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The Influence of Alcohol–Water Solvents on the Conformation of Deoxyribonucleic Acid

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Synopsis

The influence of alcohol–water solvents on the conformation of native DNA was studied by the methods of flow birefringence and viscometry. Conformational transitions of DNA were observed at low alcohol concentrations corresponding to the destruction of the water spatial structure. A change in the secondary structure of the DNA molecule was observed at high ethanol concentrations and is discussed in the paper.

INTRODUCTION

The study of environmental influences on the configuration of macromolecules allows one to consider the nature of the forces stabilizing their structure. X-ray diffraction studies of DNA fibers have shown that the macromolecular secondary structure depends on the DNA source, ionic content, and relative humidity of the sample.[1–4] Irrespective of the DNA source and type of counterions, at a relative humidity $\geq 95\%$ and in aqueous salt solutions, the DNA molecule assumes a B form. Changes in the temperature (or pH) of the solution result in the conformational transition within the DNA molecule due to the destruction of its secondary structure. Various aspects of this transition have been studied extensively.[5–7]

Conformational changes of the DNA molecule under the influence of different solvents are of considerable interest. Additional information about the nature of forces stabilizing the structure of the double-helical DNA molecule would be obtained. It seems reasonable to use mixed-type solvents with water as one of the components. Low-molecular-weight alcohols may be used as the second component because of their ability to mix well with water and their low polarity.

The influence of alcohol–water solvents on the hydrodynamical and optical parameters of the DNA molecule has been studied.[8–10] It was concluded that the denaturing effect of the alcohols on DNA is reversible. There are also some data regarding changes in the DNA circular dichroism spectra in the water–ethanol mixtures.[11–13] From the comparison of the circular dichroism spectra of DNA in films at different humidities[14] with

those in water–alcohol solvents it was concluded that at ~80% v/v of ethanol and 5×10^{-4} M NaCl the DNA molecule assumes an A form.[12] However, the authors[13] assume that the CD spectra for DNA in 80% v/v ethanol are distorted by the scattering of light due to aggregation of the DNA molecules. It follows that the question about the the DNA transition to A form at high ethanol concentration is still open to discussion.

In the present paper the influence of alcohol–water solvents on the DNA conformation has been studied by the methods of flow birefringence and viscometry, i.e., methods that give information about the secondary and tertiary structures of the DNA molecule.

MATERIALS AND METHODS

The calf thymus sodium salt of DNA with a molecular weight of 28×10^6 estimated by its intrinsic viscosity in a 0.15 M NaCl,[15] was used. Water–alcohol DNA solutions were obtained by mixing a water solution of DNA with alcohol, both containing equal quantities of Na^+ ions. Alcohol was added drop-wise into a DNA solution, which was stirred continuously. All the solutions used were filtered by means of a glass filter. The DNA concentration C_{DNA} was determined by the difference in absorption of the solution at $\lambda = 270$ nm and 290 nm after hydrolysis with 6% $HClO_4$.[16] The molar extinction coefficient $E_{260}(P)$ was estimated for each system. Only solutions with $E_{260}(P)$ corresponding to the native DNA state were used in these experiments.

The dependence of relative viscosity of the solution on the flow rate gradient g was studied on a modified Zimm and Crothers viscometer.[17] The intrinsic viscosity

$$[\eta] = \lim_{\substack{g \to 0 \\ C_{DNA} \to 0}} [(\eta_r - 1)/C_{DNA})]$$

was estimated for each system of a given alcohol content in the solvent and ionic strength μ.

The optical apparatus with an elliptic compensator and photoelectric reading was used to measure the flow birefringence.[18,19] For all the solutions studied the Δn flow birefringence value was negative and increased proportionally with g in the range of rate gradients used for the experiments. The dynamooptical constants $[n] = \lim_{\substack{g \to 0 \\ C_{DNA} \to 0}} [(\Delta n/g)/(C_{DNA}\eta_0)]$ where η_0 is the solvent viscosity, were determined. The values C_{DNA} were in the range 5×10^{-5}–3×10^{-6} (g/cm^3), depending on the ionic strength of the solutions studied.

It is known that the $[n]/[\eta]$ ratio is proportional to the difference in the polarizabilities $(\gamma_1 - \gamma_2)$ of the macromolecule in the solution. The value of $(\gamma_1 - \gamma_2)$ depends on both the inner optical macromolecule anisotropy $(\gamma_1 - \gamma_2)_i$[20] and the macroform anisotropy $(\gamma_1 - \gamma_2)_f$ emerging from anisotropic polarization of asymmetric particles in the electric field of the light wave.[21,22]

Various investigations show that the $(\gamma_1 - \gamma_2)_f$ value may be neglected when compared with $(\gamma_1 - \gamma_2)_i$ for DNA molecules in water solutions.[23,24] This can be judged by the absence of the concentration dependence of

$$\lim_{g \to 0} \left\{ \frac{(\Delta n/g)}{[\eta_0(\eta_r - 1)]} \right\}$$

and its coincidence with $[n]/[\eta]$.[25,26] The same relationships are also observed for alcohol-water solutions of DNA, as shown from the data obtained in the present work. As an example the result of measurements for one of the systems studied are presented in Fig. 1.

For linear macromolecules $(\gamma_1 - \gamma_2)_i \sim (a_\parallel - a_\perp)\, S$, where $a_\parallel$ and $a_\perp$ are the polarizabilities of the monomer residue in parallel and perpendicular directions towards the chain, and S is the number of the monomer residues in the Kuhn segment.[27] It follows that the value $[n]/[\eta] \sim S(a_\parallel - a_\perp)$ depends on the secondary structure of the DNA molecule. For DNA, $S = A/3.4$, where A is the length of the Kuhn segment.

In measurements of the concentration dependence of the parameters studied the isoionic dilution was maintained. The activity of the ions in the solvent for dilution coincided with that in the stock solution.[28,29] All the measurements were performed at 21°C.

RESULTS AND DISCUSSION

The dependence of the intrinsic viscosity of DNA on the percent of ethanol and *tert*-butanol in the solvent at different ionic strengths is presented in Figures 2 and 3. The concentration in vol % was chosen for ethanol (Figure 2) and in mol % for *tert*-butanol (Figure 3). In the text

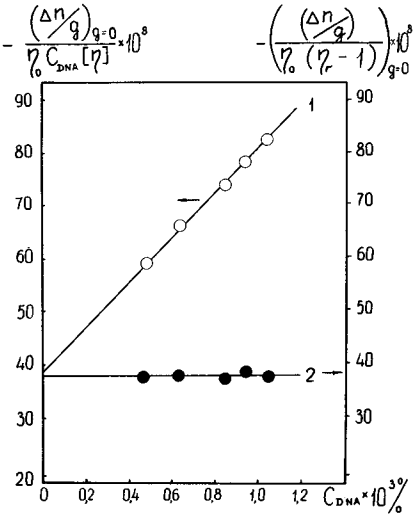

Fig. 1. Dependence of $(\Delta n/g)_{g=0}/(C_{DNA}\eta_0[\eta])$ on C_{DNA} (*1*) and $\{(\Delta n/g)/[\eta_0(\eta_r - 1)]\}_{g \to 0}$ on C_{DNA} (*2*) for DNA solution at 5.85% of *tert*-butanol, $\mu = 0.001$.

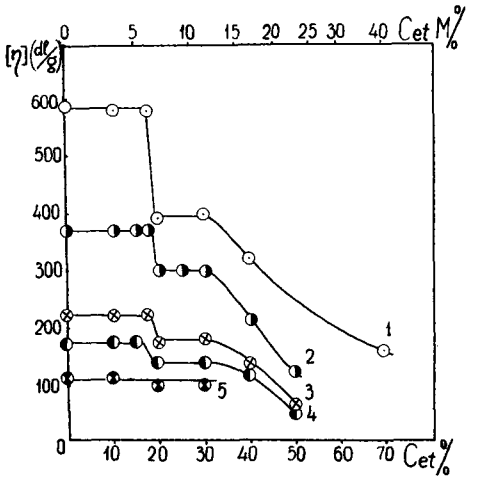

Fig. 2. Dependence of $[\eta]$ for DNA on the percent of ethanol (vol %) in the solvent at different ionic strengths: *1*—4×10^{-4}; *2*—1×10^{-3}; *3*—4×10^{-3}; *4*—1×10^{-2}; *5*—1×10^{-1} *M* NaCl. The upper scale corresponds to mol % of ethanol.

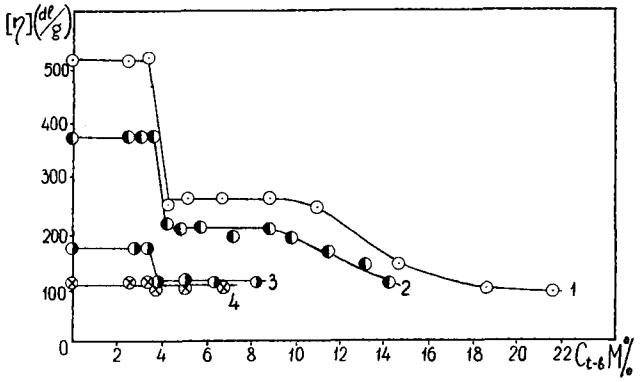

Fig. 3. Dependence of $[\eta]$ for DNA on the percent of *tert*-butanol (mol %) in the solvent at different ionic strengths: *1*—5×10^{-4}; *2*—1×10^{-3}; *3*—1×10^{-2}; *4*—1×10^{-1} *M* NaCl.

we use only molar concentrations. As is seen from Figures 2 and 3, the dependence of $[\eta]$ for DNA on the ethanol and *tert*-butanol concentration is similar. The value $[\eta]$ for DNA remains constant over a certain range of alcohol concentrations. When the molar ethanol concentration reaches ~7% and that of *tert*-butanol ~4% there is a cooperative decrease of $[\eta]$, which is greater in solutions of lower ionic strength μ. After the cooperative decrease, the value $[\eta]$ for DNA again remains constant in the range of the molar concentrations $C_{et} \sim$ 7–12%, $C_{t\text{-}\beta} \sim$ 4–9%. Further addition of alcohol leads to a gradual decrease of $[\eta]$.

It must be noted that at high alcohol concentrations a partial DNA precipitation is observed, especially in solutions of high ionic strength.

This is evidenced by the value of C_{DNA} in solution. We used the range of *tert*-butanol concentrations, where no DNA precipitation was observed. For ethanol-water solutions of DNA a wider range of alcohol concentrations was used and a noticeable DNA precipitation occurred at $C_{et} > 40\%$.

The dependence of $[n]/[\eta]$ for DNA on the alcohol content in the solvent is presented in Figures 4 and 5. Actually, as shown in these figures with a wide range of alcohol concentrations, the alcohol does not influence the value of $[n]/[\eta]$. The DNA solutions with high ethanol content (46 and 57.7%) were studied. Because of a considerable DNA precipitation and small values of η_r at ethanol molar concentrations >40%, one cannot obtain the dependence of $(\eta_r)_{g=0}$ on C_{DNA}, which is necessary for determining the value of $[\eta]$. That is why $(\eta_r)_{g=0}$ and $(\Delta n/g)_{g=0}$ were measured at 46.0 and 57.7% of ethanol and $\mu = 10^{-3}$ for only one DNA concentration. As has been already mentioned the value $\{(\Delta n/g)/[\eta_0(\eta_r - 1)]\}_{g\to 0}$ measured at finite DNA concentration in solution equals $[n]/[\eta]$ (Figure 1). This relationship can be used for estimating $[n]/[\eta]$ of DNA at 46 and 57.7% ethanol. At such values of ethanol concentration the absolute values of $[n]/[\eta]$ are reduced threefold (Figure 4).

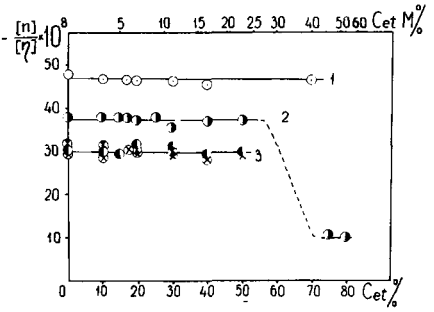

Fig. 4. $[\eta]/[\eta]$ dependence on the percent of ethanol (vol %) in the DNA solution at different ionic strengths: *1*—4×10^{-4}; *2*—1×10^{-3}; *3*—4×10^{-3} (⊗), 1×10^{-2} (◐), 1×10^{-1} (⧗) *M* NaCl. The upper scale corresponds to mol % of ethanol.

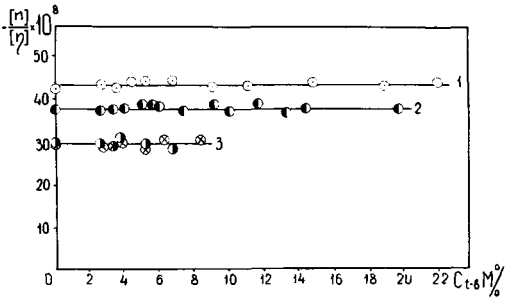

Fig. 5. $[\eta]/[\eta]$ dependence on the percent of *tert*-butanol (mol %) in the DNA solution at different ionic strengths: *1*—5×10^{-4}; *2*—1×10^{-3}; *3*—1×10^{-2} (◐), 1×10^{-1} (⊗) *M* NaCl.

The experiments have shown that the values $[n]/[\eta]$ in the whole *tert*-butanol concentration range and in the range of C_{et} <40% coincide with $[n]/[\eta]$ for DNA in water solutions. It means that the presence of alcohol in the solvent exerts no influence on the value of Kuhn segment (on the short-range interactions in the DNA chain) and on the $(a_{\parallel} - a_{\perp})$. Therefore the observed dependence of $[\eta]$ for DNA on the alcohol concentration in the solvent (Figures 2 and 3) is caused by the alteration of the long-range interactions in the DNA chain. Since alcohol is a thermodynamically poor solvent for DNA, one may expect a monotonous fall of $[\eta]$ with an increase in alcohol concentration in the solution. However the dependence of $[\eta]$ for DNA on C_{et} and $C_{t\text{-}\beta}$ suggests a somewhat more complicated case. We propose that the behavior of the DNA molecule is essentially connected with the alteration of the water structure by the alcohol.

The physical properties of alcohol–water mixtures are known to show a complicated dependence on the alcohol concentration. At certain alcohol concentrations (which depend on the chain length and branching of the hydrocarbon portion of the alcohols) there are extremes of isotropic light scattering intensity,[30] of the position of the maximum ($\nu = 5180$ cm^{-1}) of infrared absorption spectra,[31,32] of ultrasonic absorption and velocity,[33] and of specific heat,[34] of the alcohol–water mixtures. This is observed for ethanol–water and *tert*-butanol–water mixtures at the molar concentrations $C_{et} \approx 7\%$ and $C_{t\text{-}\beta} \approx 3.5\%$ and is explained by the disruption of the ordered spatial water structure. The endothermic maximum of partial molar heat for dissolving different organic compounds in ethanol–water mixtures is explained by the same reason.[35] Lower concentrations of alcohol stabilize the water structure.[32,36]

Figures 2 and 3 show that the stabilization of the water structure by low alcohol concentrations exerts no influence on the value of $[\eta]$ for DNA. This may be explained by the high stability of the molecular structure of DNA in water solution. When the molar concentration of ethanol reaches ~7% and that of *tert*-butanol reaches ~4% there is a cooperative decrease of $[\eta]$, which is connected with the conformational transition in the tertiary structure of the DNA molecule. Comparison of these data with the data on the properties of alcohol–water mixtures undoubtedly shows that the structural transition in the solvent exerts a considerable influence on the conformation of the DNA molecule. However, at present it is rather difficult to express an unambiguous opinion about the mechanism whereby structural changes in the solvent affect the hydrodynamic behavior of the DNA molecule. One may suppose that at the above-mentioned alcohol concentrations, when the ordered spatial water structure becomes disrupted, there is a cooperative change of long-range electrostatic interactions between the phosphate groups of the DNA chain and hence the macromolecular dimensions. This assumption accounts for the diminution of the influence of alcohol at high μ, when charged DNA groups are essentially shielded. To some extent it correlates with the results of Spivey and Shedlovsky,[37] where it was shown that the mobility of Na^+ ions in water–ethanol mixtures attains the extreme value at about 7% of ethanol.

Our experiments have shown that the value $[\eta]$ for DNA remains constant over a certain range of alcohol concentrations ($C_{et} \sim 7$–12%, $C_{t\text{-}\beta} \sim 4$–$9\%$). We assume that in this range of alcohol concentrations there are regions of structured water, which do not depend on the alcohol content. The same conclusion was drawn on the basis of investigation of the infrared absorption band ($\nu = 5180\ cm^{-1}$) in alcohol–water mixtures.[32]

It is likely that at alcohol concentrations $C_{et} > 12\%$, $C_{t\text{-}\beta} > 9\%$ the water structure undergoes considerable destruction. In this region the addition of alcohol leads to a monotonous fall of $[\eta]$ for DNA due to the poor quality of the solvent. We might assume that the conclusion about the influence of the solvent structure on the conformation of the DNA molecule is out of the question. However the molecular nature of this influence requires further investigations.

It is interesting to note that the slightest addition of urea (0.003%) to the water solution of DNA leads to a decrease of $[\eta]$. At the molar concentration of urea $\sim 0.15\%$ and $\mu = 0.5 \times 10^{-3}$ the value $[\eta]$ for DNA decreases twofold. This result correlates with the assumption that urea intensively disrupts the water structure.[38]

Let us consider the results of investigation of the DNA solutions at high ethanol content ($C_{et} = 46\%$ and 57.7%) and $\mu = 10^{-3}$ (%). A considerable DNA precipitation was observed in these solutions. The DNA concentration was determined after filtration of the DNA solution; in different experiments it was in the range $(1.1$–$1.2) \times 10^{-3}$ (%). Relative viscosities of these solutions were considerably lower than those of solutions of the same C_{DNA} and μ, but lower alcohol content ($C_{et} \leq 40\%$). The small value of η_r of the stock solution does not permit us to study the dependence of η_r on C_{DNA}, necessary to determine the value of $[\eta]$ for DNA.

The decrease of the viscocity of the DNA solution at high alcohol concentrations may be due to various factors. The decrease of the molecular weight of DNA in solution, due to its partial precipitation, may play a considerable role, as this process is accompanied by the fractioning of the polymer and precipitation of high-molecular fractions. In our experiments, at maximum DNA precipitation, the DNA molecular weight ($M = 28 \times 10^6$ daltons) changes approximately two times. This can be seen from the intrinsic viscosity of the DNA measured in solution dialyzed from ethanol ($C_{et} = 57.7\%$). Nevertheless, it does not compensate for the observed viscosity change of these solutions, which may be accounted for by changes in both the long-range and short-range interactions in the DNA molecule. It is worth noting that in some cases these effects can be separated from each other by the value of $[n]/[\eta]$ which does not depend on the long-range interactions in the macromolecule.

As has been already mentioned, $[n]/[\eta]$ for DNA at $C_{et} = 46$ and 57.7% was determined with the help of the relationship

$$[n]/[\eta] = \lim_{g \to 0} \left\{ \frac{(\Delta n/g)}{[\eta_0(\eta_r - 1)]} \right\}$$

The validity of the latter can be seen from Fig. 1.

Data of Figure 4 show that at high ethanol concentration (46 and 57.7%) the absolute value of $[n]/[\eta]$ is reduced threefold. It should be noted that the possible decrease of the molecular weight of the DNA due to its partial precipitation would not influence the value of $[n]/[\eta]$, since $[n]/[\eta]$ depends on the DNA molecular weight only at $M \leq 2 \times 10^6$ daltons.[39] Thus, the observed decrease of $[n]/[\eta]$ may be the result of the decrease of either the persistent length of the DNA molecule or of $(a_\parallel - a_\perp)$ with a maximum value for DNA in the B form.

Some authors believe that under the above conditions the DNA molecule assumes an A form;[12] others think it necessary to take into consideration the scattering of light due to aggregation of DNA in the investigations of DNA circular dichroism before reaching any conclusions.[13] The aggregation of DNA would have been unlikely in the studies by Ivanov et al.[12] where the molecular weight was $\sim 10^6$ daltons.

Since in the range of high molecular weights the value of $[n]/[\eta]$ for DNA does not depend on M, the possible DNA aggregation in the solutions studied (after filtration) exerts no influence on the value of $[n]/[\eta]$. To find out whether the decrease of $[n]/[\eta]$ is connected with the B → A transition, we estimated the change of $(a_\parallel - a_\perp)$, corresponding to this transition.

The decreased contribution in $(a_\parallel - a_\perp)$ of the DNA base pairs at B → A transition can be estimated with the help of the following relationship

$$(a_\parallel - a_\perp)_A' = (a_\parallel - a_\perp)_B' \times (3\cos^2\beta - 1)/2 \qquad (1)$$

where β is the angle between normal to base pair planes of the DNA in the B and A forms. One can evaluate the polarizability difference of the monomer residue for DNA in A form, assuming that the positive contribution in $(a_\parallel - a_\perp)$ of the polarizabilities of the valent bonds of the main chain does not change at the B → A transition, $(a_\parallel - a_\perp)_B' = 18.5 \times 10^{-24}$ cm^3 being known. The evaluation has shown that $(a_\parallel - a_\perp)_A$ is approximately 1.5 times lower than $(a_\parallel - a_\perp)_B$. It follows that the decrease of $(a_\parallel - a_\perp)$ at the B → A transition does not compensate for the observed change of $[n]/[\eta]$. If the decrease of $[n]/[\eta]$ is connected with the B → A transition, it must be assumed that the DNA molecule in the A form is more flexible. Small values of η_r correlate to some extent with this assumption. However, it is worth noting that at $C_{et} = 46$ and 57.7%, $\mu = 10^{-3}$ the value of $E_{260}(\mathrm{P}) \simeq 7100$ but at $C_{et} \leq 40\%$ and in DNA water solutions of the same ionic strength $E_{260}(\mathrm{P}) \leq 6800$. It is not clear whether the increased value of $E_{260}(\mathrm{P})$ indicates partial DNA denaturation, which may result in the decrease of $[n]/[\eta]$. Probably the increase of $E_{260}(\mathrm{P})$ is connected with the conformation transition in the DNA molecule.

We conclude that the results of investigations of DNA solutions at low alcohol concentrations clearly show the influence of the water structure on the tertiary structure of the DNA molecule. The secondary structure of the macromolecule appears to be insensitive to the structural alterations in the solvent. In the range of the mean alcohol concentrations (12% <

$C_{et} < 40\%$; $9\% < C_{t\text{-}\beta} < 22\%$), when the water structure is essentially disrupted, the fall of $[\eta]$ for DNA is due to the decrease of the volume effects. This is evidenced by the constancy of $[n]/[\eta]$ dependence on the alcohol content. The supposed transition of the DNA molecule into the C form at the ethanol concentrations used by Girod et al.[13] would exert no influence on the value of $[n]/[\eta]$. Probably, it is due to the small change of $(a_{\parallel} - a_{\perp})$ at the B → C transition [Eq. (1)].

A conformational transition in the secondary structure of DNA is observed at high ethanol concentrations (C_{et} = 46 and 57.7%). Our data do not permit us to express an unambiguous opinion about the nature of this transition.

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