Phase Behavior of Single DNA in Mixed Solvents

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Abstract: Compaction of single large *ds*DNA chains in aqueous solution in the presence of primary alcohols, acetone, and ethylene glycol has been studied experimentally with the use of a fluorescence microscopy technique. It is found that in the presence of all studied organic solvents single DNA molecules exhibit a discrete phase transition from an elongated coiled to a compacted globular conformation. Interestingly, DNA phase transition occurred at various weight fractions of organic solvents in aqueous solution, but at similar dielectric constants of mixed solvent for all studied primary alcohols and acetone. On the other hand, the dielectric constant of ethylene glycol−water mixtures corresponding to the collapsing transition in single DNA differed from that for the other studied systems. The explanation of this phenomenon comes through consideration of the existence of ethylene glycol conformers with various polarities in aqueous solution. Thus, the dielectric permittivity of the solvent is a key factor that determines the conformational behavior of DNA in solution. The compaction of a single DNA molecule when the dielectric permittivity constant is lowered is thought to be due to the increased importance of ion−ion correlation. Monte Carlo simulations for a single polyelectrolyte chain also show that the dimensions of the chain diminish when the electrostatic coupling is increased, i.e., by decreasing the dielectric constant. The experimental result can be rationalized with a simple free energy model balancing the counterion entropy and the ion−ion correlation energy.

Introduction

It is well established that within living cells DNA molecules exist in a highly compact conformation, which is characterized by an eminently regular structure.[1,2] The degree of compaction of a globular DNA chain can reach 10^6 in comparison to the DNA in a coiled state,[3] and the density of encapsulated phage DNA reaches 450 mg/mL.[4] The investigation of DNA folding is important for the understanding of DNA packing in vivo. DNA condensation in the presence of various cosolutes has been extensively studied during the last three decades.[5−11] However, all previous studies have dealt with relatively highly concentrated DNA solutions, and aggregation of DNA chains took place under those conditions.[12] Conventional techniques do not allow experiments at low DNA concentration in the solution, where aggregation does not occur. Thus the mechanism of condensation for a single DNA chain is still uncertain.

Recently, a fluorescence microscopy method was proposed to study the conformational behavior of a single large DNA chain (several tens of kilobase pairs (kbp)) in aqueous solution in the presence of various condensation agents.[13−17] These studies were performed in an extremely diluted DNA solution (up to 10 nM DNA in nucleotide units),[18] and the effect of the aggregation between single DNA chains during the folding process was negligibly small. The term "compaction", instead of "condensation" which implies the formation of multimolecular aggregates, has been proposed for the description of the results at the single-molecule level.[19] In the past several years, significant progress has been achieved in this field and single DNA chains have been seen to fold into compact globular structures by neutral and charged polymers,[19−24] multivalent ions,[25,26] surfactants,[27−31] and alcohols.[18] In the present study

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we examined the effect of various water-miscible organic solvents on the DNA compaction in extremely diluted aqueous solutions with the use of the fluorescence microscopy method and by Monte Carlo simulations of the corresponding model system.

To our knowledge, there have previously only been two reports on the phase behavior of large single DNA molecules in alcohol−water mixtures. Ueda and Yoshikawa[18] have found with the use of fluorescence microscopy technique that single giant T4 DNA macromolecules exhibit a first-order phase transition with the increase of the alcohol volume fraction in 2-propanol−water mixtures. However, in their study various chemicals were added at fairly high concentrations to prevent the oxidation of the chosen fluorescence marker, YOYO (dimer of the asymmetric cyanine dye, oxazole yellow), i.e., the saccharose concentration in the examined solutions was equal to 0.4 g/mL. Such a complexity of the studied solutions strongly affects the dielectric permittivity of the solvent, and obscures the interpretation of the results. Also, the mechanism of interaction between this tetravalent dye and DNA is not clarified yet, as well as the influence of the dye on the persistence length of DNA molecules. In our study we have chosen a simpler experimental system and used a conventional fluorescent dye, DAPI,[32] to stain the single phage *ds*DNA molecules in a highly diluted solution. The effect of DAPI binding on the properties of single DNA molecules, as well as the charge of the resulting DNA−DAPI complex, has been thoroughly examined by Matsuzawa et al.[33−36]

Recently, Sergeyev et al.[37] have studied the compaction of DNA with ethanol and 2-propanol and the effect of those alcohols on the stability of DNA−surfactant complexes. Among other results, it has been found that large DNA molecules exhibit a first-order phase transition with the increase of alcohol concentration, and above 50% (v/v) of 2-propanol and 60% (v/v) of ethanol all DNA molecules exhibit a compact globular conformation in the solution.

In the present study we do not use any specific condensation agent (e.g., multivalent cations) while varying the dielectric permittivity of the solvent. Therefore, the conditions chosen for this study should be more closely related to the actual environment of DNA molecules in a living cell. Besides, the relative simplicity of the system (e.g., the absence of buffer or excess salt) also makes it suitable for a qualitative comparison between experiment and theory.

The condensation of DNA induced by hexaammine cobalt(III) in the presence of primary alcohols, methanol, ethanol, and 2-propanol, has been extensively studied by Arscott et al.[38] The critical concentration of cobalt(III) hexaammine required to induce DNA condensation has been found to decrease with the decrease of the dielectric permittivity of the solvent. DNA condensation and precipitation induced by multivalent cations, spermidine^{3+} and spermine^{4+}, has also been studied[39] in the presence of different aminocarboxylic acids, which are known to increase the dielectric permittivity in an aqueous solution.[40] It has been shown that the increase in the dielectric permittivity of the solvent leads to an increase in the concentration of multivalent ions needed to induce precipitation of DNA molecules. Thus, a decrease in the dielectric permittivity of the solvent lowers the critical concentration of cations needed to induce DNA condensation and makes possible the collapse of DNA macromolecules in the presence of divalent and even monovalent cations.[7,8,38]

However, there has been no systematic study on the behavior of single DNA compaction in the presence of monovalent counterions and organic solvents of different chemical nature. Moreover, the previous studies never dealt with the single-molecule compaction of DNA. The purpose of the present work is to make clear the general features of single DNA compaction in media with varying dielectric permittivity. In the experimental part, we have chosen organic solvents of various chemical structures: primary alcohols (methanol, ethanol, 1-propanol, 2-propanol, *tert*-butyl alcohol), as well as a ketone (acetone) and a diol (ethylene glycol). Fluorescence microscopy was successfully applied to monitor the conformational behavior of single large DNA molecules in solution.

In the above discussion we have mentioned that the experimental conditions in our study are relatively simple and well controlled. The DNA sample is fairly monodisperse and we did not notice any short fragmented DNA under the present experimental conditions. This unique property of DNA, in contrast to other synthetic and natural polymers, is important for successful theoretical modeling. Thus, the selected experimental setup facilitates the qualitative comparison between the experimental data and the results of theoretical simulations.

DNA compaction is brought about by its own counterions with the change of the dielectric properties of the solution. This type of attractive interaction in highly charged macromolecular solutions has been demonstrated for several geometries in the past. The mechanism as such goes back to the work of London[41] on quantum mechanical dispersion forces. The attractive interaction between two rare gas atoms is the quantum mechanical analogue of the DNA compaction in the present study. The attractive interaction in the macromolecular context has been discussed in qualitative terms by several authors.[42,43] The first numerical study was for the attractive interaction of two planar charged surfaces.[44] Simulation techniques and sophisticated

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integral equation techniques have been used for other geometries (e.g. spheres and cylinders) as well.[45] All these theoretical studies have been performed assuming a dielectric continuum model, i.e., the solvent is a structureless medium characterized by its dielectric permittivity only. It now seems well-established that there exists an attractive component of purely electrostatic origin in the interaction between two macroions. With these results at hand, it is reasonable to assume that the same mechanism should be operative also within a single DNA chain. In fact, a recent Monte Carlo simulation shows that a polyelectrolyte chain with multivalent counterions actually folds, be it sufficiently charged.[46] In the present study we extend these simulations to include also a variation of the dielectric permittivity.

After a brief description of the experimental and simulation methods follows a section where the different results are described and compared. Finally, we discuss the results and present an explanation for DNA compaction due to changes of solvent.

Experimental Section

Materials. Coliphage T4 DNA ($M = 1.1 \times 10^8$ D, ca. 167 kbp, contour length 57 μm)[35] was supplied by Sigma (stock solution was prepared in 0.5 × TBE buffer: 45 mM Tris-borate, 1 mM EDTA, pH = 8.0). The DNA concentration was determined spectrophotometrically, assuming the molar extinction coefficient of DNA bases to be equal to 6600 M^{-1} cm^{-1};[47] the ratio of absorbency of DNA stock solution at 260 nm to that at 280 nm was found to be 1.8. The fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), and the antioxidant, 2-mercaptoethanol (ME), were obtained from Sigma. Organic solvents—methanol (p.a., >99.8%, Merck), ethanol (absolute, >99.5%, Kemetyl), 1-propanol (p.a., >99.5%, Merck), 2-propanol (p.a., >99.7%, Merck), *tert*-butyl alcohol (p.a., >99.7%, Fluka), acetone (p.a., >99.5%, Merck), ethylene glycol (anhydrous, 99.8%, Aldrich)—were used without further purification. Bidistilled water used for the preparation of samples was filtered through Millipore filters with a pore size of 0.22 μm.

Sample Preparation.[48] DNA stock solution was diluted with distilled water containing 1% (v/v) ME and the fluorescent dye. The content of ME in the solution was reduced compared to the standard protocol[26] from 4% (v/v) to 1% (v/v) to minimize the effect of ME on the dielectric permittivity of the solutions. With this protocol, the fluorescence intensity from DNA molecules stained by DAPI was still high enough for a distinct observation of the sample. The concentration of ME was kept low and constant through the series of experiments with various alcohols, and its presence was assumed to be negligible and was not taken into account in the calculations of the dielectric permittivity of the solutions.

The DNA solution, containing DAPI and ME, was gently mixed with the organic solvents to prevent mechanical damage of the giant DNA macromolecules and was then kept for 1 h before observation.

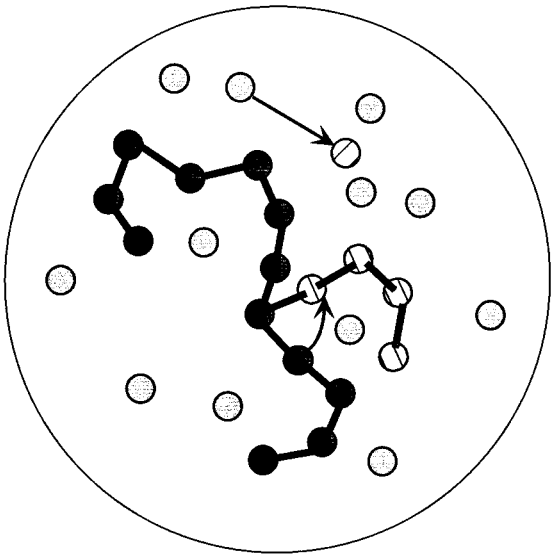

Figure 1. A schematic picture of the Monte Carlo model used. The upper arrow indicates a traditional MC move, while the lower arrow schematically shows a pivot move.

The final concentrations were as follows: DNA in nucleotide units, 0.5 μM; DAPI, 0.5 μM. These were kept constant throughout the experiment. Under these conditions, the binding number of DAPI per one DNA base-pair in an aqueous buffer solution is estimated to be equal to 0.05 and the persistence length of the DNA chain is expected to remain nearly the same as in the absence of DAPI.[33,36]

Microscope Glasses. Special care was taken to clean the microscope glasses (No. 0, Chance Propper, England) thoroughly before the observation to prevent a DNA degradation as well as a precipitation to the glass surface. Glasses were soaked in a conventional detergent solution for at least 1 day, washed with distilled water, immersed into concentrated hydrogen peroxide for several hours, washed repeatedly with distilled water, and then immersed in ethanol for at least 1 h. Finally they were washed with Millipore water and dried at 50 °C.

Methods. A fluorescence microscopy study was performed as follows: the samples were illuminated with a UV-mercury lamp; the fluorescence images of single DNA molecules were observed with a Zeiss Axioplan microscope, equipped with a filter set 01 (excitation 365 nm, beam splitter >395 nm, emission >397 nm) and a 100× oil-immersed objective lens, and digitized on a computer through a high-sensitive SIT video camera MTI VE1000 (DAGE-MTI, USA). The observations were carried out at 25.0 ± 0.1 °C, and the temperature was kept constant with a specially designed microscopic cell connected to a TS-4 ER thermocontroller (Physitemp Instruments, USA).

Simulations. (a) The Cell Model. DNA was modeled as a polyelectrolyte with a linear chain of monomers, each with a hard sphere, connected by freely jointed rigid bonds. The middle monomer was fixed in the center of a spherical cell (see Figure 1). The idea behind the cell model as applied to polyelectrolyte solutions[49] is to divide the total volume V of the isotropic solution into M equal cells of fixed volume $V_c = V/M$. Every cell contains one polyelectrolyte chain (consisting of N monomers) and the counterions of the polyelectrolyte. If wanted, "salt" can also be added. A requirement is that the total cell is electroneutral. If the charge of each monomer is z_m, and the charge of each counterion is z_c, electroneutrality requires that

$$Nz_m + N_c z_c = 0 \qquad (1)$$

where N_c is the number of counterions in the cell. In the present case all charges are set equal to ±1 and there are exactly the same amounts of counterions as monomers. While the middle monomer of the macroion was fixed in the center of the cell, the other monomers and the small ions were allowed to move in the cell. In the cell model there are no interactions ranging outside the cell, i.e., the interaction

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between species belonging to different cells is neglected. The polyelectrolyte concentration determines the cell volume V_c and hence the radius R_c.

Thus, the system corresponds to a solution with the polyelectrolyte concentration $1/V_c$ (or monomer concentration $c_m = N/V_c$) and the counterion concentration N_c/V_c. The solvent was treated as a dielectric continuum and was represented in the simulations by the dielectric permittivity ϵ_r. The interactions between the particles were of Coulomb and hard sphere type and the interaction potential $u_{ij}(r_{ij})$ between two species is

$$u_{ij}(r_{ij}) = \begin{cases} z_i z_j e/4\pi\epsilon_r\epsilon_0 r_{ij}, & r_{ij} \geq r_i + r_j \\ \infty, & r_{ij} < r_i + r_j \end{cases} \quad (2)$$

where r_{ij} was the separating distance. r_i and r_j are the hard sphere radii of particles i and j. To allow for thicker polymers, the hard sphere interactions between nearest neighbors in the chain were excluded from the simulations. The total interaction energy U was given by

$$U = \sum_j \sum_{i<j} u_{ij}(r_{ij}) \quad (3)$$

(b) The Monte Carlo Method. The Monte Carlo simulations were performed with the traditional Metropolis algorithm[50,51] in a canonical ensemble. Two different types of moves were performed, the chain was pivoted and the small ions were translated. The pivot algorithm introduced by Lal[52] makes the sampling of the chain conformations highly effective. Its efficiency for self-avoiding walks has been thoroughly discussed by Madras and Sokal.[53] Gordon and Valleau[54] discuss the problems of applying the pivot algorithm to simulations including both flexible polyions and the free ions of the supporting electrolyte.

In a pivot move one monomer was chosen to divide the chain in two parts. The shorter part was moved as a rigid body a random angle around the *x*-, *y*-, or *z*-axis. For every attempted configurational change the energy difference ΔU was computed by summing the pair interactions (eq 2) that have changed and comparing with their old energies. Although this algorithm involves more interaction calculations ($\sim N^2$) than the traditional method where only one monomer is translated at a time ($\sim N$), substantially fewer moves are needed to obtain independent configurations. The convergence is generally much faster and the net effect is a greatly reduced simulation time for a given degree of precision.[55] For every pivot move half of the free ions were translated. The total number of pivot moves were around 10^7-10^8. Every run was preceded by an equilibration run, of about 10^7 pivots, in which the initial configuration of the macroion was a two-dimensional spiral.

Results

To characterize the effect of the dielectric permittivity of the solvent on the compaction of single DNA molecules in an aqueous solution, a series of measurements with the use of methanol, ethanol, 1-propanol, 2-propanol, *tert*-butyl alcohol, acetone, and ethylene glycol as cosolvents were performed at 25 °C.

To quantitatively describe the conformational behavior of a large single DNA in the mixed solvents, the values of the long-axis length L of T4 DNA molecules, which was determined as a longest distance in the outline of a single DNA image, were measured for at least one hundred T4 DNA molecules for each studied solvent composition. The weight fractions of organic solvents were calculated from their volume fractions with the use of data in the *Handbook of Chemistry and Physics*.[56] Considering the decrease in the fluorescence intensity from the DNA-DAPI complex at high weight fractions of organic solvents, our measurements were performed up to 80 wt % concentration of organic solvents (except for the water−ethylene glycol system, where the highest studied concentration of organic solvent was equal to 90 wt %). Since the resulting plots are quite similar for all of the studied systems, we present only the results for the *tert*-butyl alcohol−water−DNA system.

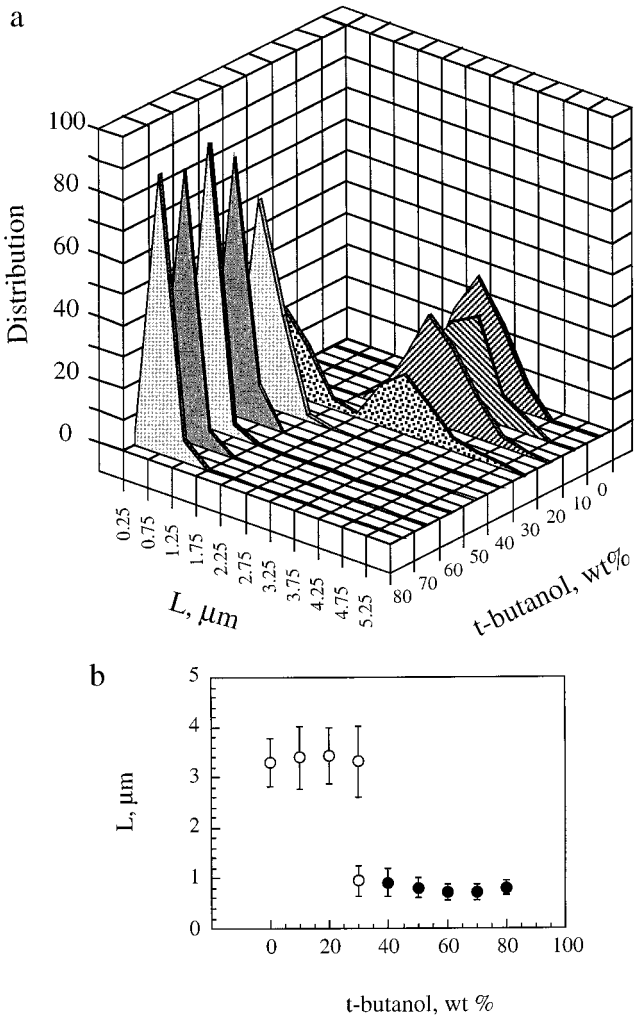

Figure 2. (a) Distribution of the long-axis lengths L of T4 DNA molecules vs the weight fraction of *tert*-butyl alcohol in aqueous solution. 100 single DNA molecules were measured for each *tert*-butyl alcohol concentration. (b) Long-axis length of T4 DNA molecules vs the weight fraction of *tert*-butyl alcohol in aqueous solution. The error bars indicate the standard deviation in the distribution.

Figure 2a displays the distribution of L values of T4 DNA macromolecules in an aqueous solution at various weight fractions of *tert*-butyl alcohol. It was found that at low weight fractions of the organic compound in the solution all T4 DNA molecules feature a relatively slow Brownian motion, while existing in an elongated coiled conformation. The values of the parameter L of T4 DNA molecules in those conditions are similar to the L values of T4 DNAs in a pure aqueous solution without any condensing agent,[26] and are found to be around 2.7−3.7 μm (Figure 2a).

Further increase in the weight fraction of the organic compound in the solution leads to a single-molecule compaction

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of DNA. We found for all systems at intermediate organic solvent concentrations that individual DNA molecules exhibit either elongated coiled or compact globular conformations, i.e., two populations of DNA molecules with different rates of Brownian motion and different fluorescence intensities are clearly distinguished. The distribution of L in the region of coexistence is clearly bimodal (Figure 2a) due to the existence of two conformational populations of DNA molecules. The free energy difference between single DNAs in the coiled and in the globular states is believed to be within the range of kT in the coexistence interval.[28] This observation is in agreement with the previously published results on the DNA phase behavior in water−alcohol mixtures.[18,37] In the coexistence region we also observed DNA molecules in a segregated state, where the compact globular conformation coexists with the unfolded coiled conformation within a single chain. The possibility of the formation of an intermediate conformational state of single DNA molecules has been noticed both in the water−primary alcohol mixtures[18,37] and in the presence of other condensation agents,[19,24,57] as well as seen in recent computer simulations.[58,59]

Finally, at high fractions of organic solvents, all DNA molecules were observed in a compact conformation while moving freely in the solution. A minor fraction of the DNA molecules, of the order of a few percent, was observed to precipitate at the surface of the microscope glass. Single DNA chains in a compact conformation are characterized by high fluorescence intensity and the average value of parameter L was found to be 0.7−0.9 μm (Figure 2a).

Figure 2b exhibits the dependence of the average dimensions on the alcohol content in the solution. It is clear from Figure 2b that a single DNA molecule exhibits a discrete, or first-order, phase transition at the thermodynamic segment level with increasing alcohol content. Figure 3 summarizes the variation of the conformational behavior of single DNA molecules with the weight fraction of the organic solvent in aqueous solution. It is evident from Figure 3 that single DNA molecules undergo a discrete coil-globule conformational transition in the presence of all studied organic solvents, whereas the widths of the coexistence regions as well as the critical weight fractions of organic solvent corresponding to the appearance of compact globules in the solution are different in the various systems.

To clarify the effect of the dielectric properties of the solvent on the chain conformation and generalize the results, the experimental points presented in Figure 3 are replotted with respect to the dielectric permittivity of the solvent in Figure 4. The calculation of dielectric constants of water−organic solvent mixtures at 25 °C was made in accordance with the data of Åkerlöf.[60] It is clear from Figure 4 that the chain conformation strongly depends on the ϵ_r of the solution. The quantitative features of DNA behavior in all alcohols as well as in acetone are similar. In the mixed solvents of high polarity, at $\epsilon_r > 54$, all DNA chains exhibit an unfolded coil conformation. The region of coexistence between coiled and globular DNA molecules begins at $\epsilon_r \approx 54$. Finally, at $\epsilon_r < 46$, the complete compaction of DNA takes place in the solution. In the ethylene glycol−water mixtures, however, DNA collapse occurs at a significantly lower value of the dielectric permittivity. The reasons of this different behavior will be discussed later.

In the simulation model it is possible to arbitrarily change the dielectric constant ϵ_r. The end-to-end distance of the polyelectrolyte is used as a conformational measure, and Figure 5 shows how the dimensions of the polyelectrolyte vary with ϵ_r. Chains with different monomer radii have been investigated, and as expected a polymer with larger monomer radii, i.e., a thicker chain, resulted in a more extended conformation. In all of the simulations the bond length in the polyelectrolyte was 6 Å. When the monomer radius was bigger than the bond length, the hard sphere interactions between two next-nearest neighbors introduce forbidden conformations and thus stiffen the chain. Even for this "stiff" chain a contraction in chain dimensions

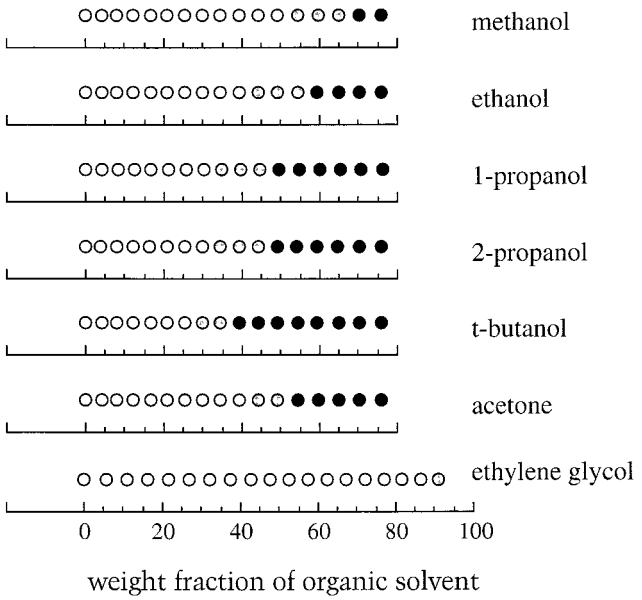

Figure 3. Dependence of the conformational behavior of single T4 DNA chains in aqueous solution on the weight fraction of organic solvent. Opened circles represent single DNA chains in an elongated coiled conformation, shaded circles represent the coexistence of single DNA molecules in coiled and in globular states, and filled circles represent compacted globular conformation of single DNAs in a sample solution.

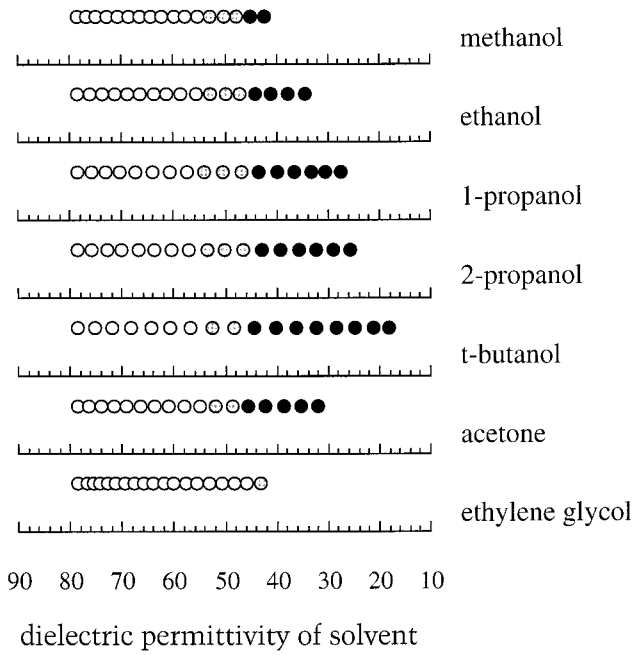

Figure 4. Dependence of the conformational behavior of single T4 DNA chains in aqueous solution on the dielectric permittivity of the mixed solvents. Definition of opened, shaded, and filled circles is the same as that in Figure 3.

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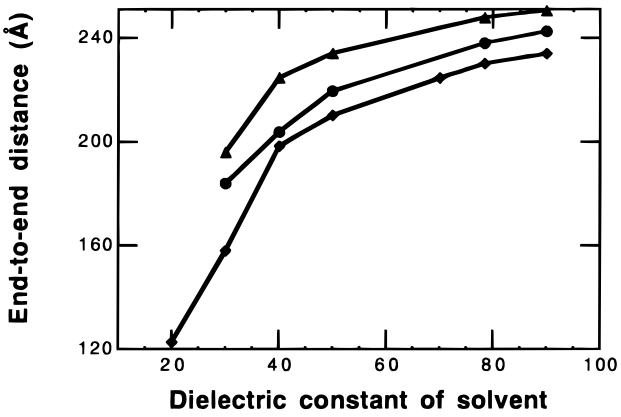

Figure 5. The end-to-end distance as a function of the dielectric constant for different monomer sizes. The results are from Monte Carlo simulations with $N = 80$ and $b = 6$ Å. The different monomer diameters are 4 (diamonds), 6 (circles), and 8 Å (triangles).

could be provoked by lowering the dielectric constant. The average size of the chain shrinks about 35% when the dielectric constant is lowered from 80 to 30.

The differences in conformation can be further illustrated by snapshots from the simulations. In Figure 6a ($\epsilon_r = 78.4$, corresponding to pure water) the polyelectrolyte is in an extended conformation and the counterions are evenly spread in the cell. The result of lowering the dielectric constant is shown in Figure 6b ($\epsilon_r = 30$), where the polyelectrolyte is compact and the counterions are accumulated near the macroion.

Figures 5 and 6 clearly show that a charged chain can be provoked to contract by decreasing the dielectric constant. Simulations for other sets of parameters have been performed and the qualitative results stand firm, i.e., when the system is dominated by electrostatic forces the chain contracts.

Discussion

It is known that DNA condensation, as well as compaction, may be induced by positively charged metal ions in aqueous solution, if the ion charge is at least 3+. Recently, Ma and Bloomfield have reported the condensation of supercoiled plasmid DNA in the presence of Mn^{2+} ions,[61] but they also noticed that a linearized plasmid DNA does not undergo condensation under the same conditions. Moreover, it has been shown that the mechanism of DNA condensation in the presence of Mn^{2+} is not primarily electrostatic.[61] Condensation of a linear DNA molecule by simple single-valent or divalent cations may be brought about by changing the quality of the solvent, i.e., by the addition of primary alcohol.[8] Besides, it has been found that the addition of primary alcohols (methanol, ethanol, and propanol) to the aqueous DNA solution in the presence of Co^{3+} cations lowers the critical condensation concentration of the metal ion.[38] All these effects are attributed to changes in the electrostatic interactions by changing the dielectric properties of the solvent through the use of alcohols as cosolvents. As ϵ_r decreases, the Coulomb electrostatic interactions become stronger and the dimensions of the DNA get smaller. Thus, the increase of the electrostatic forces leads in a nonintuitive way to attraction between the like-charged monomers. This attraction, giving rise to the compaction of single DNA molecules, is thought to be due to the increased importance of ion−ion correlations[42,46] when the electrostatic interactions become more significant. In previous simulations, it was found that the size of a single polyelectrolyte chain could decrease below the size of the corresponding neutral polymer, clearly indicating the existence of a net attractive force, that goes beyond simple mean field screening.[46] Mean field theory neglects the correlation between the counterions, which turns out to be an excellent approximation in most systems with monovalent ions. When the electrostatic coupling is increased by for example changing to divalent counterions or decreasing the dielectric permittivity, one cannot neglect the ion−ion correlations and the mean field approach breaks down. In fact, the term "correlation effects" has come to describe non-mean-field effects. The correlations act in two ways: (i) by compressing the double layer reducing the entropic repulsion and (ii) also by giving rise to a direct attractive interaction. Both mechanisms are important and of approximately the same absolute magnitude.[62] The main difference between the mean field theory, which assumes a uniform pair correlation function, and the simulations is that the latter allows for a "Coulomb hole" in the pair correlation function. Important correlation effects have been reported in several other systems.[44,63,64]

We believe that the behavior shown by DNA and the theoretical model chain in this study is a general property, which

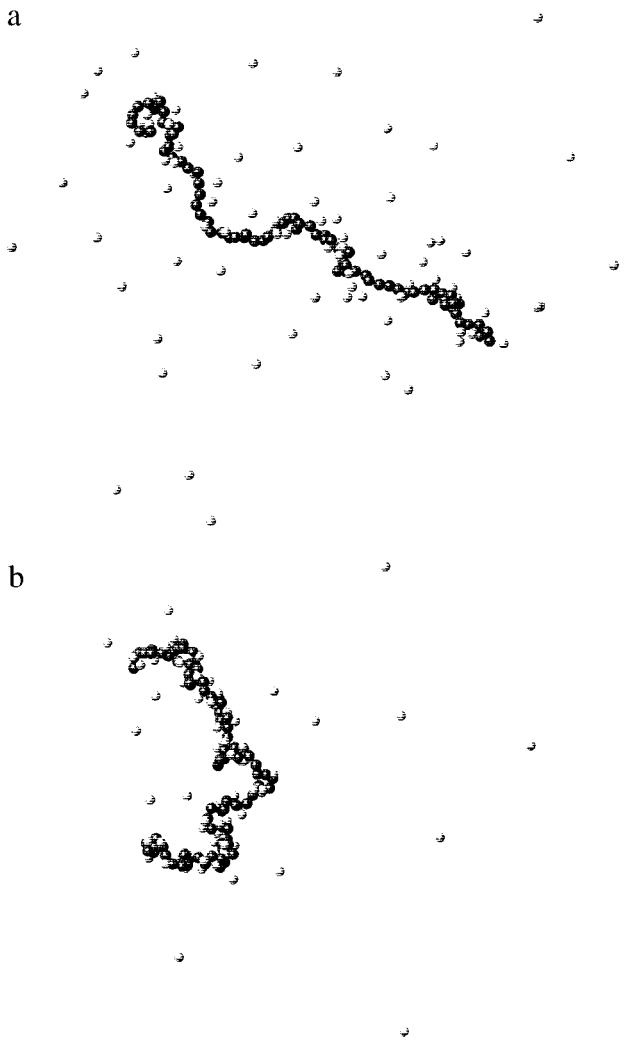

Figure 6. Snapshots from Monte Carlo simulations with $N = 80$, $r_{monomer} = 4$ Å, and $b = 6$ Å. (a) For a high dielectric constant ($\epsilon_r = 78.4$) the macroion is extended in an almost rodlike conformation. In this snapshot $R_{ee} = 222$ Å ($\langle R_{ee} \rangle = 230$ Å). (b) For a low dielectric constant ($\epsilon_r = 30$) a more compact form of the polyelectrolyte is observed. In this snapshot $R_{ee} = 156$ Å ($\langle R_{ee} \rangle = 157$ Å).

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should be present in other polyelectrolyte solutions as well, in the limit of strong electrostatic interactions. The possibility of a compact chain structure should not be surprising—after all sodium chloride does crystallize. A simple analysis of different free energy contributions should suffice to illustrate the situation.

Let us approximate the chain with its charged monomers with a uniformly charged sphere of radius R, R being a characteristic dimension of the chain, e.g., the end-to-end distance. The oppositely charged counterions are restricted to move on the surface of the sphere. Thus, we can divide the electrostatic energy into three components; monomer−monomer (U_{mm}), monomer−counterion (U_{mc}), and counterion−counterion (U_{cc}) interactions. The two former are trivial to evaluate while U_{cc} contains correlations. Assuming that the correlation energy is (attractive and) proportional to U_{cc} (with the constant α), we may write,

$$U_{mm} + U_{mc} + U_{cc} = \frac{1}{2}\frac{(Nze)^2}{4\pi\epsilon_0\epsilon_r R} - \frac{(Nze)^2}{4\pi\epsilon_0\epsilon_r R} + \left(\frac{1}{2} - \alpha\right)\frac{(Nze)^2}{4\pi\epsilon_0\epsilon_r R} = -\alpha\frac{(Nze)^2}{4\pi\epsilon_0\epsilon_r R} \quad (4)$$

Next, consider the counterion entropy. It seems to be a reasonable level of approximation to assume an ideal gas entropy for the counterions,

$$S_{cc} = Nk\left(-\ln\frac{N}{R^2} + \text{constant}\right) \quad (5)$$

The chain dimension R is of course limited and we approximate the restraining force with a harmonic potential, $\beta R^2/N$. For small R several terms of short-range repulsive character appear and we assume that these may be represented with a term $\gamma N/R^2$. The total free energy is then given by

$$A_{tot} = \gamma\frac{N}{R^2} - \alpha\frac{N^2z^2}{\epsilon_r R} - Nk\ln\frac{N}{R^2} + \beta\frac{R^2}{N} \quad (6)$$

The coefficients α, β, and γ are positive quantities and we have left out some constants which have no qualitative influence. The four terms above are a packing free energy (hard core), electrostatic correlation free energy, the ideal counterion entropy, and a chain elasticity term, respectively. The functional behavior of the different terms only represents the leading behavior and is not meant for quantitative calculations. Figure 7 shows a possible set of curves for varying parameter values. There exist two different minima corresponding to a compact globule and an extended coil. For certain parameter values they can coexist, but for others either of the minima will disappear. For example, lowering the dielectric permittivity will favor the compact structure, while increasing N (keeping Nz fixed) will lead to an elongated chain. The latter situation is the typical one encountered in an aqueous polyelectrolyte solution with monovalent counterions.

Our experimental results clearly show that for both alcohols and acetone the phase diagrams (Figure 4) of single DNA chains are similar, despite the variance in chemical structures between alcohols and ketone. At the same time, the phase diagram of DNA in ethylene glycol−water mixtures differs quantitatively from the others. The explanation of this anomaly is to be found from the structural conformational changes in ethylene glycol molecules accompanying the increase of water fraction in the solution.

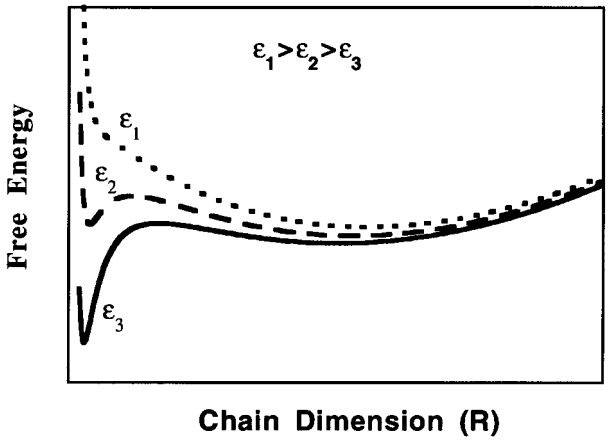

Figure 7. The free energy for a model chain according to eq 6.

The dependence of ϵ_r of alcohols and acetone on the weight fraction of organic solvents is approximately linear in a wide range of concentration while an ethylene glycol−water mixture shows deviation from the linear behavior. This effect is probably due to conformational changes in ethylene glycol molecules. It is well-known from mixtures of ethylene glycol with other solvents that the ethylene glycol conformation drastically depends on various factors, i.e., temperature, polarity of cosolvent, etc. The peculiarities of this phenomenon have been studied in the past both experimentally[65,66] and theoretically.[67,68] In aqueous solutions of ethylene glycol, the diol molecules are present in different conformational states, whose dipole moments are significantly different. We believe that the dielectric permittivity in a DNA−ethylene glycol−water mixture is somewhat inhomogeneous. For all other solvents in this study when ϵ_r reaches a value of about 46, the same value of ϵ_r characterizes both the bulk solution and the environment of the DNA molecules. A different process seems to take place in the ethylene glycol−water mixture. The polyelectrolyte DNA molecules accumulate ethylene glycol molecules in more polar conformations in the surroundings. Even if the ϵ_r value becomes as low as 46 for the whole solution, still the solution close to DNA has a somewhat higher value due to the presence of polar conformers of ethylene glycol. This local variation of ϵ_r could explain the deviation of ethylene glycol in comparison to the other solvents. When the ethylene glycol concentration is further increased the local dielectric permittivity of the solvent reaches a critical value for the DNA compacting, and all DNA molecules exhibit globular conformation in the solution.

Conclusions

In the present report we have shown that the dielectric permittivity of the solvent is a key factor that determines the conformational behavior of an isolated DNA chain in solution. The compaction of a single DNA molecule when the dielectric permittivity constant is lowered is thought to be due to the increased importance of ion−ion correlation when the electrostatic interactions are increased.

JA981491E

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