Sedimentation of DNA in ethanol-water solutions within the interval of B→A transition

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ABSTRACT

Sedimentation of DNA ethanol-water solutions has been studied over the range of ethanol concentrations corresponding to the B→A transition (65-80% ethanol, v/v). High ethanol concentrations (more than 75%) have been found to promote aggregate formation in solution. The molecular weight of DNA under fixed ionic conditions in solution (5×10^{-4} M NaCl) has been shown to influence the value of ethanol concentration at which aggregates appear. On the other hand, the fact that DNA molecular weight has not been found to exert any influence on B→A transition curves obtained from CD measurements suggests that the changes observed in DNA CD spectra on adding ethanol to the solution are independent of aggregate formation. The data obtained show that, first, aggregation is not a necessary condition for the DNA transition from the B to the A-conformation and, second, changes in CD spectra of DNA under the influence of ethanol are not related to the process of aggregation.

INTRODUCTION

It was found in (1-4) that the addition of ethanol to a DNA solution causes marked changes in the circular dichroism (CD) spectra of DNA. Since DNA CD spectra in highly concentrated ethanol-water solutions are similar to the known CD spectra of double-stranded RNA (5), as well as to the CD spectra of DNA film at 75% relative humidity (6,7) some authors (1-4, 6,7) assumed that DNA undergoes the B→A transition within the 70-80% ethanol concentration interval, while the CD spectrum of 80% ethanol solution corresponds to that of DNA in the A-conformation. However, studies on the sedimentation and viscosity of DNA in highly concentrated ethanol-water solutions (8-13) as well as electron micros-

copy data of Lang (14) attest that aggregation of DNA molecules takes place in highly concentrated ethanol-water solutions. On the basis of these facts, it is suggested in (3,15) that the changes observed in CD spectra on adding ethanol to the DNA solution may be a reflection of DNA aggregation induced by ethanol and, consequently, the CD spectrum of DNA in 80% ethanol may not correspond to the CD spectrum of the A-conformation. Furthermore, Erfurth et al. (16), who studied the B $\rightarrow$ A transition in oriented fibers and unoriented samples of DNA by Laser Raman scattering and X-ray analysis as well as Skuratovskii et al. (17) have suggested that the A-conformation of DNA is stabilized due to lateral interactions between DNA double helices packed close together and, probably, aggregation (packing) of DNA molecules is a favourable or even a necessary factor for the B $\rightarrow$ A transition.

Thus it is necessary to study at great length the sedimentation behaviour of DNA ethanol-water solutions along with their CD spectra over the interval of the B $\rightarrow$ A transition in order to elucidate the following two questions:

1. Does aggregation affect the CD spectra of DNA?
2. Is aggregation necessary for the B $\rightarrow$ A transition?

MATERIALS AND METHODS

Calf thymus DNA ("Sigma") and T7 phage DNA extracted in our laboratory by the hot phenol method (18) were used. The protein content in preparations as determined by the Lowry method was less than 1%.

The samples of DNA in 1xSSC were subjected to extensive sonication at 0°C. The molecular weight of DNA preparations was determined from their sedimentation coefficients (19). DNA samples of about 300 μ g/ml concentration were carefully dialyzed against 5x10⁻⁴M NaCl before preparing ethanol-water solutions of DNA.

DNA ethanol-water solutions were obtained in two ways: by adding an appropriate amount of 96% ethanol to the DNA-water solution followed by shaking the test-tube and by slow pouring of 96% ethanol into the test-tube with continuous mixing.

The B $\rightarrow$ A transition was tracked by the magnitude variations in CD spectra at 260 nm caused by the addition of ethanol to the DNA solution.

CD spectra were measured with a Cary 60 recording spectropolarimeter with a model 6001 CD accessory under previously described conditions (4). Sedimentation experiments were carried out using a Spinco model E analytical ultracentrifuge. The photographic plates were measured in a microdensitometer IPO-451. Reduction of sedimentation coefficient S to $S_{20,w}$ (see, e.g., (20)) for each ethanol concentration in solution was carried out to allow for the viscosity and density of the corresponding ethanol-water solutions. The value of partial specific volume of DNA at $x\%$ ethanol concentration was calculated by means of linear interpolation within 0.50 ml/g (zero ion strength, no ethanol, (21)) and 0.38 ml/g (95% ethanol, (12)) as follows:

$$\bar{v}_x = 0.50 - \frac{(0.50 - 0.38)}{95} x$$

Sedimentation coefficient $S_{20,w}$ was not reduced to zero DNA concentration since there was no substantial (over several %) difference between the sedimentation coefficients for solutions with DNA concentrations of 6 μ g/ml and 48 μ g/ml. The concentration of DNA in our experiments was usually about 30 μ g/ml.

It should be noted that unlike CD spectra, the sedimentation experiment proved to be sensitive to the method of preparation of the DNA ethanol-water solution. Table I summarizes the data on the preparations including the method of mixing DNA solutions with ethanol. For DNA with a molecular weight over 680,000 Dalton at ethanol concentrations exceeding 75%, the sedimentogram demonstrated a second fraction corresponding to the aggregates. The proportion of aggregated DNA molecules depends on the storage time of the sample (see No.8,9, Table I) and on the method of sample preparation (see No.4,5, Table I). For this reason each DNA sample was characterized by its CD spectrum and then subjected to centrifugation. As shown in (4) , the CD spectra do not depend on the method of sample preparation.

Table 1

No. of preparation (sample)	Type of DNA	Molecular weight	Ethanol concentration	The time of the sample (storage)	Method of mixing	% of aggregated molecules
1	T7	170,000	75%	24 hours	fast	0%
2	T7	170,000	80%	"-	"-	100%
3	T7	283,000	70%	"-	"-	0%
4	T7	283,000	80%	"-	"-	37%
5	T7	283,000	80%	30 min	slow	17%
6	calf thymus	350,000	77%	24 hours	fast	0%
7	calf thymus	350,000	80%	"-	"-	100%
8	T7	680,000	75%	"-	"-	62%
9	T7	680,000	75%	30 min	"-	47%
10	T7	680,000	80%	24 hours	"-	100%

Fig.1. Dependence of reduced sedimentation coefficient $S_{20,w}$ on ethanol concentration for T7 phage DNA (molecular weight 170,000 Dalton) -O-, and calf thymus DNA (molecular weight 350,000 Dalton) -□- .

RESULTS AND DISCUSSION

Fig.1 presents the dependence of reduced sedimentation coefficients $S_{20,w}$ on ethanol concentration for T7 phage DNA (molecular weight 170,000) and calf thymus DNA (molecular weight 350,000). One can see that $S_{20,w}$ undergoes practically no changes in the concentration range of 65-75% and after that (in an interval of 5%) sharply rises. A comparison of Fig.1 with Fig.2, showing the B $\rightarrow$ A transition curves of the preparations studied including T7 phage DNA (molecular weight 170,000) and calf thymus DNA (molecular weight 350,000) leads to the conclusion that the B $\rightarrow$ A transition may proceed in the absence of aggregation: indeed, the B $\rightarrow$ A transition in

Fig.2. B-A transition curves for DNAs of different molecular weights: ○-T7 phage DNA, molecular weight 170,000; △-T7 phage DNA, molecular weight 680,000; □-calf thymus DNA, molecular weight 350,000; ●-calf thymus DNA, molecular weight $4.4 \cdot 10^6$. Molecular weights in Daltons.

75% ethanol is two thirds complete and the change in $S_{20,w}$ is small. Similar results were obtained for a DNA preparation with a molecular weight of 283,000 (data not shown). Thus, our data for DNA preparations with molecular weight of 350,000 or less show that aggregation is not a necessary condition for the $B \rightarrow A$ transition.

As mentioned in Materials and Methods, the method of preparation of DNA ethanol-water solutions has no influence on the CD spectra of DNA (at a fixed ethanol concentration). Hence an admixture of aggregates (see, eg., No.8,9, Table 1) formed at molecular weights of 680,000 or more at 75% ethanol in solution does not change CD spectrum, i.e. the aggregates do not significantly contribute to the CD spectra, at least up to 80% ethanol. This conclusion is supported by previously

Fig.3. Dependence of reduced sedimentation coefficient $S_{20,w}$ on ethanol concentration for two samples of calf thymus DNA: $\square$ - molecular weight 350,000 Dalton, $\circ$ - molecular weight $4.4 \cdot 10^6$ Dalton.

obtained data for *Tetrahymena pyriformis* DNA (4) , whose CD spectra remain practically unchanged within 75 - 80% ethanol, though this DNA, like others aggregates under appropriate conditions. Besides, as shown in Fig.3, an increase in the molecular weight of DNA causes the dependence curve of $S_{20,w}$ on ethanol concentration to shift towards lower ethanol concentrations, but the $B \rightarrow A$ transition curves for these preparations (Fig.2) coincide. Hence, at high molecular weights of DNA (680,000 or more) the aggregates may appear almost simultaneously with the beginning of the $B \rightarrow A$ transition, but, the $B \rightarrow A$ transition curves themselves remain unchanged.

Our results show that it is possible to obtain an ethanol-water solution of DNA at high concentrations of ethanol without DNA aggregates by using low-molecular-weight preparations of DNA under appropriate ionic conditions with a special method of solution mixing. The absence of any changes in the $B \rightarrow A$ transition curves for such preparations as compared with those containing aggregates attests that changes in the CD spectra of DNA on adding ethanol up to 80% are not associated with an aggregation process but reflect the $B \rightarrow A$ transition and, consequently, the CD spectrum of DNA in 80% ethanol solution corresponds to the A-conformation of DNA in solution.

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