BIOPOLYMERS VOL. 13, 2227–2240 (1974)

DNA Conformation in *N,N*-Dimethyl Formamide–H_2O Solutions

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Synopsis

Optical density, viscosity, and light scattering measurements for calf thymus DNA in water–*N,N* dimethyl formamide (DMF) solutions are presented. DMF content varied between 0 and 60% (v/v) and DNA molecular weight varied between 15×10^6 and 0.5×10^6. Complementary measurements of the solubility of adenine, thymine, guanine, and cytosine in H_2O–DMF mixtures are presented. The denaturation temperature of DNA, manifested by about a 35% increase of optical density, is gradually depressed by increasing DMF content. However, a significant increase of OD occurs even before (and even after) the denaturation point, when DMF content is increased isothermally. The intrinsic viscosity also exhibits a large decrease when DMF content is increased both before and after the denaturation point. Light scattering data for high-molecular-weight DNA in the predenaturation range indicate a decrease of the mean-square radius and a constant molecular weight on increasing DMF content. The results, interpreted in terms of the wormlike chain of Kratky and Porod, indicate a large decrease of the persistence length of DNA. For low-molecular-weight DNA, radius and molecular weight increase with DMF content, indicating intermolecular aggregation. The formation of compact structures of native DNA is discussed in terms of an increased solubility of uncharged bases, and a decreased solubility of phosphate and deoxyribose groups, when a less polar environment is provided by the addition of DMF.

INTRODUCTION

It is known that the addition of organic solvents such as aliphatic alcohols, formamide, *N,N*-dimethyl formamide (DMF), etc, to aqueous solution of deoxyribonucleic acid (DNA) may cause the disorganization (denaturation) of the double-helical conformation of DNA.[1–7] The mechanisms by which organic solvents reduce DNA stability have not, however, been adequately clarified.

In an attempt to extend to polynucleic acids our work on the denaturation of biopolymers in organic solvent–water solutions,[8–10] we observed some conspicuous DMF-induced alterations of optical and hydrodynamic properties of DNA. These alterations took place in a temperature region distinct

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from that where the main denaturation occurs and may therefore be regarded as predenaturation, or postdenaturation, changes of DNA conformation. Aside from their relevance to the mechanism of denaturation, these effects are of interest in connection with the occurrence of modifications of the native structure of DNA. Compact conformations of nondenatured DNA may occur in systems of biological interest, particularly when interactions between proteins and DNA are involved.[8,11]

EXPERIMENTAL

Material

The following calf thymus DNA samples were used (Table I). Sample A was purchased from Calbiochem (lot 802184). The molar extinction coefficient was determined according to Carrara and Bernardi.[12] The value was $\epsilon(P) = 6500$ cm²/mol P in H_2O at 260 nm. Protein content (determined according to Lowry et al.[13]) was less than 0.7%. Samples B and C were obtained by sonication (cf. below) of sample A. Sample D (derived from sonication of an original sample having a molecular weight about 6×10^6), was kindly supplied by Dr. C. Strazielle of the Centre de Recherches sur les Macromolecules at Strasbourg (sample V268) and had $\epsilon(P) = 6560$ cm²/mol P at 260 nm, and protein content less than 0.7%.

Stock solutions of DNA in water were prepared by dissolving DNA in an adequate amount of 0.1 *M* LiCl solution in order to obtain a DNA concentration of about 1 mg/ml. To facilitate DNA solubilization, moderate stirring at 4°C was performed by rotation of the flask containing the solution. Mixing DMF and H_2O is an exothermic process and care had to be used in order to avoid DNA degradation. One method for preparing solutions in mixed solvents (used particularly for sample A) consisted of first mixing 0.1 *M* LiCl in DMF with 0.1 *M* aqueous LiCl, followed by slow addition of an adequate amount of stock DNA solution while the two solutions were kept under refrigeration. Alternatively, a weighted amount of DNA was directly dissolved in the appropriate mixed solvent. In the latter case, the final concentration was determined using a molar extinction coefficient appropriate for the particular mixed solvent used. pH was adjusted by addition of 0.1 *M* LiOH. Composition of the mixtures is given on volume basis (v/v).

TABLE I
Characteristics of Samples Investigated

Sample	Source	Sonication	Molecular Weight in H_2O
A	Calbiochem (802184)	None	15×10^6 ($[\eta]$)
B	Calbiochem (802184)	20 kHz; 32.4 W/cm²; 30 sec	6.4×10^6 (light scattering)
C	Calbiochem (802184)	20 kHz; 32.4 W/cm²; 2 min	8.6×10^5 ($[\eta]$)
D	C.N.R.S. Strasbourg	20 kHz; 32.4 W/cm²; 4 min	4.6×10^5 (light scattering)

Sonication was accomplished with a Mullard 3100 apparatus at the frequency of 20 kHz, power 32.4 W/cm^2. Irradiation time is indicated in Table I. Solutions were kept at low temperature ($\sim$10°C) during irradiation. The sonicated solution contained DNA in distilled water, 0.1 *M* LiCl, pH 7, concentration $\sim$1 mg/ml. Following sonication, solutions were dialyzed against water so that the DNA solution was 0.01 *M* with respect to LiCl. Samples were then lyophilized and used directly (when necessary the sample, or the solution, was stored overnight at 4°C). No alteration of pH or optical density (OD) resulted from sonication.

Adenine, thymine, guanine, and cytosine bases used for solubility measurements were obtained from Fluka.

Optical Density

Optical density was measured at different temperatures using a Zeiss PMQ II spectrophotometer. Temperature control was achieved by water circulation around a water jacket surrounding the cells. 10-min equilibrations were allowed at each temperature before taking the OD reading. Since DMF absorbs in the same wavelength interval in which DNA has its maximum absorption, it was important to assure that the amount of DMF in the solvent and the solution cells were exactly the same. This was accomplished by carefully selecting quartz cells (1 × 1 cm) with equal optical path length, and by determining the DMF–H_2O composition with accurate volumetric techniques. Moreover, cells were hermetically closed in order to prevent alteration of diluent composition during measurements. The reproducibility of OD data for duplicate measurements was within ±0.002. Independent tests were performed in order to confirm that the position of maximum absorption of DNA (260 nm) remained the same when DMF concentration was changed between 0 and 60% at 14°C.

Viscosity Measurements

Reduced specific viscosity η_{sp}/c was measured using a capillary viscometer of the Ubbelohde type, equipped with four measuring bulbs at several distances above efflux point. These bulbs define average gradients from 60 to 15 sec^{-1}. Viscosity of filtered solutions (no fractionation of DNA occurred during clarification) was measured at four values of shear rate and the value at zero rate of shear was extrapolated from linear plots of η_{sp} versus gradient. The value thus obtained was plotted in a η_{sp}/c versus c plot (at four different DNA concentrations) and the value of intrinsic viscosity $[\eta]$ extrapolated. Polymer concentration was between 0.02 and 0.09 mg/ml for sample A at 14°C, between 0.15 and 0.3 mg/ml for sample A at 61°C, and between 0.1 and 0.5 mg/ml for sample C. Temperature control was precise to ±0.05°C.

Solubility of Purine and Pyrimidine Bases

Solubility of uncharged adenine, thymine, guanine, and cytosine was determined at 25°C in 0.1 *M* LiCl aqueous solutions containing 3 and 5%

DMF. Solubility of guanine and thymine was measured in the presence of 0.02 M CH_3COOK-CH_3COOH buffer at pH 5.5. Solubility of cytosine and adenine was measured in the presence of 0.02 M tris(hydroxymethyl) amino methane buffer at pH 8 and 7.2, respectively. Equilibration was obtained by rotating hermetically closed test tubes containing a base precipitate in a thermostat for about a week. After equilibration was reached, suitable aliquots of saturated solution were analyzed for base content using the OD technique.

Light Scattering Measurement

Light scattering data were obtained using a Sophica model B photometer. Measurements were made at 25°C at $\lambda = 5460$ Å. Purification of the solution was achieved by centrifugation at 14,000 rpm for 2 hr (no fractionation of DNA occurred during centrifugation). Transfer of the clarified solution to the light scattering cell was performed according to the procedure described by Fröelich et al.[14] Scattered intensity was measured for the solvents (H_2O and 40/60 DMF–H_2O LiCl 0.1 M, pH 7) and for four DNA concentrations (between 3 and 20 $\times 10^{-2}$ mg/ml for native DNA, and 20 and 120 $\times 10^{-2}$ mg/ml for the sonicated samples) in the angular range between $\theta = 30°$ and $\theta = 150°$.

The refractive index increment was measured at 25°C with a Zeiss interferometer at 5640 Å for DNA samples B, C, D in water and in 40/60 DMF–H_2O (LiCl 0.1 M, pH 7). The solution at the highest DNA concentration ($\sim$1 mg/ml) was dialyzed against the solvent. Dilutions were accomplished using as a solvent the same solvent in dialysis equilibrium with the solution at highest DNA concentration. Dialysis in H_2O was performed using dialysis tubes (A. H. Thomas Company, Philadelphia, Pa.) while for DMF–H_2O mixtures we used a glass apparatus with two compartments separated by a glass plate with porosity 5f (produced by Jena Glasswerk Scott & Gen. Veb.). Dialysis times were 2 days for H_2O and 22 hr for DMF–H_2O. Independent determinations showed that the conditions used were adequate to assure equilibrium.

RESULTS

Optical Density Data

Thermal denaturation data for DNA (sample A) in solutions containing different amounts of DMF are collected in Figure 1. About a 35% increase of OD (hyperchromism) occurs on increasing temperature irrespective of the DMF–H_2O composition. The helix–coil transition temperature T_D, taken as the temperature corresponding to the midpoint of the thermal denaturation curves in Figure 1, decreases with increasing DMF content, in line with previous results by Herskovits.[2]

The decrease of T_D with DMF content is reported in the phase diagram of Figure 2 where the fields of stability of double-helical form (H) and random

coil (RC) are indicated. Also included in Figure 2 is the equilibrium line indicating phase separation. The latter was determined by visually observing, at each temperature, the DMF concentration at which formation of a second phase (precipitate) occurred.

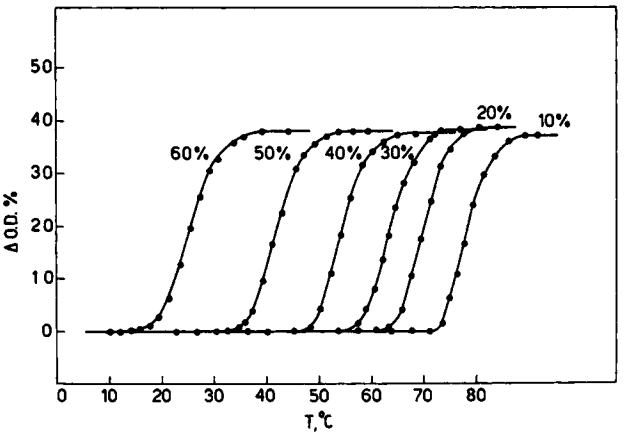

Fig. 1. Temperature variation of OD of DNA (sample A) in 0.1 *M* LiCl H_2O–DMF mixtures (pH 7) of the indicated composition. The quantity in ordinate, Δ OD is the OD observed at temperature *T* minus the corresponding value of OD at 10°C divided by the value of OD at 10°C.

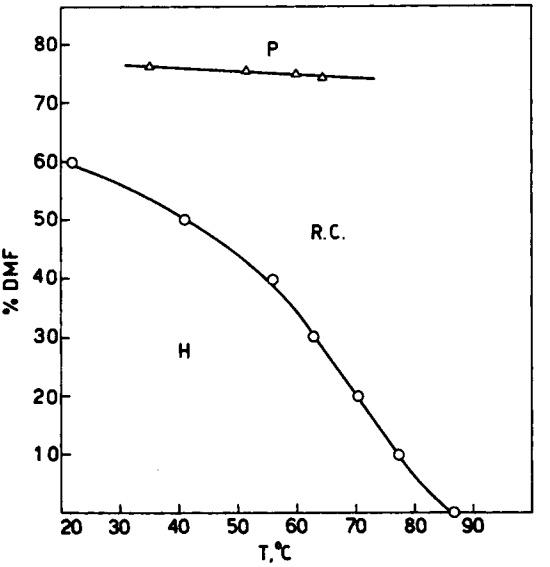

Fig. 2. Variation of the helix–coil transition temperature for DNA (sample A) with DMF content in 0.1 *M* LiCl solutions (pH 7). The field of stability of the double helix (H) and random coil (RC) is indicated. The upper line represent the points of phase separation (RC → P, DNA concentration 0.1 mg/ml).

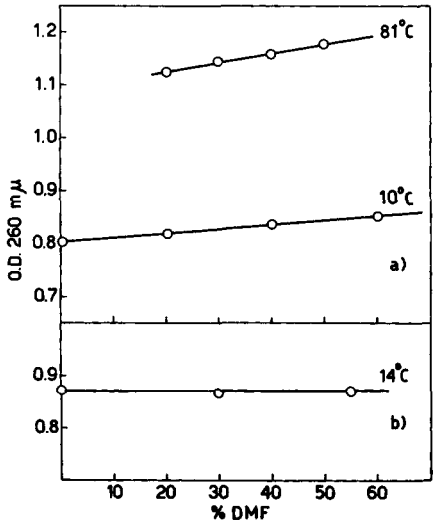

Fig. 3. *a* Isothermal variation of the optical density of DNA (sample A) in 0.1 *M* LiCl aqueous solutions (pH 7) containing increasing amount of DMF. The temperature is indicated. *b* Same plot as in *a* for an equimolecular mixture of the four bases adenine, thymine, guanine, and cytosine (pH 7, 0.1 *M* LiCl).

The type of plot in Figure 1 does not reveal an effect of DMF content on the OD of DNA, which is, instead, evidenced from the data collected in Figure 3*a*. For these data, the concentration of DNA was kept rigorously constant. It is seen that the OD of both native (10°C) and denatured (81°C) DNA increases slowly with increasing DMF content. The fact that the curves at 10° and 81°C are nearly parallel accounts for the apparent constancy of the hyperchromic effect depicted in Figure 1. The effects evidenced in Figure 3*a* were shown to be fully reversible upon reducing the DMF content by dilution, accounting for the change in polymer concentration. In Figure 3*b* we report the effect of DMF on the OD of an equimolecular mixture of the four bases, adenine, thymine, cytosine, and guanine. It is seen that no increase of OD occurs on increasing DMF concentration when the bases are not connected in a nucleic chain.

Viscosity Data

For DNA having a molecular weight less than 16×10^6 Eigner et al.[15] have shown that a capillary viscometer with a rate of shear in the order of 30 sec^{-1} allows a satisfactory extrapolation of the viscosity to zero gradient.

The variation of intrinsic viscosity for DNA samples A with DMF concentration is reported in Figure 4*a*. A particularly large reduction of $[\eta]$ by increasing DMF concentration is observed at 14°C. By comparing the range of temperatures and DMF composition pertinent to Figure 4 with the data in Figure 2, it is evident that the viscosity data of the isotherm at 14°C refer to alterations occurring in the native state of DNA. The latter data reveal, therefore, predenaturation alterations of DNA conformation.

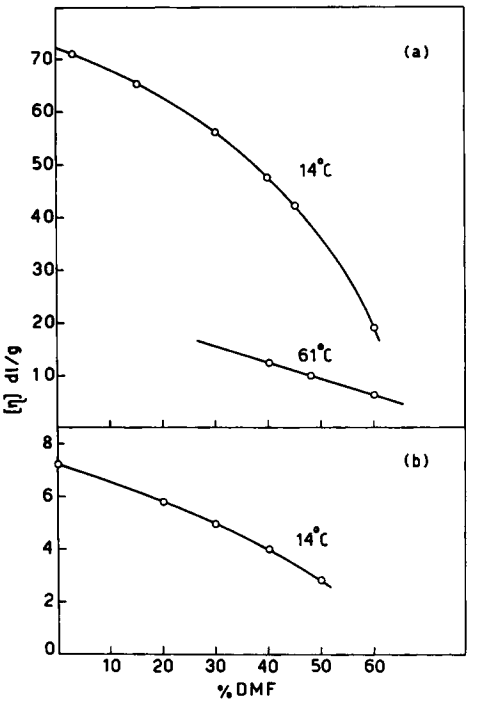

Fig. 4. *a* Variation of the intrinsic viscosity of DNA sample A with DMF content (0.1 *M* LiCl, pH 7). The predenaturation behavior is indicated by the isotherm at 14°C. The postdenaturation behavior is indicated by the curve at 61°C. *b* Same for sample B (at 14°C).

Likewise, comparison of data in Figure 4 and Figure 2 indicates that the change of $[\eta]$ with DMF concentration reported at 61°C refers to post-denaturation alterations of the (RC) DNA conformation. As an additional support to the above conclusions, we verified that (considering, for instance, thermal denaturations at 40 and 60% DMF) the change in reduced specific viscosity upon denaturation was in line with the difference between the values of $[\eta]$ reported, at given DMF composition, in Figure 4*a*. Also included in Figure 4*b* is the variation of intrinsic viscosity with DMF content for sample C. The effect of DMF on $[\eta]$ is similar to that observed for sample A.

In order to assess the influence of electrostatic effect on the intrinsic viscosity of DNA, we have measured the variation of $[\eta]$ with LiCl content for DNA in water at 14°C, using the same procedure used in the case of DMF–H_2O mixtures. The data revealed that while a reduction of $[\eta]$ from 90 to 72 dl/g occurred upon increasing LiCl content from 0.01 to 0.1 *M*, the value of $[\eta]$ up to 0.5 *M* LiCl was practically constant, and about 70 dl/g.

In order to rule out any possible contribution of irreversible effects to the large decrease of viscosity reported in Figure 4*a*, a concentrated solution (~1 mg/ml) of DNA in 0.1 *M* LiCl 30/70 DMF–H_2O was diluted with a

0.1 M 30/70 DMF–H_2O solution to the concentration range usually employed for viscosity measurements. The value of $[\eta]$ at 14°C of this solution coincided with the value indicated in Figure 4*a*. Another aliquot of the concentrated solution was instead diluted with a 0.1 M LiCl aqueous solution so that DMF composition was only 3%. The intrinsic viscosity of this solution coincided with that reported in Figure 4*a* and obtained by increasing DMF concentration from 0 to 3%. The occurrence of degradative effects resulting from the shear gradient during viscosity runs was ruled out by the invariance of efflux times of solutions during repeated measurements.

Finally, values of $[\eta]$ in 0.2 M NaCl solution were used to determine M_w according to Eigner and Doty.[16] We found $M_w = 15 \times 10^6$ for sample A and $M_w = 8.6 \times 10^5$ for sample C.

Solubility of Bases

Solubilities of adenine, thymine, guanine, and cytosine in buffered H_2O and H_2O–DMF solutions are reported in Table II. The addition of DMF increases the solubility of the four bases. Similar effects were observed in water–alcohol and water–glycol mixtures.[35]

Light Scattering Data

The light scattering data were analyzed in terms of the equation[17]

$$\frac{Kc}{R_\theta} = \frac{1}{M_w} P_\theta^{-1} + 2A_2c. \tag{1}$$

M_w and c are the polymer molecular weight and concentration (g/ml), respectively; A_2 is the second virial coefficient; R_θ is the difference of scattered light $(I - I_0)$ of the solution and of the solvent times the factor $(\sin\theta/1 + \cos^2\theta) \cdot (\tilde{n}_0^2/\tilde{n}_b^2) \cdot (R_{90}^B/I_{90}^B)$, where $\tilde{n}_0$ and $\tilde{n}_b$ are the refractive indexes of the solvent and benzene, I_{90}^B is the scattered intensity for pure benzene at 90°, and R_{90}^B is the Raleigh ratio for benzene, which was taken equal to 15.8×10^{-6} cm^{-1} at 5460 Å.[18] Data in the literature[14] indicate that the usual determination of light scattering between 30° and 150° scattering angles is accurate for molecular weight up to about 6×10^6, and $\langle \rho^2 \rangle_z^{1/2}$ up to 2600 Å.

TABLE II
Solubility of Bases in Buffered Solutions. T = 25°C.

Base	Solubility in H_2O (0.1 M LiCl) mol/l.	Solubility in 3% DMF (0.1 M LiCl) mol/l.	Solubility in 5% DMF (0.1 M LiCl) mol/l.
Thymine (pH 5.5)	0.031	0.0348	—
Guanine (pH 5.5)	3×10^{-5}	3.2×10^{-5}	3.7×10^{-5}
Cytosine (pH 8)	0.0693	0.093	0.095
Adenine (pH 7.2)	0.0068	0.0115	0.0125

For small scattering angles the function P_θ^{-1} is[17]

$$P_\theta^{-1} \simeq 1 + \frac{16}{3}\frac{\pi^2\tilde{n}_0^2}{\lambda_0^2}\langle\rho^2\rangle_z\,{}^2\sin^2\frac{\theta}{2} \tag{2}$$

where λ_0 is the wavelength in the vacuum, and $\langle\rho^2\rangle_z$ is the z average of the mean-square radius of gyration independent, for small angles, from the shape of the macromolecule. For the calculation of the optical constant of the system[17]

$$K = \frac{2\pi^2\tilde{n}_0^2}{\lambda_0^4 N_A}\left(\frac{\delta\tilde{n}}{\delta c}\right)_\mu^2 \tag{3}$$

the refractive index increment of the solution $\delta\tilde{n}/\delta c$ was determined under conditions of constant chemical potential of all solvent components. As discussed by several authors, the light scattering behavior in multicomponent solvents allows the determination of both M_w[19-23] and $\langle\rho^2\rangle_z$[24] using the equations valid for one-component solvents in spite of preferential interactions of one of the solvent components with the polymer. The only modification to be introduced is that the refractive index increment should be measured at constant chemical potential of the solvent components, i.e., using the solvent in equilibrium dialysis with the solution. Values of $(\delta\tilde{n}/\delta c)_\mu$ were 0.173 and 0.134 in H_2O and 40/60 DMF–H_2O, respectively.

The Zimm plot[17] of the quantity Kc/R_θ as a function of $\sin^2\theta/2 + Fc$ (F is an arbitrary constant) is reported in Figure 5. The extrapolation of Kc/R_θ at $c = 0$ and $\theta = 0$ yields the value of $1/M_w$ while the value of $\langle\rho^2\rangle_z$ is obtained from the slope of the $(Kc/R_\theta)_{c=0}$ curve. Data obtained from the Zimm plot for the samples investigated are collected in Table III. It appears that for sample B, M_w is the same in H_2O and in DMF–H_2O solution, while $\langle\rho^2\rangle_z^{1/2}$ exhibits a decrease of about 20%. For sample D, however, both the values of the molecular weight and of the radius are more than twice the corresponding values measured in water. The virial coefficient is negative in the case of sample D in 40% DMF.

DISCUSSION

On the basis of the data in Figures 1 and 2, the denaturation of DNA in a 40% DMF solution occurs at about 50°C. Yet, optical density (Figure 3),

TABLE III
Light Scattering Data for DNA in DMF–H_2O Solutions

Sample	Solvent	M_w	$\langle\rho^2\rangle_z^{1/2}$(Å)	$A_2 \times 10^4$ (mol·cm³·g⁻²)
B	H_2O	6.45×10^6	2207	1.5
B	40% DMF	6.25×10^6	1764	0.22
D	H_2O	4.60×10^5	578	3.15
D	40% DMF	1.10×10^6	1287	−0.75

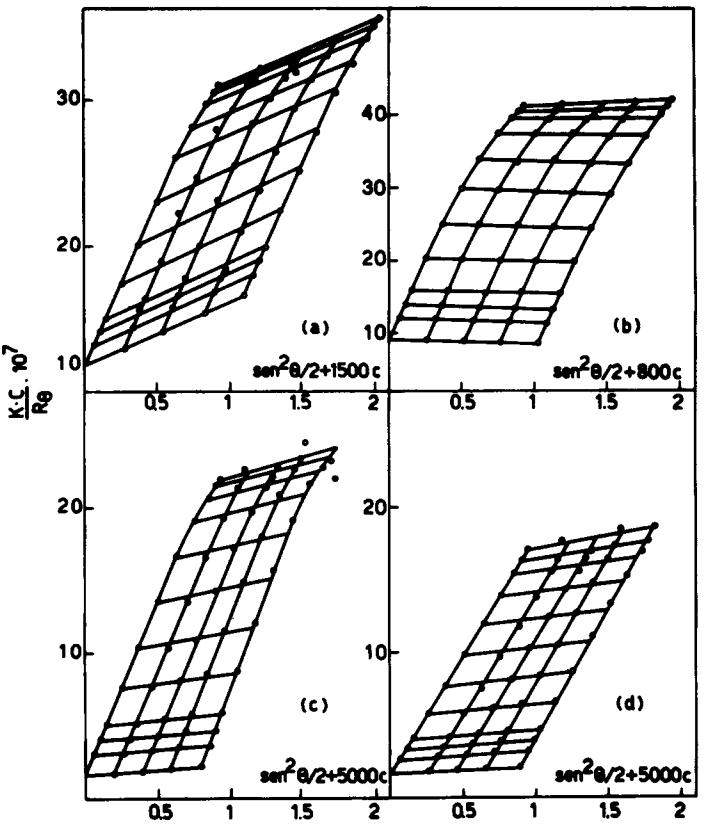

Fig. 5. Zimm plot of DNA in water and water–DMF mixtures 0.1 *M* LiCl, pH 7. *a* Sample D 100% H_2O. *b* Sample D 40/60 DMF–H_2O. *c* Sample B 100% H_2O. *d* Sample B 40/60 DMF–H_2O; T = 25°C.

viscosity (Figure 4), and light scattering data (Table III), obtained at temperatures significantly below 50°C reveal important (predenaturation) DMF-induced alterations of DNA conformation and aggregation. For the high-molecular-weight samples A and B, the alterations observed (i.e., about 5% increase of OD, 35% decrease of $[\eta]$, and 20% decrease of $\langle \rho^2 \rangle_z^{1/2}$, with respect to DNA in water) suggest that DMF favors compact DNA conformations characterized by a slight decrease of the regularity of base stacking. These alterations are essentially intramolecular, since no effect of DMF on the molecular weight of DNA (cf. sample B) is observed. In connection with the predenaturation spectral changes illustrated in Figure 3*a*, it is conceivable that local field effects might alter the OD of DNA. However, since no effect on the OD of the mixtures of free bases (Figure 3*b*) was observed, we believe that the effects observed are primarily associated with a decrease of base stacking.

The situation appears to be different when the low-molecular-weight samples (C and D) are considered. In the latter case (still considering a

40% DMF mixture), a reduction of $[\eta]$ comparable to that quoted above is observed. However, the values of radius and molecular weight of sample D are more than doubled, with respect to their values in water. Thus, significant intermolecular (predenaturation) aggregation may occur in the presence of DMF. Both the intramolecular effect observed with high-molecular DNA, and the intermolecular effect observed with low-molecular-weight samples appear to be reversible processes. Moreover, at variance with the denaturation process studied by others,[1-6] which occurs rather sharply when temperature or solvent composition is changed, the effects observed here occur continuously on a broad DMF range.

In addition to the above predenaturation alteration, DMF causes also some postdenaturation alterations of the random-coiled conformation stable above T_D. The decrease of $[\eta]$ at 61°C supports the occurrence of more compact random-coiled conformations when DMF content is increased. The occurrence of some aggregation is also suggested. In fact, the observed intrinsic viscosity is larger (by about a factor 2) than expected in terms of the intrinsic viscosity–molecular-weight dependence valid for denaturated DNA.[7,16] The increase of OD (cf. isotherm at 81°C, Figure 3) with increasing DMF content suggests a further decrease of (residual) base stacking.

The results concerning the alteration of the conformation of native DNA in DMF solutions may be put on a more quantitative basis in terms of the wormlike chain of Kratky and Porod.[25] The latter, as discussed by several investigators,[26-29] may be regarded as an appropriate model for double-helical DNA. The wormlike model is characterized by two parameters: the contour length L and the persistence length a, which characterizes the stiffness of the chain. L and a are related to the mean-square radius by the equation[25,28]

$$\langle \rho^2 \rangle = \frac{La}{3} - a^2 + \frac{2a^3}{L} - \frac{2a^4}{L^2}(1 - e^{-L/a}). \tag{4}$$

A plot of $[Kc/R_\theta]_{c=0}$ versus $\sin \theta/2$ may be used for calculating[17,30-32] the mass per unit length M/L ratio. Our experimental data for sample B in water and in 40% DMF yielded the result $M/L = 200$ daltons/Å, which is in satisfactory agreement with the value (195) commonly used.[28] Using the latter M/L ratio, and the molecular weight derived from light scattering, a contour length $L \simeq 3.3 \times 10^4$ Å was calculated for sample B. Using Eq. (4), along with the experimentally determined values of $\langle \rho^2 \rangle$ (Table III), we then calculated values of a of about 500 Å and 300 Å, in water and in 40% DMF, respectively.

As a check for the consistency of the wormlike model, we can calculate the expected decrease of intrinsic viscosity due to the decrease of the stiffness parameter determined using Eq. (4). The data of Ullman[27] allow the interpolation of the intrinsic viscosity of a wormlike chain having $L = 75 \times 10^3$ (our sample A) and variable values of a. For a friction con-

stant[27] $\alpha = 1$, we obtain $[\eta] \sim 80$ and $[\eta] \sim 40$ when $a = 500$ and when $a = 300$, respectively. The corresponding experimental data (Figure 4, sample A at 14°C in water and 40% DMF) are $[\eta] \sim 72$ and $[\eta] \sim 43$ dl/g. This agreement between light scattering and viscosity data may be somewhat fortuitous. In fact, Hays et al.[28] and, more recently, Schmid et al.[33] have discussed limitations of Eq. (4) for the determination of the persistence length using the light scattering radius of gyration. These limitations are primarily related to the incompleteness of the angular range covered by conventional light scattering instruments, resulting in the neglect of higher terms occurring in the complete expression of P_θ^{-1} [cf. Eq. (2)]. Additional difficulties arise from polydispersity, excluded volume, and anisotropy of DNA molecules.[28,33] In the absence of an evaluation of these effects, an indetermination on the absolute values of a that we have reported has to be accepted. (In fact, corrected values of a in the order of 1000 Å, have been reported for DNA in water.[28,33]) For the purpose of this investigation, it is however important to point out that, for a given DNA sample and angular range, the DMF-induced variation of a determined by light scattering was adequate to justify the corresponding trend observed from viscosity data. Thus, we conclude that both the light scattering and the viscosity results are in satisfactory agreement with the model of a wormlike chain acquiring more flexibility when DMF is present.

Predenaturation effects on the properties of DNA solutions have also been noticed by other investigators. Bloomfield[7] has summarized the earlier, scattered observations and has pointed out the need for more detailed investigations of predenaturation alteration of DNA conformation. More recently, the occurrence of predenaturation alterations of DNA in solutions of glycol, aliphatic alcohols, or dioxane have been detected through optical rotatory dispersion and circular dichroism measurements.[5,34] These structural alterations seem to involve forms (A, B, or C) characterized, for instance, by different inclinations of base pairs to the helical axis. Further work seems to be necessary in order to establish if this type of alteration of the double helix is related to the alterations that have been reported here.

In the search for a plausible interpretation of our results, we feel that the effect of electrostatic interactions (i.e., phosphate groups) should play only a minor role in the large decrease of $[\eta]$ and $\langle \rho^2 \rangle_z$ observed for high-molecular-weight DNA. In fact, our results for the variation of $[\eta]$ with LiCl concentration for DNA in water indicate that at 0.1 M LiCl the largest part of the electrostatic effect on the reduction of $[\eta]$ is screened out. Moreover, as shown by Herkovits,[2] less salt is required to suppress electrostatic destabilization effects in the less polar solvents (the dielectric constant of DMF is 37). We note that while electrostatic interactions cannot be the cause of the decrease of $[\eta]$ and $\langle \rho^2 \rangle_z$ observed for samples A and B, the lack of a strong electrostatic repulsion in 0.1 M LiCl + DMF solutions may well represent a necessary condition for the occurrence of the aggregation effects that have been observed with sample D. Intermolecular aggregation of low-molecular-weight DNA is also favored by the relatively

higher polymer concentration employed for the low-molecular-weight samples (cf. Experimental).

We suggest that the body of results that have been reported in this paper should be interpreted in terms of the occurrence of two distinct effects that DMF has on the purine and pyrimidine bases on one hand, and on the residual part of polynucleotide chain (i.e., phosphate and deoxyribose groups) on the other hand. The two effects are (1) an increased solubility of bases occurring when DMF is present (this effect is directly evidenced by the data collected in Table II); (2) a parallel tendency toward aggregation and insolubilization of the residual part of the polynucleotide chain (evidenced by the light scattering data for sample D, and by the RC → P line in Fig 2).

In the "normal" situation of native DNA in water, phosphate and deoxyribose groups are exposed and solvated by water, while bases are hydrogen bonded within the turns of the double helix. The less polar solvent would however provide a more favorable thermodynamic environment for the bases than for the phosphate and deoxyribose groups. Thus, when DMF concentration is gradually increased, a slight reduction of base stacking, accompanied by a decreased solubility of deoxyribose and phosphate residues, could cause increased flexibility and a tendency toward intra- or intermolecular aggregation. Upon further increase of DMF concentration, denaturation will isothermally occur with a large decrease of base stacking, inter- or intramolecular aggregation notwithstanding. The effects observed beyond the denaturation point suggest that the exposure of bases to the solvent, and the aggregation of the remaining part of the polynucleotide chain, continue when DMF concentration is increased in the region where the random coil is stable, until precipitation occurs.

The authors express their great appreciation to Prof. H. Benoit and Dr. C. Strazielle for their advice and cooperation during the execution of the light scattering measurements at the Centre des Recherches sur les Macromolecules.

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Received March 6, 1974
Accepted June 8, 1974