Folding transition into a loosely collapsed state in plasmid DNA as revealed by single-molecule observation

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Abstract The conformational transition of a plasmid DNA, pGEG.GL3 (12.5 kbp, circular), induced by spermine(4+) was studied through the observation of individual DNA by fluorescence microscopy. We deduced the change in the hydrodynamic radius R_H from an analysis of the Brownian motion of single DNA molecules. R_H decreases in a continuous manner with an increase in spermine(4+), in contrast to the large discrete on/off change for long linear DNA. Just after the transition to the collapsed state, a small number of DNA molecules tend to form an assembly, which disperses in the bulk solution without precipitation.

Keywords: Circular DNA; DNA condensation; Single DNA observation; Colloidal particles; DNA assembly; Spermine; DNA morphology

1. Introduction

In living cells and viruses, giant DNA molecules are folded into a tightly packed compact state. It is natural to expect that the biological activity of these giant DNA molecules should be highly dependent on their higher-order structure [1]. Not a small amount of studies on the folding transition of giant DNA molecules into condensed states, i.e., "DNA condensation", have been reported [2,3]. According to the standard understanding in polymer science concerning the conformation of a polymer chain [4], the compact state is generated under poor solvent conditions. Thus, it has been thought that the phenomenon of compaction in a single polymer chain be realized only under very dilute conditions, and that aggregation between many polymer chains is unavoidable in actual experimental conditions. Under such circumstances, DNA condensation has been regarded as a mixed process with characteristics of both single-chain compaction and multiple-chain aggregation [2]. About a decade ago, it was clarified that, from experiments on the observation of single DNA molecules, large linear dsDNA molecules larger than on the order of several tens of kbp undergo a large discrete transition between an elongated coil state and a folded compact state upon the addition of various kinds of condensing agents, such as polyamines, multivalent metal cations, hydrophilic polymer, and cationic surfactants [5,6]. As an important property of compact DNA, it has been shown that individual compact DNA molecules behave as colloidal particles with a negative charge and do not adhere to each other even after they collide [7,8]. Thus, a higher concentration of condensing agents is necessary to cause the aggregation/precipitation of multiple DNA molecules than is needed for the compaction of single DNA.

In contrast to such a significant change in linear giant DNA, it is still unclear how circular DNA folds into a compact state. A few reports on the conformation of circular DNA based on AFM observations have recently been published [9,10]. Most of the episome and genomic DNA in prokaryotes has a circular structure. In addition, even for eukaryotes, the transcriptionally active part of giant DNA is considered to be unfolded from its scaffold, suggesting the formation of a circular structure [11,12]. The present study was performed to better understand the transition in the higher-order structure of circular DNA molecules.

2. Materials and methods

2.1. Materials

Plasmid DNA pGEG.GL3 (12.5 kbp; circular DNA) was prepared as described previously [13]. 4′,6-Diamino-2-phenylindole (DAPI) was purchased from Wako Pure Chemicals. Dithiothreitol (DTT) and spermine tetrahydrochloride (SPM) were obtained from Nakalai Tesque.

2.2. Fluorescence microscopic measurements

Plasmid DNA pGEG.GL3 was dissolved in TE buffer solution (pH 8.0, 10 mM Tris–HCl, and 1 mM EDTA). The final concentrations in the sample for fluorescence microscopic observation were as follows: 0.18 μM pGEG.GL3, 0.18 μM DAPI and 5 mM DTT. Fluorescent images of DNA molecules were observed with a Zeiss Axiovert 135 TV microscope and recorded through a Hamamatsu Photonics EBCCD camera and an Argus 10 image processor.

2.3. Laser trapping

The infrared laser used for optical trapping was a Nd: yttrium–aluminum garnet, YAG, laser (SL902T, Spectron), with a TEM_{00} beam at a wavelength of 1064 nm. The laser beam was reflected by a dichroic mirror, and focused through an objective lens (Nikon Plan Fluor;

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100×, NA = 1.30) with a microscope (Nikon TE-300). The beam power was 510 mW, as evaluated just before the objective lens with a laser power meter (Neoark PM-345).

3. Results and discussion

Fig. 1A shows fluorescent micrographs of the plasmid DNA pGEG.GL3; (a) without SPM, (b) with 10 μM SPM, and (c) with 13 μM SPM, where the middle column shows the tracks of Brownian motion of the objects for up to 0.3 s. The pictures on the right are quasi-three-dimensional representations of the spatial distribution of the fluorescent intensity. DNA molecules clearly show a dramatic change upon the addition of SPM; in the absence of SPM, the fluorescence image is rather shallow and Brownian motion is mild, whereas in the presence of SPM a bright spot with large thermal fluctuation appears. From such a marked change in fluorescence, we can easily distinguish the compact state of a single DNA from an assembly of multiple DNA molecules. With an increase in the SPM concentration, the DNA molecules tend to assemble/aggregate with each other, as exemplified in Fig. 1A(c).

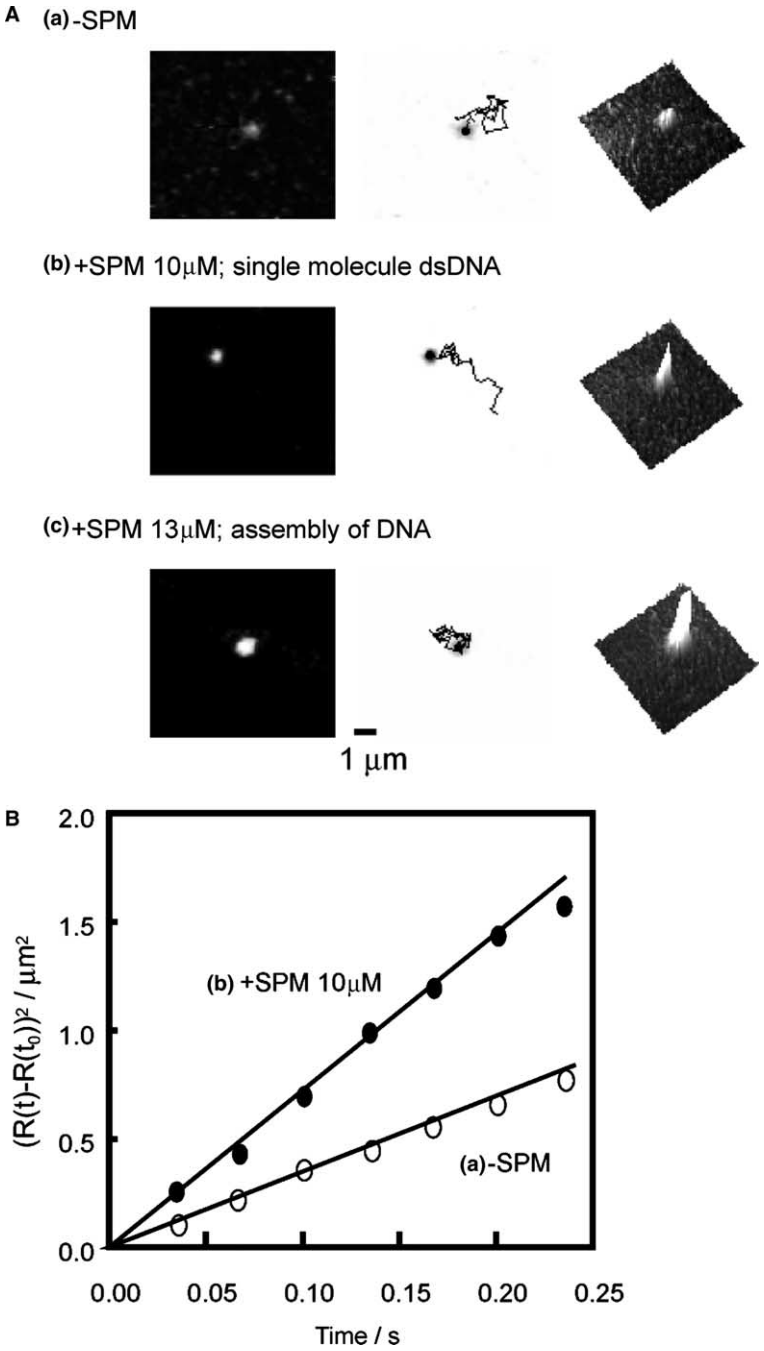

Fig. 1. (A) Differences in the elongated coil state, folded compact state and assembly of multiple DNAs. Left; fluorescent microscopic images of circular plasmid DNA, pGEG.GL3. Middle; tracks of fluorescent objects for 0.3 s. Right; quasi-three-dimensional representation of fluorescent intensity. (B) Mean square displacement of plasmid DNA without and with SPM (10 μM).