A STUDY ON THE ABSORPTION OF NUCLEIC ACIDS BY CHARCOAL

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INTRODUCTION

In 1951, ZAMENHOF AND CHARGAFF[1] discovered that RNA could be separated from DNA by adsorption onto charcoal when the nucleic acids were dissolved in 0.14 *M* NaCl. When the sodium chloride concentration was raised to 2 *M* these authors found that some of the DNA was adsorbed by the charcoal and this resulted in an *increased* viscosity of the DNA that remained in solution suggesting that molecules of low viscosity were adsorbed preferentially. DUTTA, JONES AND STACEY[2], who also investigated this effect, reported that when an excess of charcoal was used, DNA was removed from solution even in the presence of dilute salt. Since there is no *a priori* reason to suppose that charcoal is capable of discriminating among polynucleotides on the basis of their content of ribose or deoxyribose, it is probable that the separation achieved when using charcoal is based on physico-chemical characteristics. The present investigation was designed to determine the properties that are required for a nucleic acid to be adsorbed onto charcoal and whether use could be made of this procedure to separate preparations of DNA's differing in size and configuration.

MATERIALS AND METHODS

Nucleic acids

The specimen of calf thymus DNA used in this study was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. The sample was shown to be free of RNA by a colorimetric determination[3]. RNA was prepared from the liver of rats by the procedure described by LASKOV *et al.*[4]. Charcoal (Norit A) was purchased from Fisher Scientific Co., New York, N.Y.

DNA specimens (1 mg per ml of standard saline–citrate*) were sonically degraded by exposure in a 9 Kc Sonic Oscillator (Raytheon Mfg. Co.). For the preparation of thermally-denatured DNA, a solution of DNA (1 mg/ml of SSC/10) was placed into a boiling water bath for 10 min and then chilled by immersion into an ice-bath.

Experimental design

Solutions of nucleic acids (45 μg/ml of SSC) were placed into conical centrifuge tubes and portions of washed (see ref. 1) charcoal (2 mg/ml of SSC) were added. The

* Standard saline–citrate: SSC, 0.15 *M* NaCl in 0.015 *M* sodium citrate.

suspensions were shaken for 15 min whereupon they were centrifuged and 2 ml portions of the supernatant fluids were removed for further analyses. The residues were then resuspended and each tube was supplemented with more charcoal. This procedure was repeated several times.

Physico-chemical characterizations

Sedimentation coefficients were determined with a Spinco Model E analytical centrifuge equipped with an ultraviolet optical system. Solutions of DNA (0.003% in SSC) were run at 59780 r.p.m. (259, 700 *g*) and pictures were taken at two minute intervals. These photographs were traced with a Joyce-Loebl Mark III B microdensitometer. Distributions of sedimentation coefficients were calculated by the procedure of SCHUMAKER AND SCHACHMAN[5].

The banding properties of specimens in gradients of CsCl were determined essentially as described by SCHILDKRAUT, MARMUR AND DOTY[6]. Portions of the DNA together with a reference sample (*Micrococcus lysodeikticus* DNA, 1.731 g/cm^3) were placed in a cesium chloride solution, density 1.70 g/cm^3, and centrifuged in the Spinco Model E analytical centrifuge at 44770 r.p.m. for 24 h. The bands formed by the specimens at their equilibrium positions were photographed and traced as above.

Thermal transition profiles were measured with a Beckman DU spectrophotometer in the manner described by MARMUR AND DOTY[7].

RESULTS AND DISCUSSION

Adsorption of nucleic acids

The data summarized in Fig. 1 show (a) that RNA exhibited a high affinity for charcoal, and (b) that when the concentration of charcoal was increased, DNA was also adsorbed onto the charcoal, this being especially true of heat-denatured and sonically-degraded DNA. These results indicated that attachment to charcoal was probably a function of size and hence the procedure might be useful in separating DNA's on the basis of their physical configuration.

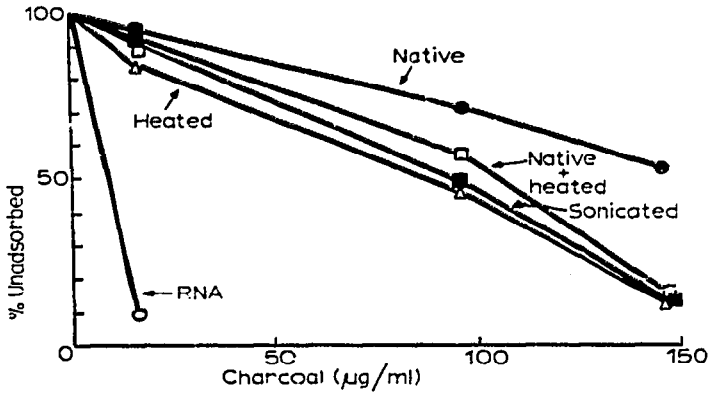

Fig. 1. Affinity of various concentrations of charcoal for nucleic acids.

Charcoal and native DNA

Native DNA can be considered as consisting of a population of molecules differing in size and shape as well as in base composition[8,9]. It was therefore of interest

to determine whether exposure of native DNA to charcoal would result in a partial fractionation of the DNA and, if so, to ascertain the basis of such a fractionation. In addition, since the buoyant density of DNA in gradients of cesium chloride is largely a function of the composition of the DNA[6,10] as well as of its state of nativeness[11], it seemed appropriate to analyse, by density gradient centrifugation, solutions of DNA from which part of the DNA had been removed by absorption with gradually increasing amounts of charcoal.

The banding property of the native DNA is shown in Fig. 2(A). In addition to the main band of density 1.703 g/cm^3, there is a satellite band of density 1.715 g/cm^3. The presence of such a satellite band in calf thymus DNA has also been reported by other investigators[6]. It should be pointed out, however, that the density value of the main band of this commercial DNA is higher than expected for calf thymus DNA, as densities of 1.698 and 1.699 g/cm^3 were reported previously by this[12] and other laboratories[6].

The banding properties of the DNA in the supernatant fluids after exposure to increasing concentrations of charcoal are also shown in Fig. 2 (B, C and D). It is of interest that by the criteria used, these DNA's appear identical to the native calf thymus DNA. Thus the specimen described in tracing D of Fig. 2, although it represented a preparation from which 50 % of the DNA had been removed with charcoal, still possessed the same banding properties as the original DNA, even including an unchanged proportion of material present in the satellite band.

From the above data it may therefore be concluded that charcoal absorption does not discriminate among DNA molecules on the basis of their composition. Since

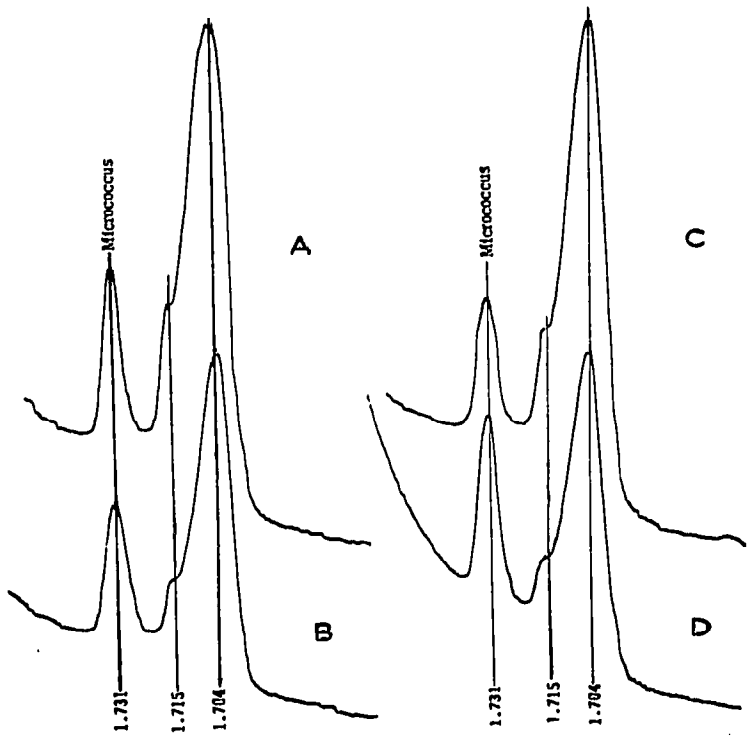

Fig. 2. Buoyant densities in cesium chloride of DNA after exposure to increasing amounts of charcoal. Curve A: original, unfractionated calf thymus DNA. Curves B, C and D: supernatant fluids after exposure to 17, 97 and 147 μg of charcoal per ml, respectively. The band at the left represents the position of the marker DNA (*Micrococcus lysodeikticus*, 1.731 g/cm^3).

the data presented in Fig. 1 indicate that the absorption might be regulated by size of the DNA, a comparison was made between the distribution of sedimentation coefficients of native DNA and of a specimen from which 50 % of the nucleotidic material was removed by charcoal. The data depicted in Fig. 3 indicate that the material

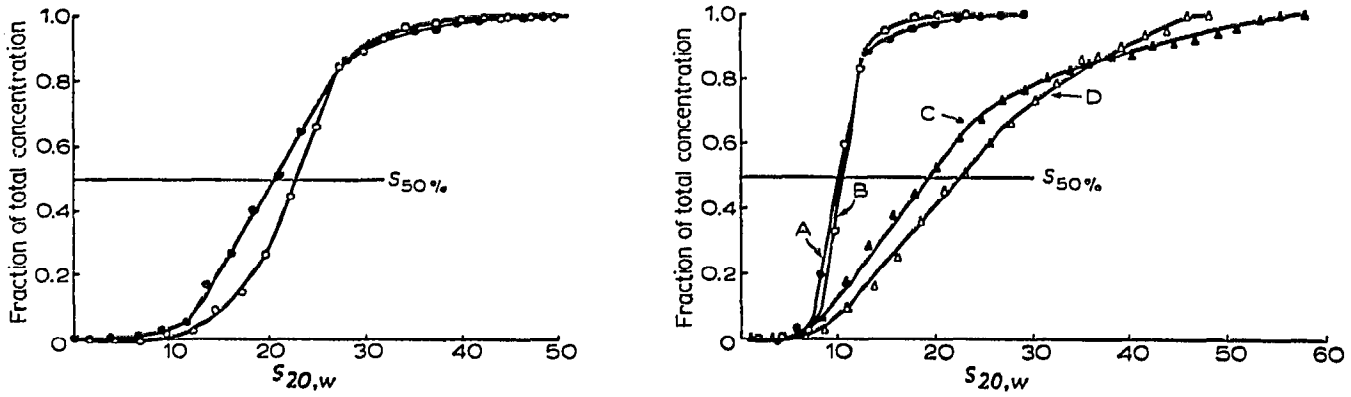

Fig. 3. Distributions of sedimentation coefficients of native (●) and charcoal-treated DNA (○). The treated sample had been exposed to 147 μg of charcoal/ml.

Fig. 4. Distributions of sedimentation coefficients of degraded and of denatured DNA before and after exposure to charcoal (147 μg/ml). Sonically-degraded DNA (●); sonically-degraded DNA exposed to charcoal (○). Thermally-denatured DNA (▲); thermally-denatured DNA after exposure to charcoal (△).

recovered in the supernatant fluid after charcoal treatment possessed higher sedimentation coefficients than the original DNA. This can be taken to mean that charcoal showed preference for the molecules of lower sedimentation coefficients since these were absent in the supernatant fluid. However the charcoal *did not* selectively absorb the smaller molecules, because the data do not show that the 50 % of the material retained by the charcoal consisted of those molecules with sedimentation coefficients below the $S_{50\%}$; indeed, only approximately 10 % of the small molecules (less than 12 S) were selectively removed by the absorbant. If the charcoal had shown some preference for the smaller molecules, then it may be expected that the distribution of sedimentation coefficients of a sonically-degraded DNA preparation, before and after exposure to charcoal, should not be altered significantly, since the molecules present in such a preparation are at the limit of selectivity of the absorbant (*i.e.* approximately 10 S). Curves A and B of Fig. 4 indicate that this expectation was fulfilled.

Charcoal and single-stranded DNA

When double-stranded DNA is heat-denatured and then quickly chilled a separation of the strands occurs, which is reflected in a modified helix-coil transition curve and an increase of *ca.* 0.015 g/cm^3 in buoyant density of the DNA[11]. Fig. 5A illustrates the banding behavior of heat-denatured calf thymus DNA in a gradient of CsCl. The specimen exhibits the increase in buoyant density expected of single-stranded DNA (compare with Fig. 2 (A)). Also shown in Fig. 5 are the tracings representing the banding characteristics of the heat-denatured DNA that was still present in the supernatant fluids after absorption with increasing amounts of charcoal. An examination of the tracings reveals that the residual DNA exhibited unaltered buoyant densities; the tracings representing the specimens exposed to charcoal,

however, have narrower distributions when compared to the one obtained with heat-denatured but "unfractionated" DNA. An inverse relationship has been shown between the width of bands in gradients of CsCl and their molecular weights[13]. It would thus appear from an examination of Fig. 5, that samples after exposure to charcoal possessed higher molecular weights than did the "unfractionated" single-stranded DNA. An analysis of the distributions of sedimentation coefficients of "fractionated" and "unfractionated" DNA (Fig. 4, C and D) supports this finding. However it must be pointed out once more that the charcoal exhibits only a limited selectivity, as the small shift towards higher sedimentation coefficients hardly parallels the amount of DNA that is absorbed onto the charcoal (approximately 90 %).

Recent findings in this laboratory[14] and elsewhere have indicated that denatured calf thymus DNA contains a species of molecules which is more readily renaturable than the bulk of the heat-denatured DNA (ref. 15, see also ref. 16). In order to de-

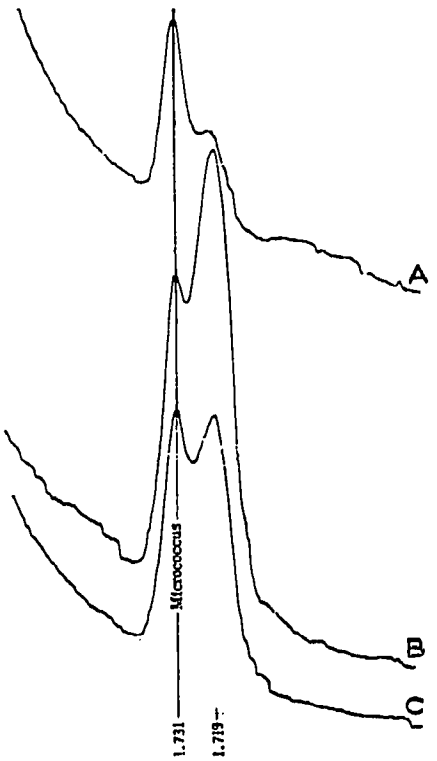

Fig. 5. Buoyant densities in cesium chloride of thermally-denatured DNA before and after exposure to charcoal. Curve A: heated DNA not exposed to charcoal. Curves B and C represent the behavior of thermally-denatured DNA exposed to 17 and 97 μg of charcoal/ml, respectively.

termine whether charcoal could discriminate between these two types of molecules, the thermal denaturation profiles of the DNA specimens described in Fig. 5 were examined. The data so obtained reveal (Fig. 6) that as the concentration of charcoal was increased, the DNA remaining in the supernatant fluid became enriched with a component exhibiting a sharp thermal transition. This result can be taken to mean that charcoal does indeed discriminate among heat-denatured molecules by showing a greater affinity for the molecules with unmodified single-stranded configurations and leaving those that are reversibly denaturable in solution.

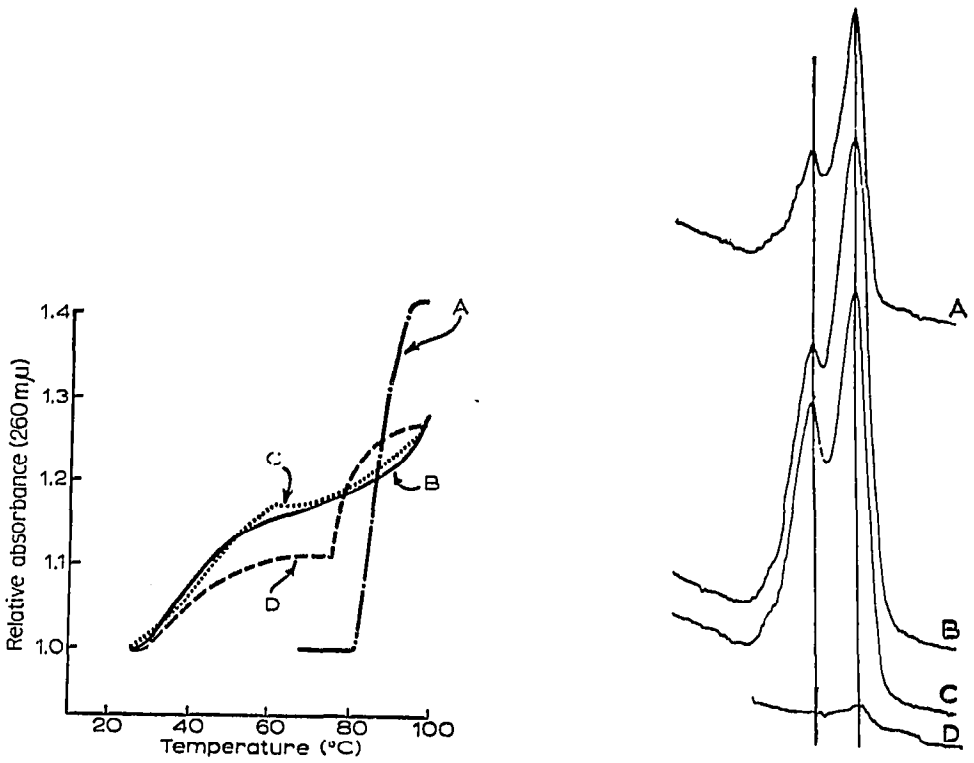

Fig. 6. Thermal denaturation profiles of heat-denatured DNA before and after exposure to charcoal. Curves A and B represent the behavior of native and heat denatured DNA's. Curves C and D represent the behavior of heat-denatured DNA after exposure to 17 and 97 μg of charcoal per ml.

Fig. 7. Buoyant densities in cesium chloride of a mixture of native and heat-denatured DNA before and after exposure to charcoal. Curve A: original mixture. Curves B, C and D: after exposure to 17, 97 and 147 μg of charcoal per ml. In this experiment marker DNA was not included. The band to the right represents the position of the native DNA.

Charcoal and a mixture of native and denatured DNA

A direct measure of the ability of charcoal to differentiate between DNA's of different configurations was obtained by studying its absorptive effect on a mixture of native and heat-denatured DNA. This effect could be assessed readily by subjecting the supernatant fluids of such mixtures to CsCl density gradient centrifugation. In the tracings so obtained, the areas underneath each peak correspond to the concentration of DNA present therein. An analysis of the tracings (Fig. 7) indicates that there was no significant shift in the proportion of material in each peak until only 15 % of DNA remained unadsorbed by charcoal. At that point only double-stranded DNA could be detected in the supernatant fluid (Fig. 7, D). These results, therefore, conform to those obtained with individual preparations of native and denatured DNA and indicate that charcoal is selective to only a small extent.

CONCLUSIONS

Although charcoal absorption effectively separates RNA from DNA, it does not separate DNA molecules varying in size and configuration. This lack of discrimination among forms of DNA may indicate that the ribose moiety of RNA indeed contributes

to the selectivity of absorption. Certainly in view of the data presented herein, charcoal absorption would not appear to be useful as a fractionation procedure for separating different molecules of DNA.

ACKNOWLEDGEMENTS

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SUMMARY

Although charcoal has a high affinity for denatured and for degraded DNA, this property is not useful in separating such molecules from high molecular weight double-stranded (native) DNA.

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