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Controlling the Intrachain Segregation on a Single DNA Molecule

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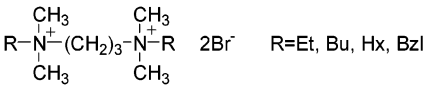
DNA compaction is an important process because the majority of genomic DNA found in microorganisms is in a compact state,[1] and controlling the DNA condensation is currently of intensive interest for the development of approaches to gene transfection.[2,3] On the other hand, DNA compaction caused by a variety of condensing chemicals[4] has attracted much interest for physicists and chemists as an experimental system of the typical transition between the coil and globular state in a linear polyelectrolyte.[5] Early reports described DNA compaction as an all-or-none type transition over a whole molecular chain.[6,7] However, recent studies have revealed a more detailed scenario of DNA compaction with both aspects of thermodynamics and kinetics. As for the kinetic aspect, it was found that DNA compaction is initiated by the formation of a nucleation center on DNA.[8−10] However, from careful single chain observations, it has been confirmed that intrachain segregation between the coil and globule parts is generated as a stable state from the viewpoint of thermodynamics.[11] Actually, it has been shown that, depending on the concentration of condensing agents, the most stable state changes from an elongated coil to an intrachain segregated molecule and, finally, to the fully collapsed state in a stepwise manner.[12−14] In the present study, we investigated the phenomenon of the intrachain segregation on DNA for a more profound understanding of the mechanism of DNA collapse using structurally designed condensing agents. We demonstrate that this process can be controlled by changes in size and chemical nature of hydrophobic groups in condensing agents.

We used quaternary diammonium compounds as condensing chemicals synthesized from *N*,*N*,*N*′,*N*′-tetramethyl-propanediamine and the corresponding bromides by the Menshutkin reaction. The synthesized dications have the general structure as shown below and abbreviated herein as RPrR.



It had been considered that cations with charges above 3+ are necessary to induce condensation/precipitation of DNA.[15,16] Contrary to this, it has recently been revealed that diammonium ions (2+) can cause compaction of individual DNA molecules.[17,18] In the present study, we have performed careful observation on the conformational changes of individual T4 DNA (166 kbp) molecules induced by synthesized diammonium compounds with fluorescence microscopy. We have found that with the increase of dication concentration, individual DNA molecules undergo the discrete transition from the elongated state to the intrachain segregated state and then to the fully compact state in a stepwise manner with

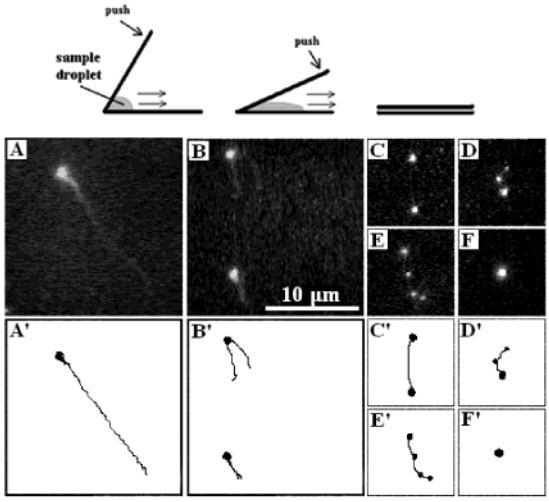


Figure 1. Above scheme: Experimental procedure to obtain stretched DNA molecules by hydrodynamic flow. The photographs are fluorescence images of T4 DNA after interaction with diammonium salts at the concentrations corresponding to the highest probability of the appearance of the intrachain segregated state: (A) [BzlPrBzl] = 1×10^{-3} M, (B) [HxPrHx] = 1×10^{-3} M, (C) [BuPrBu] = 2×10^{-3} M, (D) [EtPrEt] = 1×10^{-3} M, (E) [EtPrEt] = 1×10^{-3} M, (F) [EtPrEt] = 5×10^{-3} M, on a glass surface stretched by hydrodynamic flow. Pictures A′−F′ are drawn to facilitate the perception of the above corresponding photographs.

essentially no change in the number of mini-globules after the transition to the fully compact state has started. Fluorescent microscopy observations in bulk solution were not successful in clearly revealing the conformational changes in individual DNA molecules due to the blurring effect[11−14] of the fluorescent image of DNA chains, exhibiting translational and intrachain Brownian motion. Thus, we employed a technique which allows one to observe the stretched DNA molecules on a glass surface. Treating the sample solution droplet as shown in Figure 1 and fixing on a glass surface caused the elongation of the giant DNA molecules by the hydrodynamic current of the solution.

Typical fluorescent images of the segregated DNA structures are exemplified in Figure 1, where individual DNA molecules are stretched on a glass surface through the procedure represented as the top scheme in Figure 1. Besides globules as the final product of DNA compaction (Figure 1F), unfolded DNA chains with one or more globule-like units were observed (Figure 1A−E). We have confirmed that the number of the mini-globules along a chain remains essentially the same in the bulk solution and on the surface.

Distributions of the number of mini-globules on the individual DNA chains for various diammonium salts are shown in Figure 2, indicating that the average quantities of the intrachain segregation centers are markedly different from each other. At the studied conditions, after DNA interaction with BzlPrBzl and HxPrHx, we

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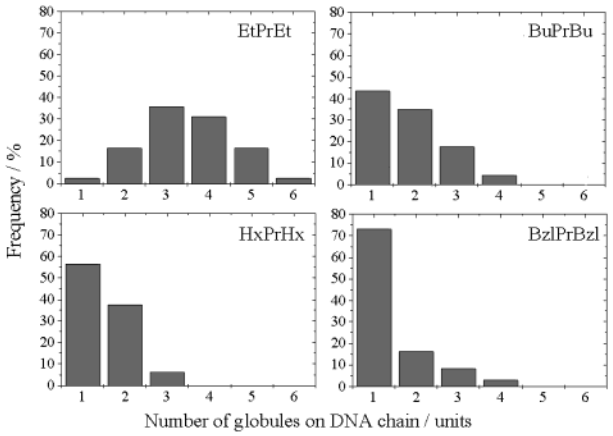

Figure 2. The distribution of the number of mini-globules on DNA individual chains after partial collapse by diammonium salts and stretching by hydrodynamic flow on a glass surface. Concentrations of diammonium salts: [EtPrEt] = 1×10^{-3} M, [BuPrBu] = 2×10^{-3} M, [HxPrHx] = 1×10^{-3} M, [BzlPrBzl] = 1×10^{-3} M. For each histogram, at least 50 partially segregated DNA molecules were analyzed.

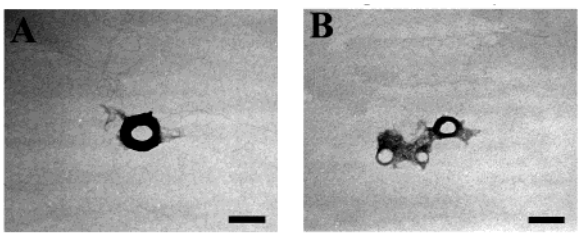

Figure 3. Examples of transmission electron micrographs of intrachain segregated states in a single T4 DNA molecule induced by HxPrHx at the concentration of 1×10^{-3} M (A), and EtPrEt at the concentration of 1×10^{-3} M (B). The scale bar is 0.1 μm.

mostly observed one segregated center on the DNA chain and a fewer number of DNA chains with two or more segregated centers. In contrast, the EtPrEt homologue induces the formation of numerous segregation centers on the DNA chain with a large distribution. The distribution in the case of BuPrBu appeared as an intermediate between HxPrHx and EtPrEt. Thus, the morphology of the "mini-globules on a string" structures is highly sensitive to the changes in the chemical structure of the quaternary dication.

To gain insight into the microscopic structure of the intrachain segregated DNA, we utilized a transmission electron microscope to study the fine structure of the partially collapsed DNA by dications. In Figure 3A, a typical electron micrograph of DNA partially collapsed by HxPrHx in the conformation of a single globule with an unfolded coil part is shown. The image of DNA collapsed by EtPrEt is presented in Figure 3B, where the DNA molecule consists of three toroidal centers. The inner radii of three toroids are close to the radius of the "big" toroid in Figure 3A, but their thickness is significantly smaller. These results correspond well to the fluorescence microscopy data, where DNA compacted by HxPrHx has one segregation center as the most probable conformation, while DNA collapsed by EtPrEt is observed as a molecule with a plural number of segregation centers. Here, we mention that similar structures with small toroids have been recently reported.[19]

Now, it has become clear that DNA molecules exhibit a unique intrachain segregated conformation depending on the chemical structure of the condensing agents. It is to be noted that the DNA used in the present study is rather long; that is, the contour length, or the full stretched length, amounts to 57 μm.[7,8] On the other hand, DNA is rather stiff; that is, the persistence length is ca. 50 nm, or ca. 130 bp, for the double helical B-form, much larger than the size of those condensing chemicals which are on the order of nanometers. This means that many cationic condensing molecules are incorporated in the formation of the mini-globules. Therefore, the stability of the mini-globules should be highly dependent on the manner of interaction between the condensing molecules bound to DNA. It is natural to expect that the increase in the associating ability of hydrophobic groups causes the larger stability of the mini-globule. For a more precise interpretation on the intrachain segregation, consideration of the size and steric structure of the condensing chemicals, besides the hydrophobicity, would be important in addition to the effect of the Coulombic interaction. As for the detailed theoretical consideration of the Coulombic effect on the stability of the intrachain segregated state, we have discussed this elsewhere.[11,14]

The results presented in this report suggest how one can control the morphology of partially collapsed DNA molecules by making the appropriate changes in the condensing chemicals' structures.

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Supporting Information Available: Experimental procedures of fluorescent and transmission electron microscopy, characterization of synthesized diammonium salts (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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