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Collapse of single DNA molecule in poly(ethylene glycol) solutions

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The compactization of a single DNA molecule in polyethylene glycol (PEG) solution was investigated both theoretically and experimentally. A theory is proposed taking into account the polyelectrolyte effect and redistribution of PEG within DNA coils. This approach makes it possible to describe the dependence of critical value, c, of PEG concentration at the point of DNA collapse on the degree of PEG polymerization, P, and on the concentration of low-molecular salt, n_s. Observation of single DNA molecule in solution of PEG has been carried out by means of fluorescence microscopy which allows one to observe the conformation of individual DNA directly. Direct evidence that the coil–globule transition of DNA occurs as first order phase transition was obtained. It was confirmed that the critical concentration of PEG decreases with an increase of the degree of PEG polymerization and salt concentration. The width of the coexistence region of coil and globule was found to be dependent on salt concentration and degree of polymerization of PEG. It was found that DNA undergoes re-entrant globule–coil transition in concentrated solution of high-molecular weight PEG. These experimental results correspond well to the theoretical predictions. © *1995 American Institute of Physics.*

I. INTRODUCTION

The phenomenon of the collapse of DNA macromolecules in an aqueous solution of polyethylene glycol (PEG) has been discovered by Lerman.[1] It was shown that DNA coils swollen in water shrink abruptly when a suitable amount of PEG is added to the solution. Since that time this effect attracted considerable attention both from experimentalists[1–3] and theoreticians[4–8] because of its fundamental significance and important applicability for molecular biology.

The general physical explanation of DNA collapse in PEG solutions is very simple. The contacts between DNA and PEG are considered to be thermodynamically unfavorable. Therefore the solvent quality for DNA becomes poorer upon the addition of PEG, that is, the effective attraction between the segments of DNA macromolecule increases. As a result, at some critical concentration of PEG the abrupt contraction of DNA coil occurs (coil–globule transition). The theoretical consideration of collapse of DNA has been developed in Refs. 4–8, analogously to the theory of coil-globule transition in the solutions of neutral macromolecules (see Refs. 9 and 10).

However, it is well-known that for the other problems of statistical physics of macromolecules, the presence of even a small fraction of charged links drastically changes the picture of conformational transitions.[11,12] This should be especially important for DNA macromolecules, because they are strongly charged in aqueous solutions. We have found only one paper, namely Ref. 6, where the fact that DNA coils are polyelectrolytes is explicitly taken into account. The corresponding theoretical analysis involves the consideration of Donnan equilibrium between the interior of the DNA coil and the outside solution. However, another important factor, namely the difference in PEG concentration inside DNA coils in comparison with that in the outer solution, was practically not taken into account in Ref. 6. On the other hand, from our experience in the theory of the collapse of polyelectrolyte networks,[11,12] we know that this factor is very essential and its accounting leads to drastic changes in the transition behavior. This was in fact initial motivation for the present study; we have discovered that our present understanding of the collapse phenomenon in other polyelectrolyte systems[11–13] (mainly in polyelectrolyte gels) does not correlate with the older theories of DNA collapse. One of the aims of the present work is thus to develop the simple theory of DNA collapse, taking into account the factors which play the most important role in the related problems of the collapse of polyelectrolyte gels and polyelectrolyte flexible macromolecules.

In the next section we will derive the free energy of DNA coils in PEG solutions. The results of the calculations for DNA collapse transition are presented in Sec. III.

Recently we have published the result on the direct observation of DNA collapse in PEG solution by using fluorescence microscopy.[14] Observation of the conformational changes of individual DNA has given the direct evidence of

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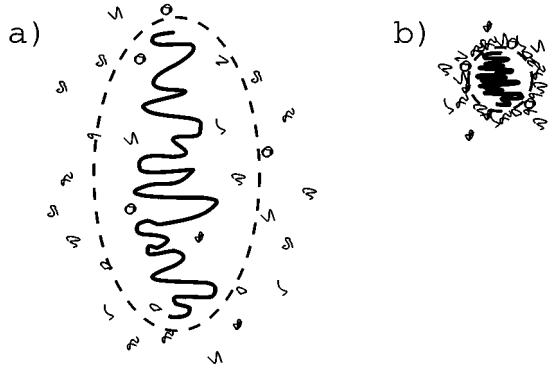

FIG. 1. Schematic representation of conformation DNA in a solution of PEG. The broken line indicates the effective volume of the DNA; (a) regime of good compatibility; (b) regime of perfect segregation.

the discrete character of coil–globule transition. Before this study, it had been obscure whether the coil–globule transition of DNA is discrete or diffuse, since under use of another methods, such as light scattering or viscosity measurement, the values of radius of gyration, for example, were obtained as averaging over ensemble of the molecules (see Ref. 15). In the present paper we have extended the experiment to know the dependence of critical PEG concentration at the transition point c on the degree of PEG polymerization P and on the concentration of low-molecular salt. These experimental results are presented in Sec. IV, where the comparison with theoretical results of Sec. III is given as well. The comparison shows, in particular, that to understand experimental results it is necessary to take into account some specific features of the behavior of PEG in aqueous solutions.

II. FREE ENERGY OF DNA IN AQUEOUS PEG SOLUTIONS

We will consider DNA macromolecules as a long stiff polyelectrolyte chain with a persistent flexibility mechanism. Let us suppose that this chain is immersed in the solution of linear flexible polymer (i.e., PEG) and low-molecular salt.

Following the approach used in our theory of collapse of polyelectrolyte networks,[12] we divide the total volume of the system into two parts, the volume occupied by DNA coil and the external polymer solution (see Fig. 1). Flexible (PEG) chains can penetrate inside DNA coils but their concentration in this region is different from that in the external solution. To describe DNA collapse we will write the free energy of DNA and free energy of external solution. The equilibrium size of the DNA coil will be determined from the condition of the minimum free energy.

Let us introduce the following notation: N is the total number of Kuhn segments of length l and d is the characteristic diameter of DNA double-helical chain. As to PEG molecules, we will assume that they are flexible and have the degree of polymerization P. To consider the effect that DNA macromolecules are strongly charged, let us denote the number of charges per DNA chain as Q. Of course, Q counterions should be also present in the vicinity of each DNA coil to compensate its charge.

The free energy of DNA coil, F, can be written as a sum of three terms (cf. Refs. 11 and 12),

$$F = F_{\mathrm{mix}} + F_{\mathrm{el}} + F_{\mathrm{tr}}, \tag{1}$$

where F_{mix} describes the free energy of mixing of DNA and linear polymer, F_{el} describes the elastic part of the free energy for DNA chains, and F_{tr} is the translational entropy of small ions in the system (i.e., counterions and ions of low-molecular salt). Of course, direct electrostatic interactions between charged species should be also taken into account, however, as it has been shown in Ref. 12 the direct electrostatic contribution to the free energy is always negligible in comparison with F_{tr}. Direct calculations show that this is true for the present case as well.

To write down the term F_{mix} we will use the Flory–Huggins lattice model,

$$\begin{aligned}\frac{F_{\mathrm{mix}}}{kT} = \Bigg[& \frac{\Phi_P}{P} \ln \frac{\Phi_P}{P} + (1-\Phi-\Phi_P)\ln(1-\Phi-\Phi_P) \\ & + \chi_{NP}\Phi\Phi_P + \chi_{NS}\Phi(1-\Phi-\Phi_P) \\ & + \chi_{PS}\Phi_P(1-\Phi-\Phi_P)\Bigg] \cdot \frac{V}{d^3},\end{aligned} \tag{2}$$

where T is the temperature, k is the Boltzmann constant, V is the volume of the DNA coil, and d is the elementary spacing in the Flory–Huggins model which was defined to be equal to the width of the DNA Kuhn segment. In Eq. (2) Φ is the volume fraction of DNA monomer links inside the DNA coil, and Φ_P is the volume fraction of PEG molecules; due to the incompressibility condition, the volume fraction of the solvent is thus equal to $1-\Phi-\Phi_P$. The values χ_{ij} $(i,j = N,P,S)$ are the Flory–Huggins interaction parameters between DNA chains (N), PEG molecules (P), and solvent molecules (S). Of course, Eq. (2) is based on a very simplified model. However, any other equations of state for polymer solution can be used instead of Eq. (23); this will not change the main qualitative results. Therefore we prefer to remain within the Flory–Huggins approach which is most familiar to polymer physics.

Following Ref. 10 the elastic free energy F_{el} can be written in the form

$$\frac{F_{\mathrm{el}}}{kT} = \frac{3}{2} \cdot \left(\alpha^2 + \frac{1}{\alpha^2} \right), \tag{3}$$

where α is the expansion factors of the DNA coil with respect to its ideal size, $R_0 = N^{1/2} l$: $\alpha = R/R_0$ (R being the actual average size of the DNA coil).

The first term in Eq. (3) is responsible for the entropy loss of DNA coils when they expand with respect to the ideal size, while the second term describes the entropy change under strong contraction of the coils. The interpolation formula (3) was first proposed in Ref. 16 and later successfully used to describe the collapse of polymer gels. By taking into account the following relationship:

$$\Phi = \frac{Nl/d}{\frac{4}{3}\pi R^3} d^3 = \frac{Nld^2}{\frac{4}{3}\pi\alpha^3 N^{3/2} l^3} = \frac{3d^2}{4\pi N^{1/2} l^2 \alpha^3}, \tag{4}$$

the formula for F_{el} can be expressed as

$$\frac{F_{\rm el}}{kT}=\frac{3}{2}\cdot\left[\left(\frac{3d^2}{4\pi N^{1/2}l^2\Phi}\right)^{2/3}+\left(\frac{4\pi N^{1/2}l^2\Phi}{3d^2}\right)^{2/3}\right]. \tag{5}$$

The expression for $F_{\rm tr}$ representing the translational entropy of small ions can be written in the form

$$\frac{F_{\rm tr}}{kT}=\left[\left(\frac{Qd}{Nl}\,\Phi+n_s\right)\ln\left(\frac{Qd}{Nl}\,\Phi+n_s\right)+n_s\,\ln(n_s)\right]\cdot\frac{V}{d^3}, \tag{6}$$

where the first term gives the translational entropy of counterions [it is easy to understand that the concentration of counterions inside the DNA coil is equal to $(Qd/Nl)\Phi/d^3$] and the salt ions with the same positive sign (for simplicity we assume that these ions are indistinguishable from the counterions), while the second term describes the translational entropy of the low-molecular salt ions of the opposite sign. In Eq. (6) n_s is the concentration of salt ions inside the DNA coils in dimensionless units, i.e., the actual concentration is multiplied by b^3, where b is the characteristic size of an ion.

In conformity with the above-stated assumption the free energy of the external solution (outside DNA coils) should be written in the form

$$\frac{F^{\rm ext}}{kT}=\left[\frac{\Phi_P^0}{P}\ln\left(\frac{\Phi_P^0}{P}\right)+(1-\Phi_P^0)\ln(1-\Phi_P^0)+\chi_{PS}\Phi(1-\Phi_P^0)+2n_s^0\,\ln(n_s^0)\right]\cdot\frac{V'}{d^3}, \tag{7}$$

where V' is the volume of the solution outside the coil (the limit $V'\gg V$ is taken in the final formulas), Φ_P^0 and n_s^0 are the PEG volume fraction and salt concentration in the outside solution. We will assume here and below that Φ_P^0 and n_s^0 are also average values for all solution which is the case if volume fraction occupied by DNA coils is very low.

Equilibrium values of Φ, n_S, and Φ_P are determined by the following three equilibrium conditions:[11]

(1) the equality of osmotic pressure,

$$-\frac{F}{V}+\frac{\partial(F/V)}{\partial\Phi}\,\Phi+\frac{\partial(F/V)}{\partial n_s}\,n_s+\frac{\partial(F/V)}{\partial\Phi_P}\,\Phi_P=-\frac{F^{\rm ext}}{V'}+\frac{\partial(F^{\rm ext}/V')}{\partial n_s^0}\,n_s^0+\frac{\partial(F^{\rm ext}/V')}{\partial\Phi_P^0}\,\Phi_P^0; \tag{8}$$

(2) the equality of flexible polymer (PEG) chemical potentials,

$$\frac{\partial(F/V)}{\partial\Phi_P}=\frac{\partial(F^{\rm ext}/V')}{\partial\Phi_P^0}; \tag{9}$$

(3) the equality of low-molecular salt chemical potentials,

$$\frac{\partial(F/V)}{\partial n_s}=\frac{\partial(F^{\rm ext}/V')}{\partial n_s^0}. \tag{10}$$

By substituting Eqs. (1)–(7) into the conditions (8)–(10) the following system of three equations is obtained:

$$\frac{\Phi_0^{2/3}\Phi^{1/3}d}{Nl}-\frac{\Phi^{5/3}d}{\Phi_0^{2/3}Nl}+\Phi\left(1-\frac{Qd}{Nl}\right)+(\chi_{NS}+\chi_{PS}-\chi_{NP})\times\Phi\Phi_P+2(n_s-n_s^0)+(\Phi_P-\Phi_P^0)(1-1/P)+\ln\left(\frac{1-\Phi-\Phi_P}{1-\Phi_P^0}\right)+\chi_{\rm NS}\Phi^2+\chi_{PS}[\Phi_P^2-(\Phi_P^0)^2]=0, \tag{11}$$

$$\frac{1}{P}\cdot\ln\left(\frac{\Phi_P}{\Phi_P^0}\right)-\ln\left(\frac{1-\Phi-\Phi_P}{1-\Phi_P^0}\right)-(\chi_{NS}+\chi_{PS}-\chi_{NP})\Phi+2\chi_{PS}(\Phi_P^0-\Phi_P)=0, \tag{12}$$

$$\ln(n_s)+\ln\left(n_s+\frac{Qd}{Nl}\,\Phi\right)=2\cdot\ln(n_s^0), \tag{13}$$

where

$$\Phi_0=\frac{3d^2}{4\pi N^{1/2}l^2}. \tag{14}$$

This system is solved numerically for different values of the parameters. The results of calculations are presented in the next section.

III. NUMERICAL RESULTS

The mathematical analysis of systems (11)–(13) show that, depending on the parameters of equations, the system has either one or three solutions; in the latter case only two solutions are of physical meaning, since the third solution corresponds to the maximum of free energy. Nevertheless the solutions with physical meaning (irrespective of number of solutions) always obey two limiting situations. The first situation is the regime of good compatibility between PEG and DNA [see Fig. 1(a)]. PEG molecules practically freely penetrate inside the DNA coil, so that the composition of water/PEG within the DNA coil is the same as the composition of water/PEG in the external solution. In this regime DNA has swollen coil conformation. It should be pointed out that existence of this regime is caused by the availability of free-moving counterions creating osmotic pressure exerting DNA. On the other hand, the presence of counterions leads to the improvement of compatibility between two different polymers since in the case of phase separation translational loss is very high.[13] The second limiting situation is the regime of practically perfect segregation between the DNA chain and PEG molecules; $\Phi_P\ll\Phi_P^0$ [see Fig. 1(b)]. PEG molecules segregate from the DNA coil and impose additional osmotic pressure which induces contraction of the DNA to the globular state with an increase of Φ_P^0. If systems (11)–(13) have three solutions, the transition from first regime to second one occurs as a phase transition with a discrete jump in DNA size. The phase transition point is determined according to the Maxwell rule.

Now let us discuss the results of numerical calculations.

Clearly the exact shape of the curve $\Phi(\Phi_P^0)$ can be calculated only with precise knowledge of values of all parameters of these equations. It is known from the literature that some of these parameters, for example χ_{PS}, depend on the

volume fraction of PEG and temperature (see e.g., Ref. 17). But to understand the most essential features of DNA in PEG solutions we would like to discuss some limiting cases as the first step of theoretical analysis. Thus, let us suppose that $\chi_{PS}=\chi_{NS}=0$ because of good compatibility of DNA and PEG with water at room temperatures.

Parameters which characterize DNA molecules were chosen to be typical for the experimental investigation of DNA compactization. We took data from Refs. 5 and 18 reported to us that the radius of gyration h of T2DNA ($M=1.24\times10^8$) in 0.2 M NaCl is equal to 2.52×10^{-4} cm. Contour length L_c of DNA can be roughly estimated as $L_c\sim Md/660$ Å; the diameter of DNA double-helical chain is about $d\sim20$ Å. Thus volume fraction Φ of DNA links within the DNA coil can be estimated as $\Phi\sim L_c d^2/h^3\sim10^{-4}$. We took for our calculations, $Nl/d=M/660\sim10^5$, $\Phi_0\sim N^{-1/2}\sim0.008$, $Qd/Nl=0.1$. Under these values of parameters in the solution of low-molecular salt with dimensionless concentration n_s equal to 0.0054, the system of Eqs. (11)–(13) gives an equilibrium value of Φ approximately equal to 10^{-4}, i.e., we varied parameters Q and Φ_0 to obtain a desirable value of Φ. For the case of NaCl, dimensionless concentration of salt $n_S=0.0054$ corresponds to 0.2 M. As for parameter χ_{NP}, it was chosen as 0.4; $\chi_{NP}=0.4$.

In Fig. 2 the dependencies of $\alpha=(\Phi_0/\Phi)^{1/3}$ (a) and Φ_P (b) on Φ_P^0 for different values of the degree of polymerization P are presented. At small values of P (curve 1) the regime of good compatibility is realized even at high values of Φ_P^0. In this case the DNA coil exhibits only a small decrease of the size with an increase of Φ_P^0. At higher values of P (curves 2–4), systems (11)–(13) have three solutions and curve $\alpha(\Phi_P^0)$ consists of the two parts with sharp transition between them; the first part corresponds to good compatibility and swollen DNA coil; the second part describes compressed DNA and regime of perfect incompatibility. As P increases the point of transition $\Phi_P^{\rm cr}$ shifts to smaller values of Φ_P^0. At the end, in the case of very high values of P PEG and DNA are incompatible at all values of Φ_P^0 (the system has only the solution corresponding to perfect segregation even at extremely small values of Φ_P^0). DNA contracts monotonously and reaches a globular state at very low values of Φ_P^0. These results are natural since incompatibility of stiff and flexible polymers is a well-known entropic effect,[19] and this effect becomes more pronounced as the degree of polymerization increases.

Figure 3 illustrates the influence of low-molecular salt concentration n_S on $\alpha(\Phi_P^0)$. Here, the less the salt concentration, the higher the value $\Phi_P^{\rm cr}$ at the transition point, and the less the contraction of DNA before the discrete transition into globule. This is due to the fact that the role of osmotic pressure of counterions of DNA molecules is relatively smaller in the presence of other small ions.

These theoretical results qualitatively coincide with the experimental data reported in Refs. 1, 2, 5, and 20–22. Namely, compactization of DNA is generated at sufficiently high values of degree of polymerization P of PEG and low-molecular salt concentration n_S. The transition point shifts to lower values of Φ_P^0 with the increase of n_S.

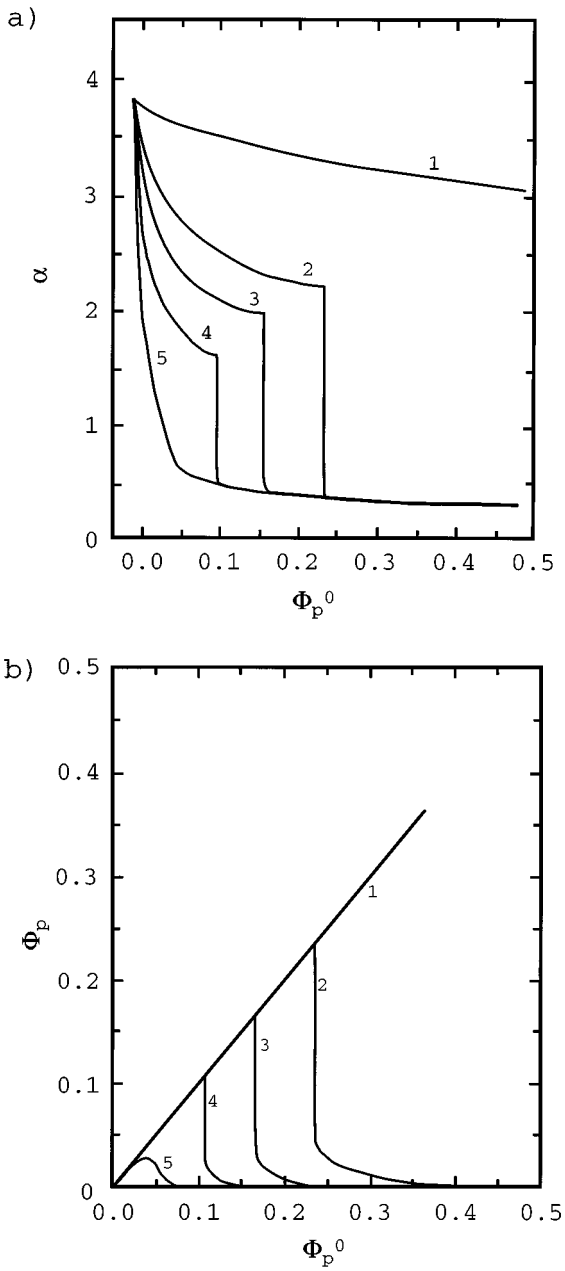

FIG. 2. Dependencies of α (a) and Φ_p (b) on Φ_p^0 at $n_S=5.4\times10^{-3}$ and $P=4(1)$, 60(2), 150(3), 450(4), 2000(5).

Figure 4 illustrates quantitative comparison of our theoretical results with experimental data and the theoretical curve obtained in the framework of Frisch and Fesciyan theory (see Ref. 6). It should be pointed out that both the experimental data and the theoretical curve (it is drawn as a dashed line) are taken from the same paper.[6] In Fig. 4, the dependence of PEG critical concentration corresponding to DNA compactization on ionic strength μ is presented. This dependence was obtained for λ DNA with the molecular weight $M=3\times10^7$ in solution of PEG with the degree of polymerization $P\sim100$ and NaCl. Our calculations were performed for the following parameters chosen to be in correspondence with experimental conditions: $Nl/d=45\ 000$; $P=100$; $\chi_{NP}=0.45$; $\Phi_0=0.01$; $Qd/Nl=0.1$. One can see

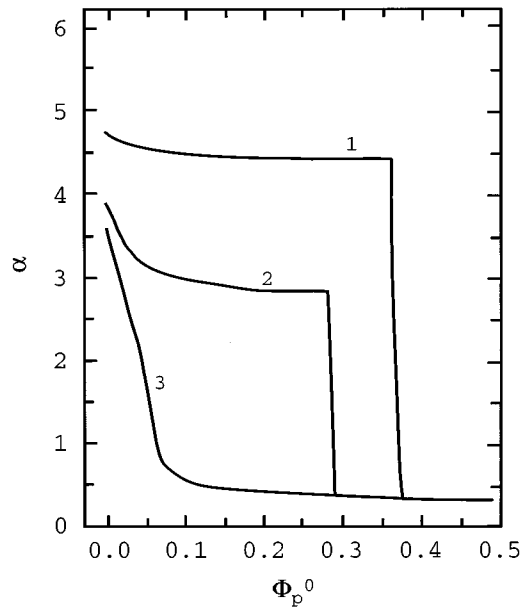

FIG. 3. Dependencies of α on Φ_p^0 for $P=60$ and $n_S=10^{-3}$ (1); 4×10^{-3} (2); 10^{-2} (3).

that the theory proposed predicts more pronounced dependence $c(\mu)$ which at low values of μ deviates strongly from the nearly linear dependence $c(\mu)$ obtained in Ref. 6; this fact has better correlation with the experimental data. On the other hand, at high values of μ our theory predicts stronger dependence c on μ and lower critical value c in comparison with experimental data. It suggests that to describe the dependence $c(\mu)$ quantitatively it is necessary to take into account also the nonzero values of the parameters χ_{PS}, χ_{NS}, and additional factors such as condensation of counterions

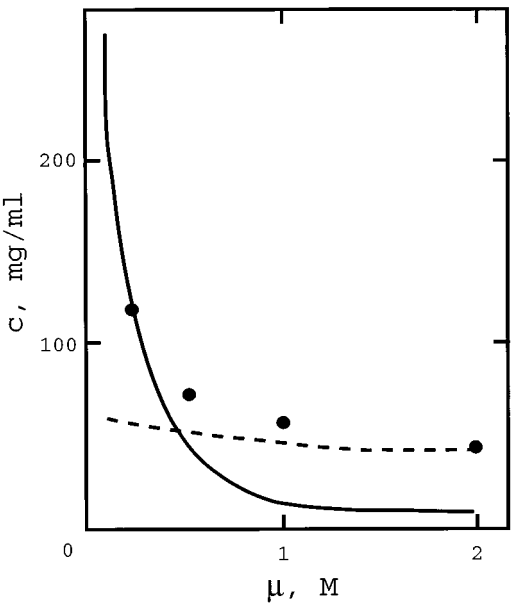

FIG. 4. Comparison of theoretical and experimental data. Dependence of critical PEG concentration c on ionic strength μ; closed circle, experimental data from Ref. 6; dashed line, theoretical curve from Ref. 6; solid line, theoretical curve calculated by Eqs. (11)–(13).

which leads to a decrease of the effective charge of DNA (parameter Q). Besides, our experiments on direct observation of DNA conformation (see Sec. IV) show that at high salt concentration the coexistence region of coil and globule states is rather wide. Thus, the discrepancy between experimental and theoretical results at high salt concentration can be explained by the fact that experimental determination of the transition point is somewhat ambiguous. To conclude, although the fit of experimental data of Ref. 6 to the theoretical predictions of the present paper shows rather strong deviations, however, the qualitative behavior is described quite satisfactory.

To obtain the experimental verification of theoretical results and to receive deeper insight into the features of DNA coil–globule transition in PEG solution, we have carried out an investigation of DNA conformation with fluorescence microscopy. In the next section we discuss the results of our experimental exploration.

IV. EXPERIMENT

T4DNA ($M=1.08\times10^8$) was obtained from Takara Shuzo Co., Ltd., and from Nippon Gene. PEG was partly purchased from Kishida Chemical Co., Ltd., and in part was gifted by Nihon Oils and Fats Co., Ltd., and it was used without additional purification. The fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), and antioxidant, 2-mercaptoethanol (2-ME), were purchased from Wako Pure Chemical Industries, Ltd.

The sample solutions were prepared in accordance with following procedure. At first, PEG was dissolved in distilled water (~55 °C); then other components were added to the aqueous solution of PEG on the following consequence: DNA, DAPI, 2-ME, and NaCl. The final concentration of DNA, DAPI, and 2-ME were kept constant in all experiments; DNA in nucleotide, 0.6 μM; DAPI, 0.6 μM; 2-ME, 4% (v/v). The concentrations of PEG and NaCl were adjusted as desired. The sample prepared stood still for 35 min before direct investigation and then observations were carried out at room temperature (~20 °C) in buffer solution to keep *p*H.

Fluorescence images of DNA molecules were observed by use of Nikon TMD microscope equipped with a 100× oil-immersed objective, and were recorded on videotapes with a high sensitive Hamamatsu SIT TV camera (see Fig. 5, *I*). The data were analyzed with an image processor, Argus 10 (Hamamatsu Photonics).

To characterize the DNA sizes we have measured the length L of long axis of DNA defined as the longest distance in the outline of the DNA image. To find the distribution of L the values of L were averaged over a hundred different molecules. In the region of coil–globule transition of DNA this distribution has two maxima and it is this fact that proves directly the discrete character of DNA conformational transition.[14]

Figure 5 shows fluorescence images of the DNA molecule (*I*) and the corresponding quasi-three-dimensional representation (*II*), where *I* is the intensity of fluorescence light. Given here are the imprints of DNA staying in coil (a), globule (c) conformation, and in the situation when both coil and

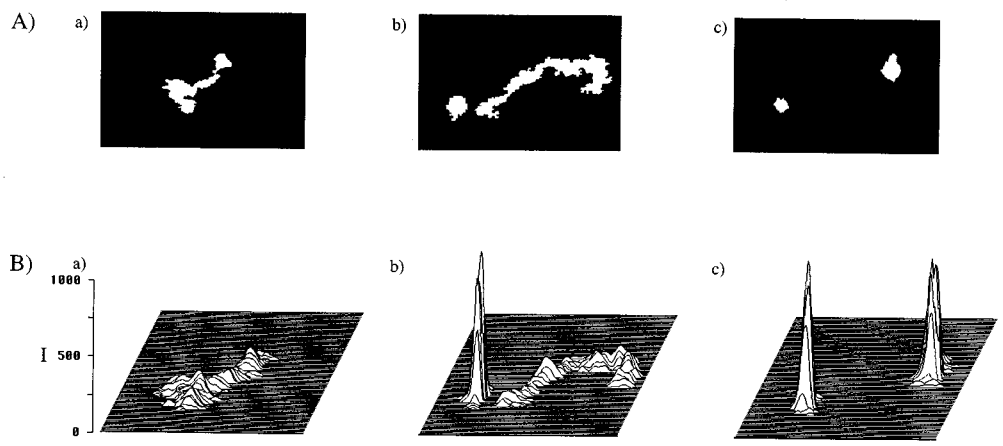

FIG. 5. Fluorescence images of T4DNA (A) and corresponding three-dimensional representation (B). I is the intensity of fluorescence light; (a) coil conformation; (b) coexistence of coil and globule conformation; (c) globule conformation.

globule states coexist (b). To make sure that each obstacle in the fluorescence image actually represents the single DNA molecules, we have integrated the intensity of fluorescent light over individual obstacles. From this procedure, we have found that the total intensity of fluorescence light of separated obstacles approximately (within experimental error about 10%) equal to each other for different obstacles which show up DNA on coil and globule states. It confirms that we really observe the compactization of single DNA molecules, and that the transition of DNA from coil to globule state is not accompanied by the process of intermolecular aggregation.

Figure 6 shows the dependencies of DNA size L on the concentration of PEG of a different degree of polymerization. Since under high concentration of PEG the intermixing of PEG, DNA, and other species is rather slow, we have prepared the mixed sample with gentle heating. Namely, a mixed solution was kept at 55 °C for 15 min; then it stood for 20 min at room temperature (~20 °C) before observation. In this experiment we used 10 mM tris-HCl buffer (pH $=7.9$).

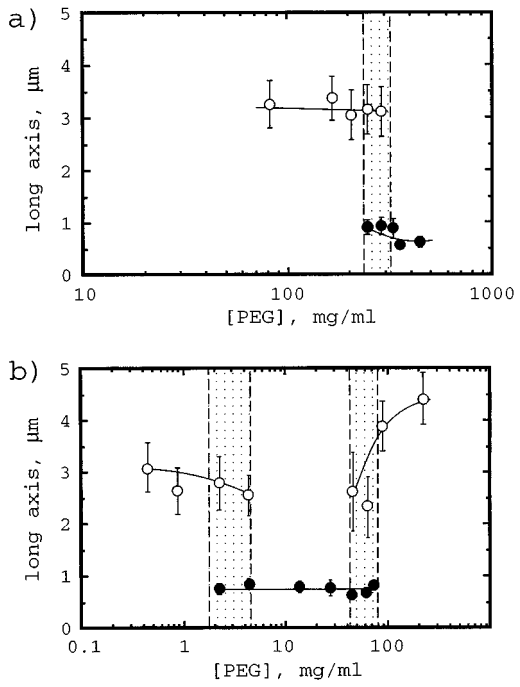

FIG. 6. The dependencies of DNA long axis L on PEG concentration with a different degree of polymerization; $P=186$ (a); 454 (b). Open circles indicate the coil state; solid circles indicate the globule state.

In the solution of PEG with a degree of polymerization $P=186$, sharp first-order phase transition of DNA to the globule state was observed at PEG concentration c equal approximately to 250 mg/ml [Fig. 6(a)]. In the case of longer macromolecules of PEG with a degree of polymerization $P=454$, the DNA collapse occurs at smaller values of PEG concentration; $c\sim 3$ mg/ml [Fig. 6(b)]. This kind of experimental trend coincides with previous experimental data,[6,20–22] and theoretical results discussed in Sec. III.

Figure 6(b) has another interesting and striking feature; DNA returns to the expanded coil state at high values of PEG concentration. This transition is realized also in a jumplike manner as first order phase transition. To our knowledge the effect of re-entrant decollapse of DNA in concentrated PEG solution has never been observed until now. Experimental methods used allows an estimate of the width of the region of the coexistence of coil and globule state; in this region distribution of the size of DNA coils has two maxima. One can see that the region of coexistence of coil and globule DNA is somewhat wider in the case of longer PEG macromolecules. On the other hand, it is worthwhile to mention that, with an increase of time the coexistence region becomes narrower in both cases; metastable coils and metastable globules gradually change to more stable globule and coil states, respectively.

We also studied the dependence of critical PEG concentration c of the DNA collapse on the NaCl concentration. We investigated DNA in solution of PEG with degree of polymerization P equal to 200 and of 10 mM phosphate buffer (pH=7.2). Obtained results are shown in Fig. 7; here theoretical dependence is given as well. The theoretical curve was calculated by Eqs. (11)–(13) with the following values

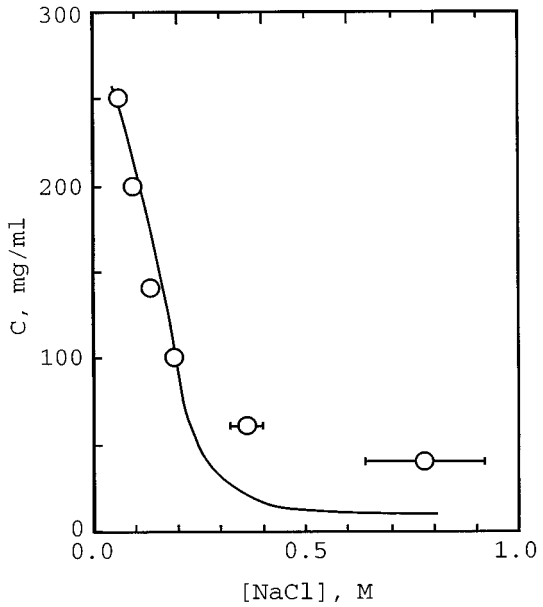

FIG. 7. The dependence of the critical concentration c of PEG with $P=200$ on NaCl concentration; circle, experimental data; line, theoretical curve calculated by Eqs. (11)–(13). The horizontal bar at high concentrations of NaCl indicates the width of the coexistence region of coil and globule states at a fixed PEG concentration.

of parameters: $Nl/d=10^5$; $\Phi_0=0.008$; $Qd/Nl=0.1$; $\chi_{NP}=0.45$. In this experiment we have determined the concentration of NaCl at which DNA undergoes collapse in the solution with given values of PEG concentration. To define critical concentration we obtained distribution DNA over length for different salt concentration. Critical concentration was estimated as concentration at which distribution of DNA over length has two maxima, i.e., coil and globule states coexist. At low PEG concentration region of salt concentration where this distribution has two maxima is rather wide; the width of this region is indicated by horizontal bars. In the case of higher PEG concentration a region of coexistence of coil and globule is rather small; its width is smaller than size of a printed circle. In correspondence with results of Sec. III, it was obtained that the more the salt concentration is the less the value of PEG concentration at the point of DNA collapse transition (compare Figs. 4 and 7). The dependence c on salt concentration was found to be rather strong at small values of NaCl concentration, and these experimental results are consistent with theoretical calculations and experimental data of Ref. 20. However, at high salt concentration, the fit of experimental data to the theoretical prediction is not satisfactory; theory gives smaller values of critical concentration. We have already discussed possible reasons for this difference in Sec. III. In addition, just point out that the experimental ambiguity on the definition of the transition point on the high values of NaCl concentration is rather high.

Thus experimental results on the dependence of DNA collapse on PEG and NaCl concentration are in good agreement with data discussed in Sec. III. As to the re-entrant transition of DNA from globule to coil state in the concentrated solution of high-molecular weight PEG, this effect can be caused by the change of χ_{PS} with the increase of Φ_P^0 and n_S (or ionic strength μ). The fact that χ parameters are not constant is familiar to polymer science, for example, this is the main feature of the so-called "new Flory theory."[23] However, in the present case the dependence of χ_{PS} on various parameters should be very complex because in addition to the usual dependence of χ_{PS} on Φ_P^0 we should take into account the influence of polyelectrolyte (DNA and DAPI) on χ_{PS}. Indeed, it is known that PEG is compatible with water because in aqueous solution water molecules associate with oxygen groups of PEG and form a solvation layer.[24] This layer is destroyed if a low-molecular electrolyte is added in solution[25–28] and compatibility of PEG and water becomes poorer. One can expect that the similar effect is observed for PEG molecules near the DNA coil because DNA is a strong polyelectrolyte. This effect can be taken into account by simple renormalization of the parameter of the interaction of PEG and water. Namely, we should introduce the additional parameter of interaction of PEG and water within the coil of DNA which will be different from χ_{PS}; $\chi_{PS}^{\text{in}} > \chi_{PS}$. The tendency of destruction of the solvation layer for high enough values of molecular weight between PEG can lead to the fact that the lower critical temperature of separation PEG and water becomes lower than the room temperature; PEG and water become incompatible in the vicinity of DNA coil at room temperatures. As soon as water and PEG are incompatible PEG molecules will try to segregate from the regions in the vicinity of DNA coils. Therefore, the osmotic pressure of PEG will decrease drastically and DNA can undergo a sharp reentrant decollapse. Thus, the PEG–water interaction may be of crucial importance for the re-entrant decollapse effect.

In fluorescence microscopy measurements we have used DAPI as a dye strongly attached to DNA.[29,30] Another important effect is that the complexation of the ions of DAPI which contain two positively charged amidinium groups with DNA induces the release of monovalent H^+ counterions of DNA to the external solution. The resulting gain in the translational entropy of these counterions is very high (cf. Refs. 12, 13). Thus we should take into account this ion-exchange reaction while describing the DNA immersed in the solution containing DAPI and this can modify essentially the equation obtained above.

Recently we have shown that availability of DAPI at the concentration used in our experiment does not lead to change of DNA radius of gyration.[31] Nevertheless its presence will modify the parameters χ_{PS}, χ_{NS}, and χ_{NP} of interaction between DNA, PEG, and water. In fact, the interaction of PEG with DAPI seems to be very advantageous from the energetic point of view because of the attraction between oxygen groups of PEG and DAPI groups containing nitrogen. This effective attraction increases with the increase of the degree of polymerization of PEG and of PEG concentration Φ_P^0. At high values of P and Φ_P^0 it can lead to a considerable effective decrease of binding constant between DAPI and DNA. This factor may also contribute to the reentrant decollapse of DNA.

To make clear what is the main effect causing the reentrant transition, additional experimental and theoretical ex-

ploration should be carried out. We are planning to perform such analysis in one of the forthcoming papers.

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