Disappearance of the Negative Charge in Giant DNA with a Folding Transition

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ABSTRACT In the present study we measure the electrophoretic mobility of giant T4 DNA (166 kbp) by electrophoretic light scattering for the elongated and folded compact states at different spermidine (trivalent cation) concentrations in 50 mM sodium maleate buffer (pH 6.0). It is found that the electrophoretic mobility of elongated DNA in the absence of the multivalent cation is seven times greater than that of fully folded compact DNA, where, with the increase of the concentration of spermidine, an abrupt transition is generated after a gradual decrease of the mobility. An analysis of the electrophoretic mobility suggests that the folded compact DNA chains almost completely lose their negative charges, by taking into account the difference of friction mechanism between an elongated and folded compact state. From the single chain observation by use of fluorescence microscopy, it is found that a phase-segregated structure is generated at intermediate concentrations of spermidine. The gradual decrease of the electrophoretic mobility in the transition region is, thus, attributed to the formation of the segregated state, exhibiting partial electroneutralization in the folded part. Disappearance of the negative charges in the completely folded compact DNAs is discussed in relation to the mechanism of transition, in terms of a first-order phase transition.

INTRODUCTION

The folding transition of DNA molecules from an elongated state into a compact state has attracted much attention in the past several decades in relation to the biological function of giant DNAs in vivo, e.g., regulation of gene expression, replication of DNA, etc. (Livolant, 1991; Sikorav and Church, 1991; Murphy and Zimmerman, 1994). It is well known that giant DNA molecules with a contour length of millimeters to centimeters exist in a highly packed state in living cells. For example, the total length of DNA in a single human cell is on the order of a meter, whereas the size of the cell nucleus is in the range of several micrometers (Watson et al., 1987). From recent developments in molecular biology, it is becoming clear that cationic chemical species, such as histone octamer, protamine, spermidine, etc. play an essential role in the packing of long DNA chains into the nucleus (Shibata et al., 1990; Pennisi, 1997). On the other hand, naked DNA chains in the absence of such cations exhibit an elongated random coil behavior in an aqueous solution (Yanagida et al., 1983; Matsumoto et al., 1992). Condensation of DNA chains is induced in vitro by the addition of these cationic chemical species (Bloomfield, 1997). Based on observations by electron microscopy, it was found that various kinds of orderly morphologies such as a doughnut shape (toroidal form) and rod-like shapes are generated upon the addition of multivalent cations (Gosule and Schellman, 1976, 1978). With regard to these phenomena, it has been suggested that multivalent cations decrease the negative charges in DNA through electrostatic binding and that this decrease in the negative charge is the cause of the condensation of DNAs (Bloomfield, 1997).

Generally speaking, a change in solvent quality from good to poor decreases the effective volume of single polymer chains. It has been accepted that the folding transition, or coil-globule transition, is either mild or steep depending on the parameters of the polymer, such as chain length and stiffness, and that this transition always goes through a so-called θ-state. Actually, almost all of the experimental data, such as those from light scattering, sedimentation, viscosity measurement, UV spectroscopy, etc., have indicated that DNA condensation is continuous for the physicochemical characteristics in the ensemble of polymer chains (Geiduschek and Gray, 1956; Lerman, 1971; Post and Zimm, 1982; Thomas and Bloomfield, 1983). In relation to the term of condensation, it has been indicated that DNA condensation is generally reserved for the aggregation of several chains in orderly morphology (Bloomfield, 1997). With careful arrangements and considerations on the measurements with light scattering for the DNA chains in a dilute condition, Widom and Baldwin (1983) reported on the "monomolecular condensation of λ-DNA induced by cobalt hexammine" and concluded that 1) the transition is not a two-state reaction, and 2) the transition for monomolecular condensation is diffuse. Unfortunately, due to the limitation of the experimental methods they used, this conclusion was attained not from the actual single DNA chain observation but from the measurement on the ensemble of DNA chains. On the other hand, the possibility of the occurrence of discrete transition between elongated and

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collapsed states at the level on the individual chains has been suggested based on the theoretical discussion under the framework of mean-field theory (Lifshitz et al., 1978; Post and Zimm, 1982). To explain such a diffuse nature on the experiments for the transition in DNA condensation, Post and Zimm proposed the possibility that DNA molecules could be composed of two different domains, a compactly packed and an elongated coiled part, by introducing the Boltzmann distribution to the ensemble of DNA chains (Post and Zimm, 1982). Our research group recently reported that the folding transition of individual giant DNA chains is definitely discrete using single-chain observation techniques with a fluorescence microscope (Minagawa et al., 1991, 1994; Mel'nikov et al., 1995a; Yoshikawa and Matsuzawa, 1995; Yoshikawa et al., 1996a; Takahashi et al., 1997; Yamasaki and Yoshikawa, 1997). It has been clarified that the folding transition is a first-order phase transition, or an all-or-none switching, at the level of individual DNA molecules with a decrease in the chain volume on the order of 10^{-4}, whereas the folding transition seems to be continuous as for the physico-chemical properties of the ensemble of chains. With regard to the compaction with multivalent cations, the all-or-none character is found to be enhanced with the increase of the valence of the cation from $+2$ to $+4$, both from experimental observations and theoretical calculations (Yoshikawa et al., 1996a; Takahashi et al., 1997). With the increase of the concentration of coexisting monovalent cations such as Na^+, the critical concentrations of the multivalent cation required to induce the transition increase, and the all-or-none character tends to be weakened. In other words, the increase of the concentration of monovalent cation has the effect to shift the first-order phase transition toward the criticality. This means that the correlation length in the first-order phase transition decreases with the decrease of the valence of the multivalent cations and also with the increase of concentration of the coexisting monovalent cation. When the correlation length becomes smaller than the contour length of the polymer, phase segregation is generated for each molecular chain; i.e., elongated and collapsed parts coexist in a chain (Yoshikawa, 1997).

Thus, the discrete nature of the folding transition has been established by experiments involving single-chain observation. A remaining problem is how to explain the mechanism of compaction to account for the decrease in the large effect of the repulsive Coulombic interaction between the negatively charged segments in the DNA chains. According to the Oosawa-Manning theory, the so-called counterion condensation theory, the charge of DNA phosphates is estimated to be neutralized by 76% in the presence of the salt of a monovalent cation, almost regardless of the concentration of the counter-cation (Oosawa, 1971; Manning, 1978). The phenomenon of counterion condensation has been confirmed with the NMR measurement on ^{23}Na (Anderson et al., 1978). Due to the large electrostatic repulsion between negatively charged DNA phosphates, DNA chains exist in an elongated coiled state. With the addition of condensing agents, the repulsive interaction in the DNA chains would decrease, and as a result the chains undergo DNA condensation. Based on experimental observations, it has been reported that a charge neutralization of up to 89–90% was necessary to induce DNA condensation (Bloomfield, 1997). Similarly, Widom and Baldwin suggested that a multivalent cation with a valence of $+3$ or greater has a role to bind each of two adjacent DNA helices and that this site binding would afford the sufficient energy to overcome the segment-segment repulsive forces of the remaining unneutralized phosphate charge (Widom and Baldwin, 1983). Thus, seeing the research history of DNA condensation, the majority of the researchers seem to have regarded that several percent of negative charge survive at least at the onset of DNA condensation, in other words, considerable amount of negative charge remains even after DNA condensation. The Oosawa-Manning theory was developed based on a theoretical consideration of a highly charged straight chain with infinite length. Their theory has been shown to be useful for explaining the physico-chemical characteristics of elongated polyelectrolytes dissolving in an aqueous solution, such as electrophoretic mobility. On the other hand, DNA condensates have been found to be highly packed (Gosule and Schellman, 1976, 1978). Thus, the framework of the Oosawa-Manning theory is not straightly applicable for the transition of polyelectrolytes into a compact state. The fact that the folding transition of individual DNA chains into a compact state is a first-order transition indicates that the free energy exhibits bimodality or double minima. Therefore, it is necessary to characterize the properties in the highly compact globular state, apart from the theoretical framework of Oosawa-Manning. In this paper, we interpret an experimental procedure to evaluate the residual charges of DNA in the fully folded compact state and show that almost complete charge neutralization is necessary for DNA macromolecules to assume a folded compact structure.

MATERIALS AND METHODS

Materials

Bacteriophage T4 DNA (166 kbp) was purchased from Takara (Kusatsu, Japan). Spermidine hydrochloride and the fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) were obtained from Wako Pure Chemical Industries (Osaka, Japan) and used without further purification.

Sample preparation

Sodium maleate buffer solution (50 mM, pH 6.0) was used, and the pH value was adjusted by adding NaOH aqueous solution. Buffer solution with a desired amount of spermidine was mixed with the DNA buffer solution. All solutions were prepared with Millipore water (18.3 MΩ).

Electrophoretic light scattering

The electrophoretic mobility of T4 DNA was measured using an Otsuka Electronics (Hirakata, Japan) ELS-800 spectrophotometer (Oka et al., 1991; Takagi, 1993) at a constant voltage of 85 V. All measurements were carried out at 20.0 ± 0.1°C using a NESLAB RTE-111 circulator. When the DNA concentration is below 5 μM (in phosphate), no aggregation or association of multiple DNA chains is noted. Thus, we used a DNA concentration of 2 μM throughout the experiments. Just before each experiment run, we newly prepared the respective sample, and the observation was made after the sample was allowed to equilibrate for 30 min. To avoid contamination of the sample solution by dust, all of the preparatory solutions except for DNA solution were filtered through a 0.22-μm Millipore filter before preparation.

Fluorescence microscopy

To visualize individual DNA molecules by fluorescence microscopy, a fluorescent probe, DAPI, was used (Larsen et al., 1989; Kapuscinski, 1995). The concentration of DAPI was 0.2 μM. Under this condition, it has been confirmed that the contour length, 57 μm, and the persistence length, 50 nm, remain essentially constant (Yoshikawa et al., 1992). The number of DAPI molecules per base pair is estimated to be 0.05 (Matsuzawa and Yoshikawa, 1994), suggesting that DAPI has little effect on the charge neutralization of DNA. The observation was carried out at room temperature, ~20°C. Other experimental conditions were the same as those for the ELS measurements. The system used for fluorescence microscopy was equipped with a video camera and has been described previously (Yoshikawa et al., 1996b; Yamasaki and Yoshikawa, 1997). The apparent length of the long axis, L, which was defined as the longest distance in the outline of a DNA image, was evaluated with an image processor. Due to the blurring effect of fluorescent light, DNA images were thicker than the actual chains by ~0.3 μm (Mel'nikov et al., 1995b). To estimate the hydrodynamic radius, R_H, a diffusion coefficient for each DNA molecule was evaluated from the mean square displacement of the fluorescence images (Mel'nikov et al., 1995a; Yamasaki and Yoshikawa, 1997). The viscosity was measured by a TOKIMEC Visconic ELD viscometer calibrated with JS 2.5 calibration liquid (Showa Shell Co., Tokyo, Japan).

RESULTS

Fluorescence microscopy

It has been well established that, depending on the concentration of spermidine, individual DNAs exhibit an all-or-none transition between the elongated and folded compact states and that there exists a rather wide region in which the two states coexist (Minagawa et al., 1991, 1994; Mel'nikov et al., 1995a; Yoshikawa and Matsuzawa, 1995; Yoshikawa et al., 1996a; Takahashi et al., 1997; Yamasaki and Yoshikawa, 1997). To identify experimental conditions suitable for measuring the compaction with ELS, we examined the effect of spermidine on the conformation of DNAs using fluorescence microscopy. As for the measurement with ELS, we had the intention to minimize the effect of electro-osmotic flow by choosing a buffer solution containing a sufficient amount of small electrolyte. After the efforts to determine an appropriate buffer solution suitable for the measurements both with fluorescence microscopy and ELS, we have chosen 50 mM sodium maleate (pH 6.0) solution. Fig. 1 shows typical fluorescence microscopic images of T4 DNAs with spermidine concentrations of 0 mM (*a–c*), 10 mM (*d–f*), and 12 mM (*g–i*) in 50 mM sodium maleate buffer solution, where individual DNAs exhibit both translational and intra-chain Brownian motion. In this series of pictures, the time interval between successive frames is ~0.1 s. In the absence of polyamine, DNA chains take an elongated coiled conformation, as in Fig. 1, *a–c*, indicating that the aqueous solution is a good solvent for DNA. When the spermidine concentration is high enough, as in Fig. 1, *g–i*, DNA chains show a folded compact state. In the compact state, DNAs appear as a bright dot, indicating a high density of segments in a narrow space. Between the elongated and folded compact states, DNAs exist as a partially unfolded conformation, as in Fig. 1, *d–f*. At the solvent conditions chosen in the present study, the partially unfolded structure is stable and the individual DNAs never tend to aggregate with each other for at least several hours.

Fig. 2 shows the distribution of the long-axis length, L, of T4 DNA at different spermidine concentrations. At each concentration of spermidine, we measured L for 100–200 DNA molecules, and all histograms were normalized to be unity. At a low concentration of spermidine, all DNA macromolecules exist as elongated random-coiled chains (Fig. 1, *a–c*) with a mean L value of ~3 μm. On the other hand, when the spermidine concentration was higher than 10 mM, all of the DNA chains show a folded compact state. At intermediate concentrations, an intra-chain segregated conformation, or partially unfolded structure, is observed. As the probability distribution of L gives a bimodal profile, we can discriminate the partially unfolded state from the fully unfolded state from the morphology in time-successive fluorescence images.

The mean values for the distribution of L, together with the standard deviation, are given in Fig. 3. The gray closed circles, open circles with oblique cross-hatching, and black closed circles indicate the mean value of L for each distribution in the elongated coiled, partially unfolded, and fully folded compact states, respectively. The error bars give the standard deviation. In the fully folded state at a high spermidine concentration, DNAs tend to form large aggregates gradually, on a time scale of several hours. Thus, the figure shows the result of measurements taken within 15 min after equilibration for 30 min.

Measurement of electrophoretic mobility

Fig. 4 shows the results of ELS measurements for T4 DNA at different spermidine concentrations, where the vertical axis indicates the position in the observation chamber perpendicular to the applied electric field. Due to the negative charge on the surface of the glass chamber, significant electro-osmotic flow toward the negative electrode is induced. On the other hand, in the middle layer, fluid flow is induced opposite the direction of the osmotic flow near the glass surface. The inverse flow is induced as compensation for convective flow in the closed

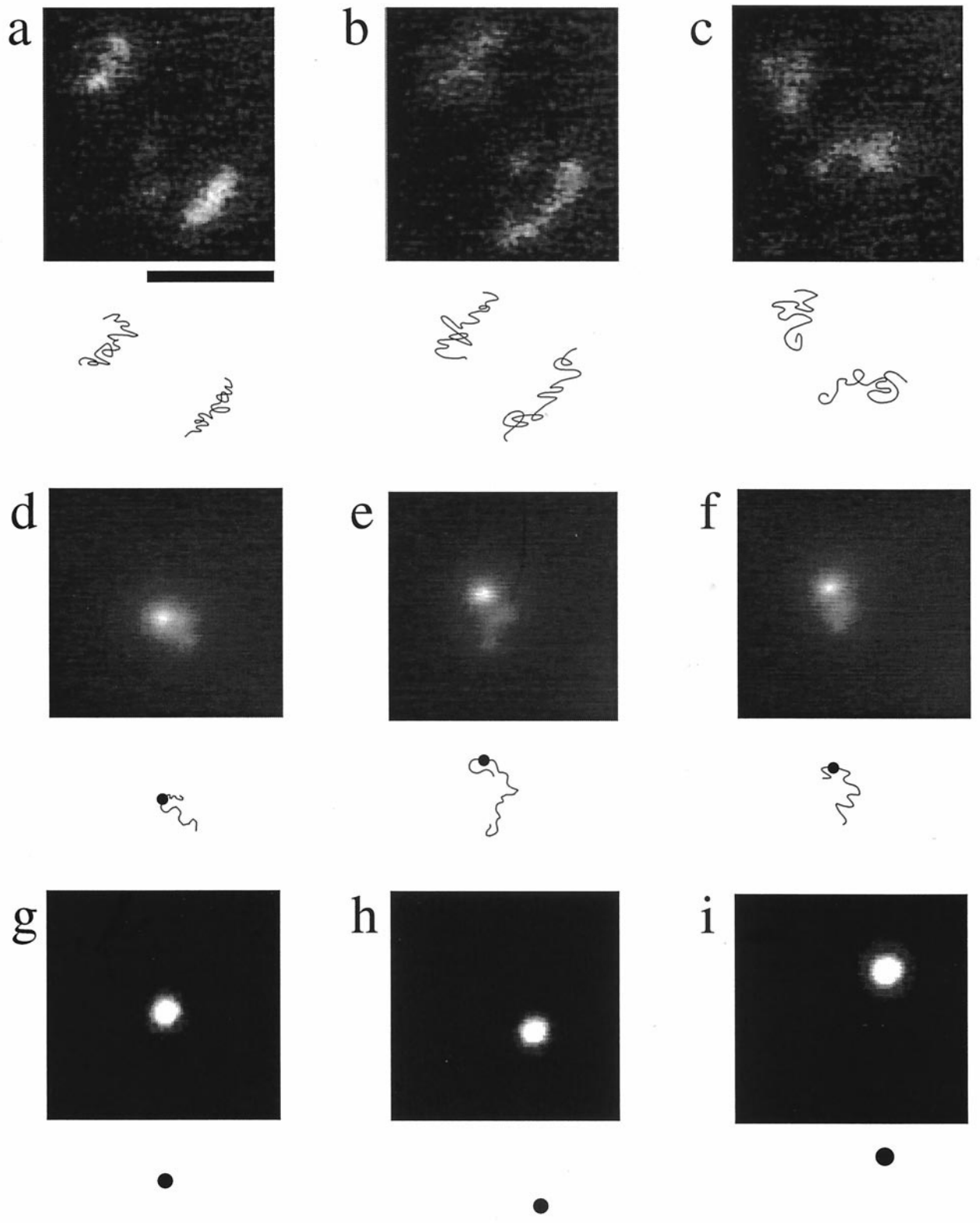

FIGURE 1 Fully unfolded (*a–c*), partially unfolded (*d–f*), and compact folded (*g–i*) T4 DNA. The upper panels are successive fluorescence images of DNA molecules stained with DAPI. Schematic representations of possible conformations corresponding to the fluorescence images are shown under each image. An interval between frames is ~0.1 s.

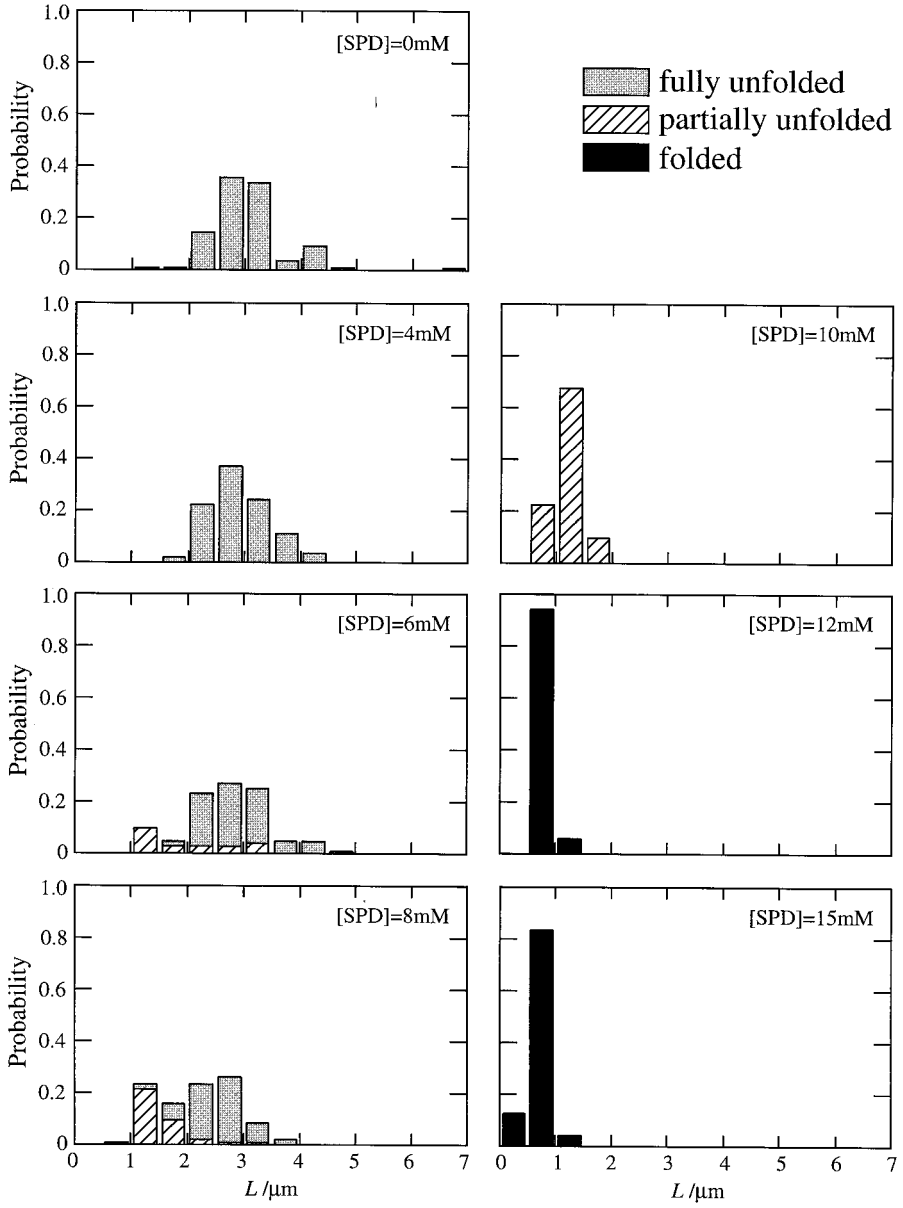

FIGURE 2 Histogram of the long-axis length, L, of T4 DNA molecules at various concentrations of spermidine. The areas in the histograms are normalized to be equal.

chamber. Thus, with the application of a constant electric field, a parabolic flow profile is generated in the chamber (Oka et al., 1991). On the other hand, the electric field is almost uniform in the chamber, and therefore, the driving force for electrophoretic motion should be uniform in the channel of the chamber. Based on the knowledge that the apparent motion of DNAs can be represented as the summation of parabolic osmotic flow and uniform electrophoretic movement, one can find the position where the osmotic flow is minimized (in our case, ~0.3 mm from the surface as indicated by the horizontal dotted line in Fig. 4). Therefore, it is possible to deduce the true electrophoretic mobility, U, from the experimental profile, as shown in Fig. 4. Actually, we obtained the true mobility by numerical fitting of the experimental results to the Mori-Okamoto equation, which combines the Smoluchowski, Komagata, and White equations (Mori and Okamoto, 1980).

The change in the electrophoretic mobility, U, of DNAs with the spermidine concentration is summarized in Fig. 5. The data for U are the average of several experimental results at each concentration, and the experimental error of all measurement is less than 4%. The right axis shows the relative electrophoretic mobility, U/U_0. A marked decrease

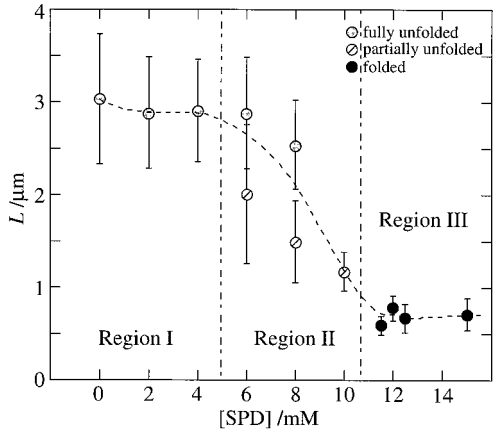

FIGURE 3 Long-axis length, L, of T4 DNA molecules versus the concentration of spermidine. The gray and black closed circles indicate the mean values of L in fully unfolded and compact folded DNA molecules, respectively. The vertical bars show the standard deviation. Open circles with oblique cross-hatching indicate the mean values in partially unfolded DNAs. The partially unfolded structure exists only in region II. The broken line indicates the ensemble average of L for 100–200 DNA molecules.

in U is noted with the addition of 2 mM of spermidine. Note that the mobility decreases only gradually at a higher concentration of spermidine.

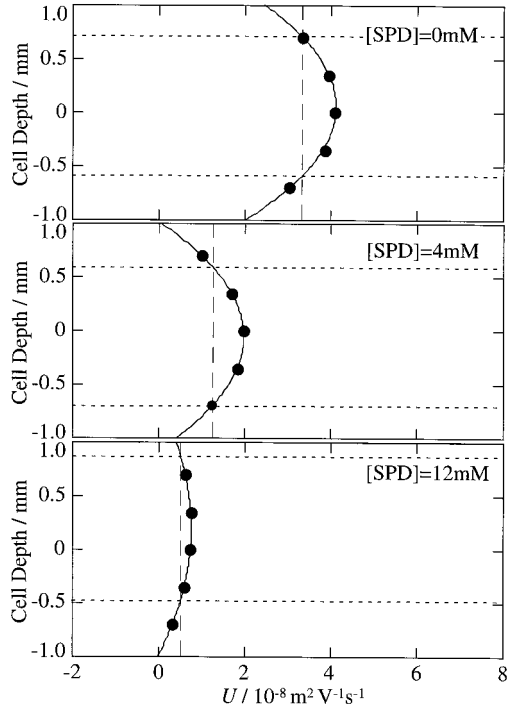

FIGURE 4 Electrophoretic mobility of T4 DNA measured by ELS spectrophotometer. The vertical axis indicates the cell depth (2 mm). Horizontal dashed lines indicate the stationary layer where electro-osmosis disappears. True mobility is determined at the intersection of the curves with the horizontal dashed lines, represented by vertical dashed lines.

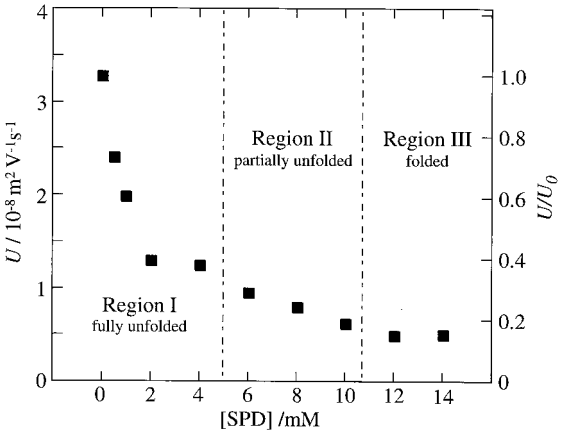

FIGURE 5 Electrophoretic mobility of T4 DNA versus concentration of added spermidine.

Fig. 6 shows the distribution of electrophoretic mobility based on the ELS measurements. The width of the band in the folded state ([SPD] = 12 mM) is about two times larger than that in the unfolded state. This experimental trend is attributable to the enhanced Brownian motion of folded compact DNAs.

Table 1 shows a comparison of the electrophoretic mobility, U, and hydrodynamic radius, R_H, between the unfolded state ([SPD] = 0 mM) and folded state ([SPD] = 12 mM). The hydrodynamic radius was obtained from the measurement of the mean square deviation versus time for individual DNAs using a fluorescence microscope. Interestingly, larger unfolded DNA exhibits greater electrophoretic mobility than smaller folded DNA.

DISCUSSION

Analysis of the electrophoretic mobility

The procedure used to obtain the net charge of the folded compact DNA is as follows. When a charged particle moves toward the opposite electrode under a steady electric field, E, an electrical force and a friction force, which is proportional to its velocity, v, balance each other. Thus, the velocity of the charged particle is represented as:

$$v = \frac{zeE}{f} = UE, \tag{1}$$

where z is the number of electric charges for the particle, e is the electric charge unit, f is a coefficient of friction, and U is the electrophoretic mobility of the charged particle. In the case of DNA electrophoresis, z is the product, $z = \xi z_p$, where ξ and z_p indicate the fractional residual charges and the total number of phosphate groups, respectively. The relationship between z, f, and U has been shown to be valid for relatively large DNA chains over 400 base pairs, based on electrophoretic measurements at high ionic strength (Ol-

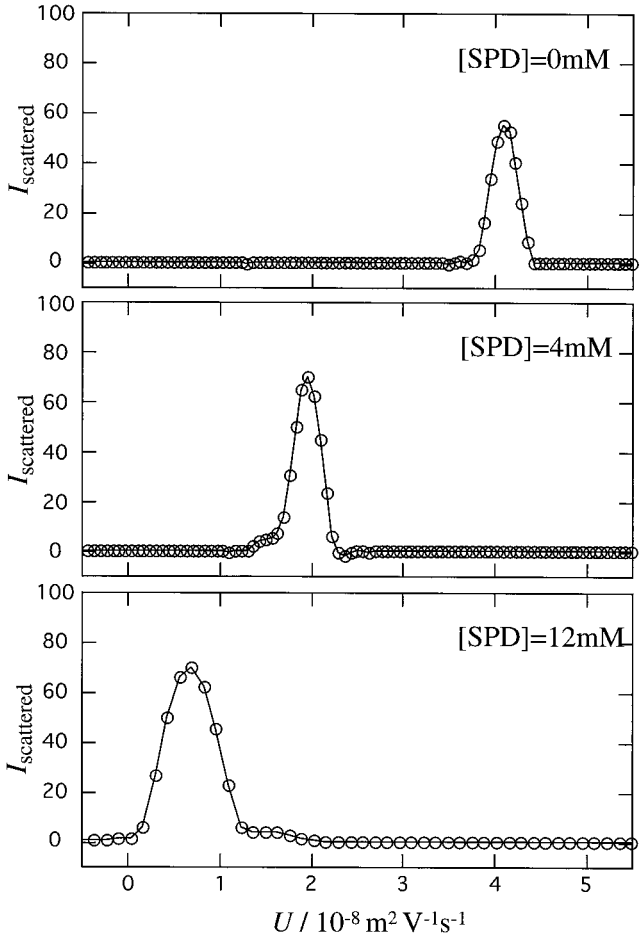

FIGURE 6 Change in the line width in ELS spectra. Significant broadening is mainly attributable to the change in the diffusion coefficient of suspended particles, indicating a conformational change of DNA into the folded compact state. Each spectrum is corresponding to the mobility at the center of electrophoretic chamber given in Fig. 4.

ivera et al., 1964; Ross and Scruggs, 1964; Ma and Bloomfield, 1995; Li et al., 1996). Generally, it has been known that the mobility is almost independent of the molecular weight of polyions in the free-draining regime (Olivera et al., 1964; Xia et al., 1993; Stellwagen et al., 1997). A validity of such a free-draining model is widely accepted in the free-solution electrophoresis of DNA, when the conformation of DNA retains an elongated state. Under this situation, f would be independent of the residual charges of DNA.

Let us evaluate the value of f for the elongated DNA from the result of electrophoretic mobility (region I). The mobility for the elongated DNA in the absence of multivalent cations is measured to be 3.28×10^{-8} m^2 V^{-1} s^{-1}. This value is in a good agreement with those obtained by other researchers (Olivera et al., 1964; Ross and Scruggs, 1964; Stellwagen et al., 1997). According to the Oosawa-Manning theory, the fractional residual charges of DNA without multivalent cations, ξ_0, is estimated to be 0.24. Thus, f is determined to be 3.9×10^{-7} Nsm^{-1} with the unit [force]/[velocity] from Eq.1. Using this value, ξ is calculated to be 0.091 for an elongated DNA at 4 mM of spermidine concentration, which is in a good accordance with the predicted value from the modified Manning theory by Wilson and Bloomfield (1979). This means that the above assumption concerning the friction mechanism is appropriate for the elongated DNA migrating under a steady electric field in region I.

On the contrary, the free-draining model becomes inadequate for the interpretation of folded compact state; i.e., the value of f for the fully folded DNA should be quite different from that for the elongated chain due to an impermeability of the compacted structure. In other words, to obtain an accurate residual charge on the fully collapsed DNA, a relaxation effect caused by a diffuse electric double layer surrounding DNA should be taken into account. In our experimental condition, however, the Debye screening length, which is evaluated to be less than 1.4 nm, is negligibly small compared with the size of folded compact DNA. In the following discussion, therefore, we neglect the relaxation effect for simplification.

With the assumption that the friction mechanism for the fully folded DNA obeys the Stokes' low, $f = 6\pi\eta R_H$, f is calculated to be 3.4×10^{-9} Nsm^{-1} from the hydrodynamic radius R_H, 160 nm (see Table 1). Using this value, ξ for the folded compact DNA at 12 and 14 mM spermidine concentration are deduced to be 3.1×10^{-4} and 3.2×10^{-4}, respectively. The change in the fractional residual charge on DNA together with the relative mobility is given in Fig. 7. The broken line is the residual charge calculated with the two-variable counterion condensation theory, where we used Eqs. 6 and 7 in the paper of Wilson and Bloomfield (1979). Thus, it has become clear that, after the correction with the change in the size of DNA chains, the actual net charge on fully folded compact DNA, which corresponds to its surface charge, becomes extremely lower than the apparent value before the correction on the electrophoretic mobility.

Mechanism of DNA folding transition

From the measurement with ELS, it is noted that just before the folding transition, the degree of charge neutralization is achieved up to ~90% with an addition of spermidine, as is indicated as the abrupt decrease of the electrophoretic mobility. The electrophoretic mobility continues to decrease gradually (Fig. 5) even before the transition into the fully folded state, retaining the profile on the band with a single peak (Fig. 6). It is to be noted that the light-scattering experiment provides only limited information on the nature of the transition, because precise discrimination on these two states has been very difficult by electrophoretic measurement with light scattering due to the large difference in

TABLE 1 Electrophoretic mobility, U, hydrodynamic radius, R_H, and viscosity, η, for T4 DNAs in the unfolded and folded states

T4 DNA	[SPD] (mM)	U (10^{-8} m^2 V^{-1} s^{-1})	R_H (nm)	η (10^{-3} Nsm^{-2})
Unfolded	0	3.28 ± 0.020	810 ± 107	1.10
Folded	12	0.47 ± 0.006	161 ± 52	1.13

Each value of U is the mean of three to six independent measurements with ELS; hydrodynamic radius, R_H, is evaluated from the linear relationship of the mean square displacement with time in the Brownian motion of individual DNAs, using fluorescence microscopy under essentially the same experimental conditions as in the ELS measurement.

scattered light intensity between the elongated and folded compact states (Widom and Baldwin, 1983).

As for the actual change in the degree of charge neutralization accompanied by the folding transition, the negative charge decreases in a discontinuous manner with the compaction, as shown in Fig. 7. It is apparent that almost complete charge neutralization is achieved in the folded compact state. The remaining small negative charge is attributable to a dissociated phosphate group of DNA at the surface of the compact state, most probably with toroidal structure (Bloomfield, 1991). Such an experimental trend corresponds well to our recent theoretical interpretation (Takahashi et al., 1997). Let us briefly explain the mechanism of the folding transition, skipping the details of the reported theoretical interpretation in our recent studies (Yoshikawa et al., 1996a; Takahashi et al., 1997; Yamasaki and Yoshikawa, 1997). As the negative charge is completely neutralized in the folded compact state except on the surface, the Coulombic repulsion between DNA segments may disappear. The complete charge neutralization of the negative charge means that almost all of the phosphate groups are bound to positively charged counterions, i.e., Na^+ and spermidines in the present experiments. Thus, the highly dense negative charges along the DNA chain are converted to a highly dense layering of dipoles along the chain. It is well known that dipole-dipole interaction can be either attractive or repulsive, depending on the orientation of the dipoles. Under ambient temperature, the resulting dipoles after the folding transition will find suitable arrangements to stabilize the system under thermal agitation. The spatial correlation of the bound counterions can make the system more stable in folded compact DNA, together with the bridging effect of the multivalent cation between neighboring segments in a DNA chain (Widom and Baldwin, 1983; Bloomfield, 1997).

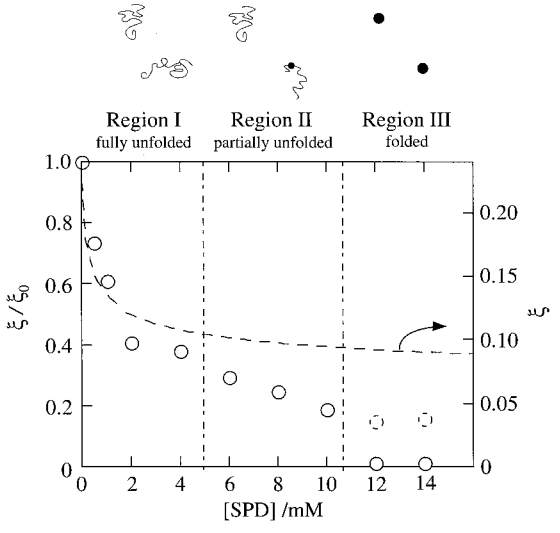

FIGURE 7 Change in the residual charge. The open circles indicate the relative change in residual charge, ξ/ξ_0, where the charge at [SPD] = 0 mM is normalized to be unity. In region III, the relative electrophoretic mobility, U/U_0, or the apparent residual charge without correction of the friction coefficient, is indicated by a broken circle. At a low concentration of spermidine, there exists no obvious difference between the open and broken circles. In other words, the position of the relative electrophoretic mobility almost coincides with that of the relative residual charges, because the friction coefficient of the DNA chain does not exhibit significant change in this region. The broken line is the fractional residual charge, ξ, calculated within the framework of the Manning theory.

Thus, the internal energy of DNA is stabilized by charge neutralization with the counterion and the multivalent cation. The next problem is how to interpret the change in the free energy of this system. With charge neutralization, many counterions should be absorbed into the folded DNA products, implying a large decrease in the translational entropy in the counterion. If we take into account the effect of ion exchange between the monovalent cations located near or bound to the negative charges in DNA and the multivalent cations, the folding transition will increase the net translational entropy driven by the enhanced binding of the multivalent cations with the transition. In fact, the ion exchange driven by the release of bound monovalent cation from the vicinity of DNA is proved by Braunlin et al. (1982). This theoretical interpretation was confirmed by our recent study, in comparison with the results of the folding transition of individual giant DNAs, caused by the addition of divalent, trivalent, and tetravalent amines (Takahashi et al., 1997). In the above theory, we assume that the residual charge of DNA in the folded compact state is negligible due to complete charge neutralization. The experimental results obtained in this work correspond well to such a hypothesis. We also examined the effect of temperature on the folding/unfolding transition of giant DNAs in the presence of a fixed concentration of spermidine and found that with an increase in temperature, elongated coiled DNA undergoes a discrete change into the folded compact conformation (Mu-

rayama and Yoshikawa, 1999). A similar trend was also reported by Widom and Baldwin (1983). These experimental observations again support our theoretical interpretation.

The failure of the counterion condensation theory to explain the folding transition of DNA in a correct manner, as represented in Fig. 7, should not be unexpected, because this theory is valid for DNA segments in the absence of interaction with other segments. It is to be noted that the Oosawa-Manning theory has been essentially advanced to explain the electrostatic interaction between an infinite rod-like polyion and its counterion (Oosawa, 1971; Manning, 1978). Although the value for the residual charges predicted by this theory can be accepted for DNA chains in the coiled state, this theory is not suitable for DNA chains in the folded compact state (Widom and Baldwin, 1983). We think that the folding transition of DNA chains should be described in terms of competition in the relative stability between the fully elongated state and the folded compact state. Thus, the balance of the free energy in the two states is the necessary starting point in the interpretation. Further detailed studies including the effect of hydration and the correlation of ions are awaited based on the bimodality in the free energy.

Formation of a partially unfolded state

In our experimental conditions, we noticed the formation of a partially unfolded state as in Fig. 1. A similar structure has also been observed in the compaction with the addition of 2-propanol (Ueda and Yoshikawa, 1996), poly(2-vinylpyrrolidone) (Starodoubtsev and Yoshikawa, 1996), cetyltrimethylammonium bromide (Yamasaki et al., 1999), and poly(ethylene glycol) derivatives with pendant amino groups (Yoshikawa et al., 1997). Also for such an intra-chain segregated state, the negative charge on the folded parts is expected to be neutralized almost completely, whereas the unfolded random-coiled parts retain the negative charge with ~10% of the dissociation in the phosphate groups. The gradual reduction of electrophoretic mobility in the intermediate region (region II) supports the above scenario. Recently, Raspaud et al. (1999) also suggested a similar phenomenon, with the term of partial electroneutrality. The formation of such a segregated structure is interpreted as a result of the competition between the correlation length in the transition and the contour length of the DNA chain as has been explained in the Introduction. In the present study, we are obliged to choose the experimental condition with 50 mM buffer to minimize the experimental error in the ELS measurement due to the electro-osmotic convectional flow. Thus, under our experimental condition, in the intermediate region (region II in Fig. 7) the individual DNA chains take an intra-chain segregated state. If, in the future, it becomes technically possible to measure the electrophoretic mobility of individual giant DNAs under very dilute salt concentrations by overcoming the effect of electro-osmotic flow, it will become possible to observe an abrupt decrease in the electrophoretic mobility accompanied by the discrete folding transition.

With regard to the bimodality in the free energy, Hill pointed out that there should be a bimodal distribution of ligand binding among host polymers when the interaction is highly cooperative and the system is small enough (Hill, 1963). Shirahama et al. (1992) reported that a surfactant binding to polyelectrolytes showed bimodality, as observed by ELS measurements. Based on the results in our studies together with those of these other studies, it is expected that the phase transition in a single molecular chain, or the bimodality in free energy, is a rather general phenomenon, being not limited to DNA molecules.

Conclusion:

It has become clear that nearly complete charge neutralization is achieved in the fully folded compact DNA molecule, whereas just before the folding transition into the compact state, DNA chains retain 10% of the negative charge. Disappearance of the negative charge is related to the unique nature of the folding transition as a first-order phase transition in a finite system.

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