried out on columns of ECTEOLA (capacity 0.39 mequiv./g, lot No. 1079, obtained from Brown Company, Berlin, New

Hampshire) using a gradient elution system as outlined by BENDICH *et al.*[3]. Heated and control DNA samples were chromatographed on parallel columns fed by a common two mixing chamber system. This was used so that ready comparison of the elution patterns could be obtained. The flow was controlled by pumping the solvents through the columns using a peristaltic pump (obtained from Harvard Apparatus Company, Inc. Dover, Mass.) at a rate of 4 ml per hour. Ten ml fractions were collected and the absorbance of each fraction at 260 mμ was determined using a Beckman DU Spectrophotometer. A complete analysis of a DNA sample by this method usually requires approximately 20 days.

Ultracentrifugal analysis

Solutions of heated and control DNA in 0.01 *M* phosphate buffer, pH 7, were analyzed at 59,780 r.p.m. in a Spinco Model E ultracentrifuge equipped with ultraviolet optics. The samples were diluted to a constant absorbance of 0.70 optical density. Average sedimentation coefficients were calculated for each sample after the ultracentrifuge patterns were converted into plots of concentration *versus* distance by use of a Spinco model R Analytrol equipped with a microanalyzer attachment.

Thin layer chromatography

Serva ECTEOLA-TLC prepared for thin layer chromatography (obtained from Gallard-Schlesinger Chem. Mfg. Co., Garden City, N.Y.) was used. Eight grams of the ECTEOLA-TLC were suspended in 50 ml of distilled water and then applied to 8 × 8 in. glass plates with Research Specialities Company equipment. It was found desirable to sieve the dry resin through a 61 μ sieve prior to use. The plates were dried at room temperature. A solution of 1.0 *M* NH_3 in 2 *M* NaCl and 0.01 *M* phosphate (pH 11.0) was found to be the solvent of choice. This is the same solvent used in the next to the last step in the column fractionation procedure. This has also been reported as the solvent of choice in paper chromatography of nucleic acids[5]. The plates were allowed to equilibrate overnight in the presence of the solvent prior to use. It was found necessary to actually prewet the plates prior to the DNA application by allowing the solvent to rise 1.5 cm above the application point. The DNA samples were applied to this prewet plate by means of a micropipet. The DNA failed to migrate to any detectable extent without this prior moistening. The plates were then developed by allowing migration of the solvent to a level about 14 cm beyond the point of application. This usually required about 90 min. All chromatography was carried out at room temperature. After drying the plates, the DNA spots were detected and marked under ultraviolet light. Although some tailing was almost always noted, the rates of migration could easily be estimated using the most dense portion of the center of the ultraviolet-absorbing areas. This value is reported as the R_F* in this study.

RESULTS AND DISCUSSION

The results of the column chromatography of the control and 60 min heat treated DNA are shown in Fig. 1. These results are consistent with those given by ROSENKRANZ AND BENDICH[6] for the heating of DNA in water. The loss of the more slowly eluted fractions indicate some loss of the higher molecular weight fractions of the DNA upon heat treatment. This heat degradation can also be seen in results of the thin-layer

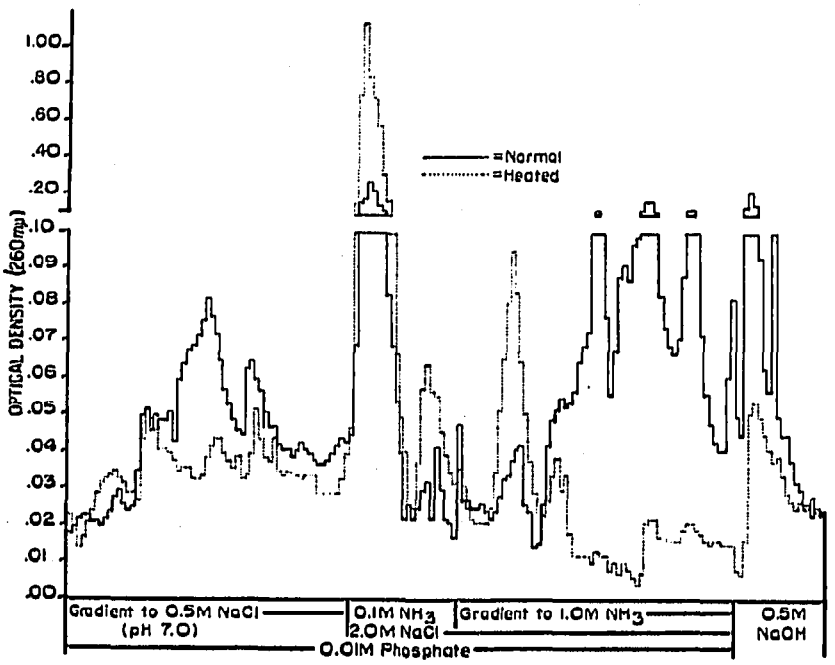

Fig. 1. Chromatographic profiles of native and heated (1 hour) solutions of calf thymus DNA using ECTEOLA cellulose.

chromatography (TLC) in Table I. The increases in heating time, to a maximum of 60 min in this study, yields a degraded DNA sample with a progressive increase in R_F* values. Native DNA showed no observed migration. The increased rate of migration can be correlated with the shift to a greater amount of more easily eluted fraction as seen in the column elution profiles of Fig. 1. The profile displacement in the column chromatography and some of the spreading observed in the ultraviolet absorbing areas which contributes to the rather broad range of the R_F* values (Table I) in the TLC is indicative of the heterogeneous nature of the DNA molecules.

TABLE I

EFFECTS OF HEATING WATER SOLUTIONS OF DNA ON R_F* VALUES OF TLC AND SEDIMENTATION COEFFICIENTS

Time of heating (min)	*Sedimentation values (ave.)*	*ECTEOLA cellulose thin-layer chromatography*	
		R_F*	*No. of samples*
0	24.2	0	24
5	12.1	0.46 ± 0.05	11
10	11.5	0.53 ± 0.12	10
15	10.1	0.54 ± 0.05	10
30	6.4	0.61 ± 0.10	12
60	5.3	0.76 ± 0.10	15

It should be re-emphasized that without the prewet procedure in the TLC no satisfactory migration could be observed for any of the DNA samples. This effect must be due to the irreversible binding of the DNA to the dry ECTEOLA cellulose. This observation is perhaps not unexpected since column chromatography is always

carried out under conditions in which the support is moistened with the initial solvent.

Table I also shows the effects of heating on the average sedimentation coefficient of the DNA. The marked change in the $S_{ave.}$ of 24.2 to 5.3 upon heating for 60 min. is reflected in the R_F* change from essentially zero, or no observable migration under these conditions, to a value of 0.76. The relationship between the calculated sedimentation coefficients and the R_F* values for the various heat degraded DNA samples is shown in Fig. 2. The changes observed in the rates of migration using TLC must then

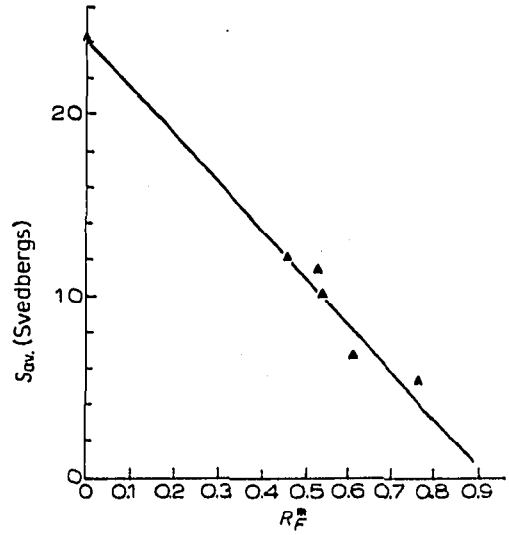

Fig. 2. Correlation between average sedimentation coefficients and R_F* values from thin-layer chromatography of a series of heat-degraded calf thymus DNA samples.

be related to changes in the physicochemical properties of the DNA as demonstrated by ultracentrifugation studies. These data are quite consistent with those correlating sedimentation rates with both column chromatography[4] and paper chromatography[5]. Thus, sedimentation values of DNA samples can be quickly and simply approximated from TLC studies.

Thin-layer chromatography has also been applied to a variety of other nucleic acid samples including commercially available herring sperm DNA and yeast RNA. An average R_F* value of 0.77 was obtained for the herring sperm DNA. The yeast RNA showed a major spot with an R_F* value of 0.81 and three much lighter spots with lower R_F* values. The differences between the lack of migration of the highly polymerized calf thymus DNA and the rapid migration of these samples reflects the ability of this system to discriminate among macromolecules. In this way, TLC might well be applied as a convenient technique in the preliminary separation of a number of physicochemically distinct nucleic acid samples.

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SUMMARY

These studies indicate that convenient thin-layer chromatography techniques may be applied to the separation of high molecular weight nucleic acid samples. ECTEOLA-cellulose was used as a support and 1.0 *M* NH_3 in 2 *M* NaCl and 0.01 *M* phosphate was the solvent. This study correlates the R_F* values of DNA using thin-layer chromatography with the behavior of DNA in studies of chromatography as well as ultracentrifugation. Progressive degradation of the DNA by heat is accompanied by an increase in R_F* values.

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