Nucleation and Growth in Single DNA Molecules

Kenichi Yoshikawa* and Yukiko Matsuzawa†

Graduate School of Human Informatics
Nagoya University, Nagoya 464-01, Japan

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In living cells and viruses, double-stranded DNA chains usually exist in a compact state. For example, in human cells, DNA chains with a total length of ∼2 m are compacted within the nucleus, which is only a few micrometers in diameter. It is now becoming clear that the higher-order structure of compacted DNA chains affects the mechanism of self-regulation of gene expression in living cells.[1] We have recently found that, with the addition of various condensation agents such as poly(ethylene glycol) (PEG),[2] cationic surfactant,[3] spermidine, and alcohol, an individual double-stranded DNA chain undergoes a first-order phase transition between an elongated coiled state and a compacted globular state. We present here the results of our observations on the time course of the collapse and decollapse of a single DNA chain. We show that a single polymer chain exhibits "nucleation and growth" in the process of forming a compacted globular structure.

With fluorescence microscopy, it becomes possible to observe individual DNA chains exhibiting translational and intramolecular Brownian motion.[4] Figure 1 shows typical fluorescence images of T4 DNA molecules in coiled and globular states with the addition of PEG to the aqueous solution. This figure also shows a schematic representation of the relationship between the fluorescence image and the microscopic structure of the corresponding DNA chain. Due to the blurring effect, the actual size of DNA is smaller by ∼0.3 μm than that observed by fluorescence microscopy.[5] In our experimental system, the time resolution was 1/30 s.

Figure 2 shows the forward and reversed transitions of a single double-stranded T4 DNA chain from an elongated random coil into a compacted globule in an aqueous solution of PEG. It has been confirmed that the integrated intensity of the fluorescence remains almost constant, ±10%, during these processes. The transition point between the coil and globule lies at [PEG] ≈ 6.0 M. Thus, at [PEG] = 6.8 M, as shown in the left side of Figure 2, the individual coil is metastable and has a lifetime on the order of several tens of minutes. During Brownian motion of the metastable coil, a bright spot appears spontaneously at a certain point on the chain. As the fluorescence intensity at the brightest spot gradually increases, the apparent contour length of the chain decreases. The process of compaction from coil to globule is completed within 10 s. The time course of this compaction has been analyzed by measuring the apparent contour length, L, and the light intensity at the brightest spot. Figure 3 shows that both L and the fluorescence intensity exhibit linear time dependence between $t = 0$ and 6 s. After the disappearance of the coil on one side, the remaining

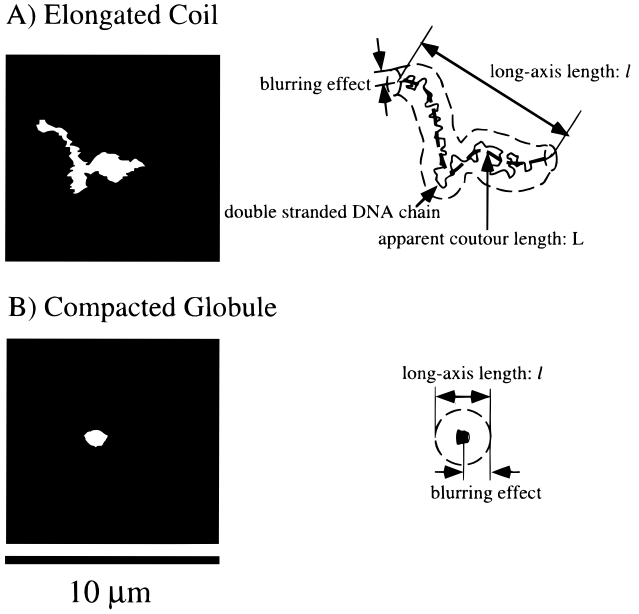

Figure 1. Fluorescence images of a T4 DNA with the coiled state (A) in aqueous solution and with the globular state (B) in 6.8 M PEG (average degree of polymerization, 186) solution. In the schematic drawings, the regions encompassed by the broken lines correspond to the white part in the original pictures on the left. The blurring effect is ∼0.3 μm.[5] The obstacles are T4 DNA molecules (0.6 μM in nucleotide), 166 kbp with a contour length of 57 μm, in aqueous solution[12] with 0.6 μM 4′,6-diamidino-2-phenylindole (DAPI) and 4% (v/v) 2-mercaptoethanol. Under these conditions, it is estimated that ∼5% of the phosphoric group in the DNA chain is electrically neutralized through binding with DAPI, as deduced from the binding constants,[12] and that both the persistence and contour lengths remain essentially constant compared to those in the absence of DAPI. Other experimental conditions are similar to those in previous studies.[5]

coil is pulled into the globule at a rather high speed ($t \approx 6-7$ s in Figure 3A). The brightest spot in the DNA chain in Figure 2 (left) may represent the highly packed DNA, or the "nucleus" of the "DNA crystal". The process of compaction from the metastable coil to the globule is similar to nucleation and growth from a metastable or supersaturated fluid state to a highly condensed or crystal state. The essential difference between the time course in the coil−globule transition of a single DNA chain as a linear string and the nucleation and growth observed in the usual first-order transition is the dimensionality of the system. We have noticed that the speed of the growth of the globule is also nearly constant in the compaction of a DNA chain induced by spermidine (data not shown), suggesting that this is a general feature of the compaction of a single chain. The constancy of the speed of growth may be explained[5] by considering the unique aspects of the nucleation and growth of a single chain, i.e., of the remaining coiled portion, only that which is just adjacent to the globule is pulled into the condensed globular "crystal".

The time profile of the reverse transition from a globule into a coil is shown in Figure 3B. In contrast to the linear change with time in the transition from metastable coil to globule, the reverse transition does not proceed at a constant speed; i.e., the process is initially slow, and then gradually accelerates. Such an asymmetric character between forward and reversed processes corresponds well with the previous observation by Widom and Baldwin for the condensation of DNA induced by $Co(NH_3)_6$.[6]

In the present study, we have described the transition of a single DNA chain. Most previous experimental studies on the

* To whom correspondence should be addressed.
† Present address: Department of Ecological Engineering, Faculty of Engineering, Toyohashi University of Technology, Toyohashi 441, Japan.

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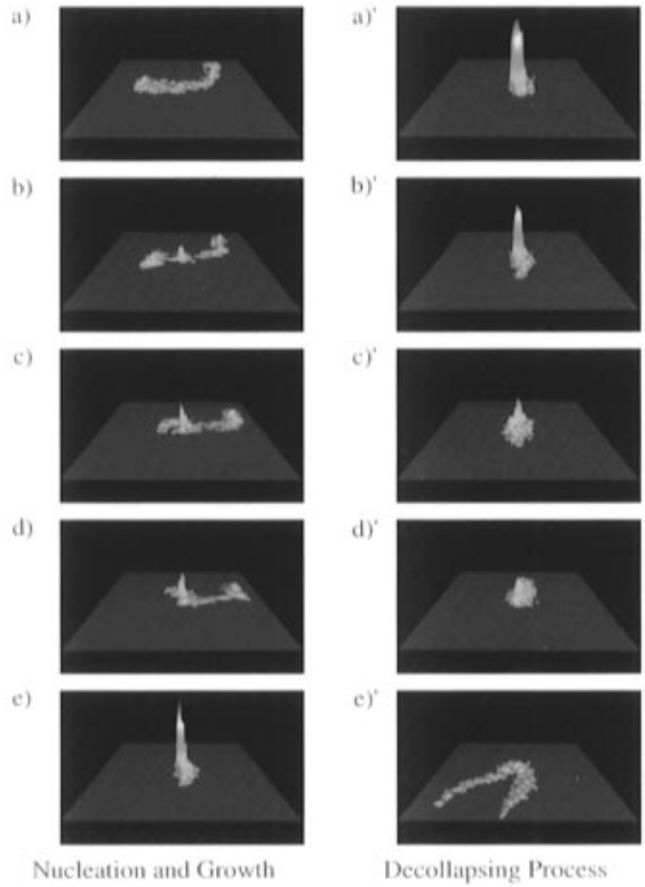

Figure 2. Dynamic process of transition of single T4 DNA molecule. The color indicates the intensity of the fluorescence, corresponding to the spatial density of the segments in a single DNA. We have confirmed that the phenomenon of the nucleation and growth is rather general, as is indicated by the observation of this process for more than 50 molecules. Among the observation, nucleation is most frequently generated at the end of the DNA chain. (Left) Transition from elongated coil to compacted globule. The kinetic process is characterized as "nucleation and growth". The time interval is 2 s. (Right) Transition from globule into coil. The time interval is 3 s, except for the period, 20 s, between d′ and e′.

condensation of DNA chains, induced not only by PEG[7] but also by polyamines,[8] multivalent cation,[6,9] alcohol,[10] etc., have concluded that the transition is steep but continuous, based on measurements of the ensemble of DNA chains. The coil−globule transition of synthetic polymers has also been regarded as continuous.[11] The discrepancy between the continuous nature of the transition in previous studies on DNA solutions and the discreteness of the transition of a single DNA chain is due to the difference in the transition of a single chain and that of an ensemble of DNA chains.

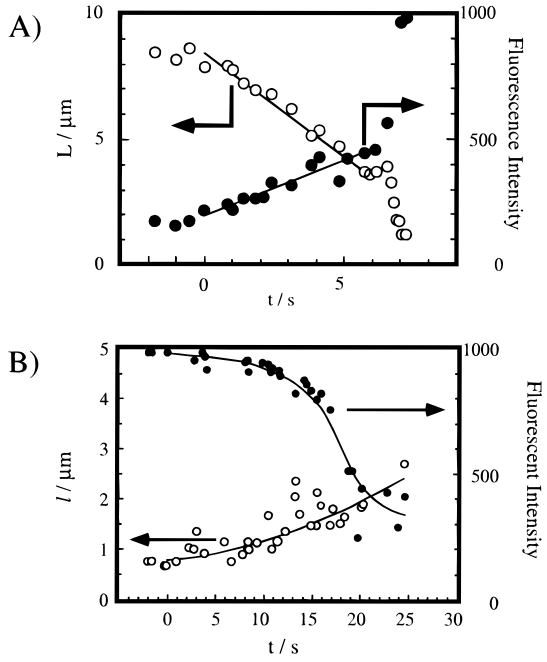

Figure 3. (A) Time course of the transition from coil to globule, showing the increase in the brightest spot at the nucleus and the decrease in the apparent contour length, L, of the chain. The speed of the growth of the nucleus and that of the decrease in the chain length are both constant. The starting point for the process of spontaneous decollapse is taken as $t = 0$. Prior to the start of nucleation, random thermal fluctuation of the DNA chain is observed, with an average apparent contour length of $L \approx 7$ μm. After absorbance of the coil on one side into the globular portion, the remaining part of the coil is pulled into the globule at a relatively high speed ($t \approx 6-7$ s), corresponding to the process between d and e in Figure 2 (left). (B) Time course of the transition from globule to coil, showing a decrease in brightest intensity at the globule spot and an increase in the long-axis length l. This observation was carried out under a concentration gradient in aqueous PEG solution between glass plates. As the PEG concentration decreases due to diffusion within the sample solution, the DNA chain exhibits spontaneous transition. The curve for the time-dependent change in l gives the relationship $l \sim t^{1.8}$, where the exponent is obtained from least-squares fitting.

The fact that long DNA chains exhibit nucleation and growth is important in both biological and physical sciences. Since the activity of transcription from DNA to RNA is expected to be closely related to the compaction or condensation of the DNA chain, the present findings may be useful in understanding the mechanism of self-regulation in gene expression. Especially, it is to be noted that the present work affords us a new insight into the problem of how a long DNA chain can avoid self-knotting *in vivo* in the process of compaction. For physicists and physicochemists, the results of the present study suggest new challenging problems: (1) Which factor determines the steric structure of the compacted globule, i.e., toroid or rod?[9] (2) What effects do various parameters, such as solvent composition, temperature, and concentrations of added chemicals, have on the speed of compaction? (3) Does a critical phenomenon exist in the transition of a long polymer? and (4) Is there any essential difference between the transition of DNA and those of other natural or synthetic polymers?

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