Observation of two forms of conformations in the reentrant condensation of DNA

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Reentrant condensation of DNA in the presence of spermidine (SPD) is studied by gel electrophoresis (GEP). It is found that the reentrant condensation of DNA induced by SPD can produce a reentrant jamming of DNA molecules at the liquid-gel interface during GEP. However, not all the DNA are jammed at the interface indicating that there are different forms of condensed DNA. A model of condensed DNA consisting of two conformations can be used to explain the experimental observations. A phase diagram of the reentrant condensation based on the jamming states of DNA in terms of the length of DNA (L) and concentration of SPD is constructed. Furthermore, no charge inversion is observed during the reentrant transition.

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Physical properties of a macromolecule in a polyelectrolyte [1] is a fascinating subject because it is not only of interest in the fundamental understanding of physics of polymers and electrolytes but also of vital importance in the biological processes such as protein folding [2], the packing of DNA [3] in cells, etc. Since DNA is a charged polymer, the packing of DNA means attractive interactions between like charges [4]. Recently quite a few studies [5,6] have been devoted to the understanding of how this attractive interaction can be generated by condensing agents [7]. However, the nature of this condensation remains unclear.

A peculiar property of this condensation is that DNA will be condensed only in a limited range of the concentration of the condensing agent. This dissolve-condense-redissolve phenomenon of DNA as the concentration of the condensing agent is being increased is known as the reentrant condensation [8–10]. Although both bulk [8,9] and single molecule [7,10] experiments have been carried out to study this reentrant condensation, many aspects of this condensation remain unknown. For example, very little is known about the conformation of DNA in the reentrant states. Although a phase diagram for the reentrant condensation is proposed by Nguyen *et al.* [11] based on a model of screening of macroions by the condensing multivalent cations, no experimental confirmations have yet been reported. Furthermore, the predicted charge inversion between the dissolved and the redissolved states has never been verified.

In this paper, we report the results of a method to study the reentrant condensation by using gel electrophoresis (GEP) of DNA. The idea is to use GEP to detect the conformations of DNA in the presence of SPD and the sign of the charge of the DNA/SPD complexes formed can also be inferred at the same time. It is found that SPD can induce a reentrant partial jamming of DNA molecules at the liquid-gel interface during GEP at concentrations similar to those of the reentrant condensation of DNA. Our findings suggest that this partial jamming is caused by the existence of two forms of condensed DNA-SPD complexes. A phase diagram of the reentrant condensation based on the jamming states of DNA in terms of the length of DNA (L) and the concentration of SPD (c) is constructed. The shape of the phase diagram is, in general, in agreement with the form proposed in Ref. [11] for the reentrant transition of DNA. However, no charge inversion is found. A remarkable feature of the phase diagram, which is not predicted by the theory, is that the dejamming or redissolve concentration of SPD is not monotonic in L, suggesting that the mechanism of the reentrant transition might be quite different for the cases of small and large L.

The gel electrophoresis [12] experiments are carried out in a conventional electrophoresis setup. All the gels used in the experiment are 0.7% (weight) agarose. DNA samples with various SPD concentrations are prepared by dissolving both DNA and spermidine (Sigma) in 0.5X TBE buffer (45 mM Tris-borate, 1–1.25 mM EDTA, pH=8.0). The fluorescence dye GelStar (CAMBEX) is used in the experiments for bulk electrophoresis observations. The two main DNA molecules used in the experiments are T4 [165.6 kbp (kilo base pairs), Wako] and λ phage (48.5 kbp, Promega). Shorter DNA molecules are either produced by PCR or purified from digestion products. Note that experiments have been carried out to confirm that the use of different fluorescence dyes and the presence of SPD with various concentrations will not affect the results based on fluorescence intensities reported below.

Figure 1(a) shows the results of the GEP of λ-DNA (8 ng/μl) in the presence of SPD for a period of 10 min in a field of 100 V/10 cm. Before the application of the field, the DNA-SPD solutions with various concentrations of SPD (c) were loaded in the wells and two fluorescence bands are formed for each well after the field has been applied. For example, in Well-D (c=2 mM), it can be seen that only a fraction (α) of the DNA originally in the well can move into

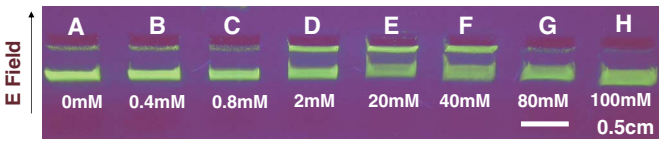

FIG. 1. (Color online) Electrophoresis of λ-DNA in 0.7% agarose gel with various spermidine concentrations in a dc field.

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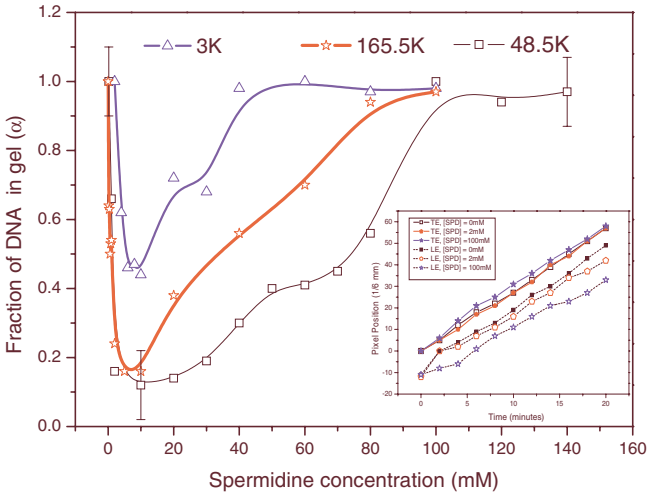

FIG. 2. (Color online) Spermidine concentration dependence of α for three different DNA lengths. The inset shows the position of both the leading edges (LE) and trailing edges (TE) of the moving bands in Fig. 1 as a function of time.

the gel to form the lower band and DNA left in the well forms the upper band close to the lower part of the well. One might think that the DNA/SPD left in the well are stuck at the pores on the interface of the gel. However, a reversal of the field direction in Fig. 1 reveals that most of the DNA/SPD left in the well of Fig. 1 move to the upper part of the well and get jammed there. Obviously, most of the DNA in the upper bands of Fig. 1 are free to move in the solution but could not move into the gel. These DNA can be thought as being jammed at the liquid-gel interface.

A remarkable feature of α is that it is a nontrivial function of c. Figure 2 shows the SPD concentration dependence of α measured from Fig. 1. There is a concentration of SPD at which α is at its minimum. This form of Fig. 2 suggests that there is a jamming and dejamming transition in the transport of DNA into the gel as c is increased. At both very low and very high concentration of SPD, there is no jamming of the DNA at the gel interface. From Fig. 2, two characteristic c, namely, c_j (jamming) and c_d (dejamming), can be defined such that $\alpha \approx 1$ when $c < c_j$ (region I in Fig. 4) and $\alpha \approx 1$ when $c > c_d$ (region III in Fig. 4). Systems with $c_j < c < c_d$ (region II in Fig. 4) can be viewed as in jammed states.

A trivial explanation for the above observation is that the DNA left in the well (upper band) are entangled to form an aggregate. This entanglement effect will be DNA concentration sensitive and the values of measured c_j and c_d would then be a function of the concentration of DNA being used. To test the validity of this simple explanation, experiments similar to those shown in Fig. 1(a) are repeated with various DNA concentrations from 0.8 to 40 ng/μl and c_j and c_d are measured for λ-DNA. The measured values of c_j and c_d show that the jamming-dejamming transition considered above is independent of DNA concentrations. This last observation suggests that jamming can occur even with one single DNA molecule. In order to check for the single molecular nature of the jamming, we have also setup an electrophoresis experiment on top of an inverted fluorescence microscope (Axiovert 200M, ZEISS) equipped with a 100× oil-immersion objective (NA=1.4) by using DNA labeled with YOYO-1 (Molecular Probes). These single molecule experiments show that phenomena similar to those shown in Fig. 1 can be reproduced. That is, within the same sample, some DNA molecules are seen to move into the gel while some of the DNA molecules get stuck in the interface during GEP. However, since both YOYO-1 and SPD will bind to DNA, the c_j and c_d for single molecule experiments are different from those reported here when YOYO-1 is used [13]. From the video images [14], we find that all DNA which can move into the gel is more or less flexible (stretched out) while most of the jammed DNA are in a very compact (a point) form.

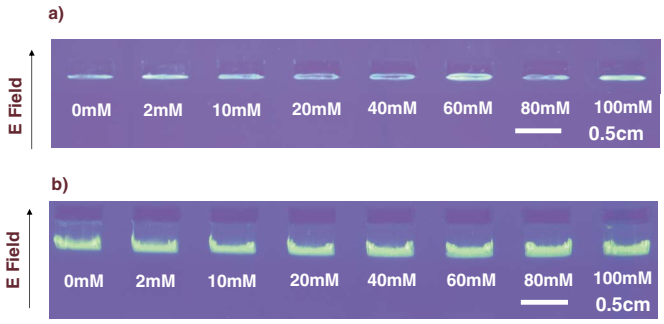

FIG. 3. (Color online) Electrophoresis of λ-DNA with various spermidine concentrations in a dc field in agarose gels with (a) 2 mM SPD and (b) 100 mM SPD.

The picture emerging from the discussions above is that there seems to be two forms of DNA-SPD complexes in the presence of SPD. One of them (referred to as the J form) is presumably not flexible enough to pass through the pores of the gel and being jammed at the interface while the other form (referred to as the F form) can somehow be driven into the gel. An interesting property of these F forms of DNA-SPD complexes is that their GEP bands move with the same speed as the DNA with no SPD as can be seen from the inset of Fig. 2, where the positions of the different GEP bands in Fig. 1 are plotted as a function of time. Although the DNA-SPD complexes from different wells are prepared with different SPD concentrations, they all seem to behave similar to SPD-free DNA when they are moving in the gel. A possible explanation for this observation is that the binding of SPD in these two forms of DNA-SPD complexes is different and therefore produces the different conformations seen in the single molecule images. In this model, the binding of SPD to DNA in the F form is weak and the DNA is more flexible. Since there is no SPD in the gel, once the F form of the DNA-SPD complexes gets into the gel, the SPD originally binding to the DNA can presumably easily unbind from the DNA and then diffuse into the gel. However, as the binding of SPD to DNA in the J form is very strong, even the SPD concentration near the interface of the well is being lowered by diffusion, the J form of the DNA-SPD complex remains intact and cannot move into the gel.

To further confirm our model that the DNA in the moving bands in Fig. 1 is free of SPD, we have also performed experiments with gels in which SPD are added. Figures 3(a) and 3(b) show the results of GEP experiments similar to that

of Fig. 1 except that 2 and 100 mM of SPD are added to the gels in Figs. 3(a) and 3(b), respectively. It can be seen clearly from Fig. 3(a) that all the DNA are being jammed at the lower part of the well. That is, even the F form of the DNA-SPD complexes cannot move into a gel with 2 mM SPD. This result supports our model that the F form of the DNA-SPD complexes can easily lose some of the SPD and turn into the J form when the DNA is now in equilibrium with a SPD concentration which is lower than the concentration when the F form was first created. Similarly, in Fig. 3(b), all the DNA can move into the gel with 100 mM SPD. Even the J form can move into a gel with 100 mM SPD. As the 100 mM SPD in the gel diffuses into the well, all the DNA-SPD complexes close to the liquid-gel interface are converted to the reentrant F form of the DNS-SPD complexes. Although the DNA-SPD complexes prepared with 100 mM SPD can move into gels with both zero and 100 mM SPD, the speed of the band in the SPD-free gel is about two times faster than that of the 100 mM gel, suggesting that there might be fundamental differences in conformations or charge distributions.

The phenomena shown in Figs. 1 and 2 are similar to the reentrant condensation of DNA [8–10]. If the jamming picture discussed above is valid, the c_j and c_d will just be the N_c (lower critical concentration of SPD below which DNA will dissolve) and N_d (the upper critical concentration of SPD above which DNA will dissolve) in the notation of Ref. [11]. In fact, the range of concentration for the jamming to occur is also close to that of the reentrant condensation [8–10]. In such a case, c_j and c_d will be determined by DNA and SPD interaction but not by the electrophoresis parameters. Indeed, experiments similar to those in Fig. 1 but with field strengths in the range of 25 V/10 cm to 150 V/10 cm and gel concentrations in the range of 0.5 to 2 % all give the same c_j and c_d within experimental uncertainties. However, from the direction of the motion of the electrophoresis bands, no charge inversion is observed.

Intuitively, the longer the DNA the easier it is for jamming to occur and a larger c is needed to redissolve (reentrant) the DNA. That is, c_j will decrease with L and c_d will increase with L which is one of the general predictions of Ref. [11]. To test this idea, experiments similar to those reported in Figs. 1 and 2 with various L are performed and the results are summarized in a phase diagram (Fig. 4). It can be seen from Fig. 4 that jammings occur only when L is larger than about 3 kbp and c_j decreases with L all the way in our experimental range. The minimal DNA length for jamming to occur can be roughly estimated by assuming the condensed DNA to form a toroidal structure [15] with n parallel segments bundled together. No jamming will occur if the n-bundled toroid is flexible enough to be squeezed into the smallest gel pores. This occurs when $n \times \xi_p \sim$ minimal pore size, where ξ_p is the persistence length of DNA ($\sim$53 nm). Near the minimal jamming length, n is small and one expects the radius of the toroid $\sim \xi_p$. For the current experimental condition, the minimal gel pore size $\sim$100 nm [16], and hence the minimal jamming length $\sim 2\pi n \xi_p \sim 2\pi 100$ nm $\sim$2 kbp, which agrees well with the extrapolation from Fig. 4. On the other hand, c_d increases with L only for $L < 48$ kbp. For both $L=86$ and 166 kbp, c_d is only a quarter

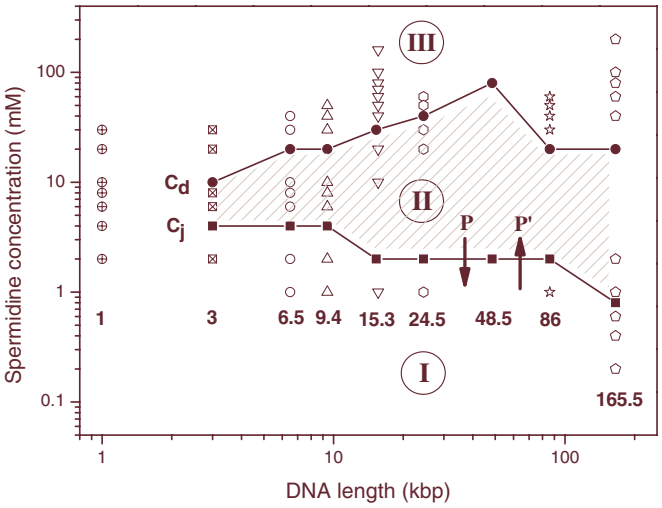

FIG. 4. (Color online) Jamming phase diagram as a function of the length of DNA and spermidine concentrations. The phase boundaries c_j and c_d are determined from $\alpha < 0.05$ and $\alpha > 0.95$, respectively. The concentrations of DNA used in the experiments are in the range of 1–5 ng/μl except for T4 which is 1.6 ng/μl. Symbols are larger than the error of the data points. With the precision shown, the phase diagram is not sensitive to the concentrations of DNA used. See text for the discussion on the paths P and P′. Small symbols: triangles, stars, etc., represent the points where actual data were taken.

of that for $L=48$ kbp. Note that all the initial concentrations in the experiments of Fig. 4 are chosen to be below the overlapping concentrations of the DNA to avoid the possibility of interchain condensation or entanglement.

The nonmonotonic behavior of c_d as a function of L suggests that the mechanism of the reentrant transition might be quite different for the cases of small and large L. One possible scenario is that when the length of DNA is long enough, a Rayleigh-like [17,18] instability can occur which will turn a single charged condensed blob into many smaller blobs. Presumably, a chain with many smaller blobs (the reentrant state) is flexible enough to reptate through the gel. This nonmonotonic dependence of c_d on L is also supported by the measured c dependence of α as shown in Fig. 2 for three different values of L. It can be seen from Fig. 2 that for a given c, the value of α for $L=165.5$ kbp is always in between those of $L=3$ and 48.5 kbp.

Another remarkable feature of Fig. 4 is that there is hysteresis when the system crosses the phase boundary of c_j as shown as the paths P and P′ in Fig. 4. During the experiments, we found that the jamming state of a DNA solution depends on how it was prepared: samples prepared from the dilution of condensed states (region II) will produce jammed states even when their final concentration is in the dissolved range (region I) if the dilution is not strong enough or waiting time is not long enough. For example, a sample of $c = 2$ mM will produce a jammed state even when it is diluted to $c' = c/2$ or $c/4$ if experiments similar to those of Fig. 1 are performed within 10 min of their preparations. It will only produce a dejammed state when it is diluted to $c' = c/10$. This last result explains our finding that although there are diffusions of SPD out of the wells into the gel in experiments

similar to those of Fig. 1, these experiments will produce the same results if they are all conducted within 10 min after the samples are loaded into the wells. However, similar experiments carried out close to c_d appear to be insensitive to the how the final concentration is reached.

From the discussions above, it is clear that the reentrant jamming observed in our experiments is directly related to the reentrant condensation of DNA. It seems that the jamming state of DNA is consisted of a mixture of the J and F forms of DNA. The difference between regions II and III of Fig. 4 might just be the difference in the ratio of these two states. It has been reported [7] that many forms of DNA can coexist in the presence of SPD. However, it is not clear what is the origin of the coexistence of these two forms. Finally, it is now possible to perform single DNA experiments with nanodevices [19]. These devices will be very useful in the detailed study of the properties of the different forms of the condensed DNA when better controls are needed. Furthermore, it might be even possible to develop DNA separation nanodevices based on the jamming and dejamming properties of DNA in the presence of SPD.

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[1] F. Oosawa, *Polyelectroytes* (Dekker, New York, 1971).
[2] V. S. Pande, A. Yu. Grosberg, and T. Tanaka, Rev. Mod. Phys. **72**, 259 (2000).
[3] O. Lambert, L. Letellier, W. M. Gelbart, and J. L. Rigaud, Proc. Natl. Acad. Sci. U.S.A. **97**, 7248 (2000).
[4] B. Y. Ha and A. J. Liu, Phys. Rev. Lett. **79**, 1289 (1997).
[5] I. Rouzina and V. A. Bloomfield, J. Phys. Chem. **100**, 9977 (1996).
[6] N. Gronbech-Jensen, R. J. Mashl, R. F. Bruinsma, and W. M. Gelbart, Phys. Rev. Lett. **78**, 2477 (1997).
[7] T. Iwataki, S. Kidoaki, T. Sakaue, K. Yoshikawa, and S. S. Abramchuk, J. Chem. Phys. **120**, 4004 (2004), and references therein.
[8] J. Pelta, F. Livolant, and J.-L. Sikorav, J. Biol. Chem. **271**, 5656 (1996).
[9] E. Raspaud, M. O. de la Cruz, J.-L. Sikorav, and F. Livolant, Biophys. J. **74**, 381 (1998).
[10] Y. Murayama, Y. Sakamaki, and M. Sano, Phys. Rev. Lett. **90**, 018102 (2003).
[11] T. T. Nguyen, I. Rouzina, and B. I. Shklovskii, J. Chem. Phys. **112**, 2562 (2000).
[12] J.-L. Viovy, Rev. Mod. Phys. **72**, 813 (2000).
[13] Y. Murayama and M. Sano, Biopolymers **77**, 354 (2005).
[14] See EPAPS Document No. E-PLEEE8-75-207703 for observations of reentrant jamming and condensation of DNA during gel electrophoresis. For more information on EPAPS, see http://www.aip.org/pubservs/epaps.html.
[15] B. Schnurr, F. Gittes, and F. C. MacKintosh, Phys. Rev. E **65**, 061904 (2002).
[16] N. Pernodet, M. Maaloum, and B. Tinland, Electrophoresis **18**, 55 (1997); M. Maaloum, N. Pernodet, and B. Tinland, *ibid.* **19**, 1606 (1998).
[17] L. Rayleigh, Philos. Mag. **14**, 184 (1882).
[18] A. V. Dobrynin, M. Rubinstein, and S. P. Obukhov, Macromolecules **29**, 2974 (1996).
[19] See, for example, O. B. Bakajin, T. A. J. Duke, C. F. Chou, S. S. Chan, R. H. Austin, and E. C. Cox, Phys. Rev. Lett. **80**, 2737 (1998); A. Balducci, P. Mao, J. Han, and P. S. Doyle, Macromolecules **39**, 6273 (2005).