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RNA switches the higher-order structure of DNA

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Abstract

By the direct observation of single duplex DNA molecules by fluorescence microscopy, we found that RNA molecules have the potential to change discretely the higher-order structure of individual DNA molecules between the compact and elongated states. We performed an experiment with a linear giant DNA (T4 DNA, 166 kbp) and a circular DNA (cosmid vector, 42 kbp), and examined the effect of single-strand RNA on their conformations under a physiological concentration of spermidine. Individual DNA chains compacted by spermidine were elongated in an abrupt manner with an increase in the RNA concentration. This finding is discussed in view of the effect of the interplay between the dynamics of chromosomal DNA and the production of RNA in the cytoplasmic environment.

Keywords: RNA; Spermidine; Discrete unfolding transition; Higher-order structure of DNA; Fluorescence microscopy; Single molecular observation

1. Introduction

It is well known that when genomic DNAs are transferred to an aqueous solution by dissecting a living cell they assume an elongated conformation, which is much larger than the cell volume [1]. Thus, genomic DNAs exist in a highly folded state in every living cellular environment. To fold a long DNA chain into a compact structure such as a chromosome, cationic substances such as histone, protamine, and polyamines are considered to play crucial roles [2–4]. They have the effect to fold giant DNAs into a compact state by neutralizing the negative charges of phosphate groups in DNAs.

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It has been reported that the in vitro condensation of DNA molecules can be achieved with these biological cationic substances and with some synthetic chemicals, including neutral hydrophilic polymer (PEG), and metal complexes [5]. In previous studies, DNA condensation in vitro has been considered a highly cooperative phenomenon [6–8]. Unfortunately, most of the previous studies have failed to observe the transition of elongated DNA into a compact state at the level of single chains. Thus, the term 'condensation' implies a mixture of single-chain collapse and multi-chain association. Quite recently, based on the observation of the conformation of individual DNA chains by fluorescence microscopy [9,10], it has been shown that a single duplex DNA molecule switches its conformation, in an all-or-none manner, from elongated coil into compact folded states with the addition of various chemical agents, such as polyamine, metal cation, alcohol and neutral polymer [11–14]: the effective volume is reduced on the order of 1:100 ~ 1:10 000. In other words, the coil-globule transition [15] of giant DNAs has been found to be markedly discrete.

DNA folding is important not only in the compaction of giant DNAs in viruses and prokaryotic cells, but also in the genomic structure in eukaryotic cells. Studies on eukaryotic chromosomes have suggested a regulatory relationship between the degree of condensation of DNA and the transcriptional activity. For instance, DNA molecules are folded accompanied by the alternate-banding pattern, which reflects the different degree of condensation on chromosomes. Interestingly, it has been noted that abundant genes are located in regions where DNA is less condensed [16]. Studies on both the puffs in the polytene chromosomes of the salivary gland of fly larvae, and the lampbrush chromosomes of amphibian oocytes have indicated that the regions in which genes are actively transcribed correspond to decondensed chromatins [17,18]. It has also been reported that mammalian female somatic cells have two X-chromosomes, one of which is so highly condensed that the genes on it are transcriptionally inactive [19]. Furthermore, from the study on the chromatin morphology in ongoing RNA synthesis, it has been suggested that RNA concerns the organization of the higher-order structure of chromatin [20].

The degree of condensation on chromosomes in eukaryotic cells has also been considered to be closely related to the regulatory mechanism of gene expression. Thus, we have started to examine the effects of various key compounds found in cellular fluid on the switching of the higher-order structure of DNAs. In this study, using single-molecular observation by fluorescence microscopy, we obtained experimental evidence that RNA molecules induce the discrete unfolding transition in both linear and circular duplex DNA chains.

2. Materials and methods

2.1. Materials

Bacteriophage T4 DNA (166 kbp), as a linear giant DNA, and the cosmid vector, Charomid DNA 9–42 (42.2 kbp), as a circular DNA, were purchased from Nippon Gene (Tokyo, Japan). A mixture of single-strand RNA molecules (1 μg/μl, Perfect RNA Markers 0.2–10 kb) was purchased from Novagen Co. Inc. (Madison, WI, USA). We used TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 7.6). A fluorescent dye that specifically binds to double-strand DNA, 4′,6-diamidino-2-phenylindole (DAPI), was purchased from Wako Pure Chemicals (Osaka, Japan). Spermidine trihydrochloride (SPD) and the antioxidant 2-mercaptoethanol (ME, 4% (v/v) in the experiment) were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). ME was used as a free-radical scavenger to reduce fluorescent fading and light-induced damage of DNA. We purified water by distillation and filtration with a Millipore filter. The water was then treated with diethylpyrocarbonate to inhibit ribonucleases chemically.

2.2. Preparation of sample solutions

To stain DNA chains fluorescently, 0.1 μM and 0.042 μM DAPI were added to very dilute DNA

solutions of 0.1 μM (in phosphate) T4 DNA and 0.042 μM Charomid DNA, respectively, where the former and latter solutions correspond to DNA molecular chain concentrations of 0.3 pM and 0.5 pM. SPD was added to the solution and adjusted to a desired concentration. After the addition of SPD, the solution was incubated at 25°C for 1 h, and then mixed with RNA. The solution was allowed to stand for equilibration at 25°C for up to 2 h after the addition of the RNA mixture.

2.3. Fluorescence microscopy

Fluorescence microscopic measurements were performed as follows. The sample solution including the dye was illuminated with 365 nm of UV-light. The fluorescence images of DNA were observed using a Zeiss Axiovert 135 TV microscope equipped with a 100× oil-immersed objective lens, and recorded on an S-VHS videotape at 1 frame per 1/30 s through a high-sensitivity Hamamatsu SIT TV camera. The observation was carried out at 25°C. It has been confirmed that the presence of DAPI under these conditions has no significant effect on the persistence length or the contour length of T4 DNA [21]. The apparent long-axis length, which was defined as the longest distance in the outline of the DNA image, was evaluated with an Argus 20-image processor (Hamamatsu Photonics, Hamamatsu, Japan). The Brownian motion of Charomid DNA was analyzed using the same system. Special care was taken to thoroughly clean the glass microscope slides and coverslips before observation.

3. Results and discussion

3.1. Analysis of unfolding a linear DNA chain

Fig. 1 shows the fluorescence microscopic images and the corresponding fluorescence intensity profiles of single linear DNA (T4 DNA) molecules at the various concentrations of RNA under a fixed concentration of spermidine, [SPD] = 500 μM. In the absence of RNA, all molecules took a compact form, as seen in Fig. 1a (see also Fig. 3). Fig. 1b shows that the elongated and compact states coexist in the solution at [RNA] = 200 μM (in phosphate). Most of the DNA molecules are unfolded into an elongated coil shape at [RNA] = 400 μM, as shown in Fig. 1c. As this figure clearly shows, it is possible to discriminate between compact and elongated DNAs from a fluorescence image, since the former has a high-intensity peak due to dense packing in the folded state while the latter has a dispersed weaker intensity due to its elongated conformation.

Fig. 2 shows histograms for the distribution of the long-axis length of single duplex DNA molecules at different RNA concentrations. With an increase in the RNA concentration, the peak of this distribution shifts toward a longer length. Moreover, the distribution shows bimodality in solution at [RNA] = 200 μM and 300 μM, indicating that the unfolding of DNA can be characterized as an almost all-or-none transition with regard to the conformation of individual molecular chains. With careful observation, we noted the appearance of partially compact DNA chains at an intermediate concentration of RNA, as exemplified in Fig. 1d. This partially compact conformation comprises only a minor population. Fig. 2 shows the fraction of partially compact DNAs is in a shaded column, indicating that these DNAs exhibit a long-axis length L of over 1.5 μm.

As noted above, we used a sub-mM SPD concentration, which corresponds to the usual physiological concentration in living cells [22]. It has been previously reported that SPD causes a discrete change in giant DNAs from an elongated state into a compact state [11]. Since the effect of SPD on the higher-order structure of DNAs is markedly dependent on the ionic environment of the solution, we re-examined the effect of SPD on the conformation of T4 DNA in the buffer used in the present study (see Fig. 3). In the shaded region in Fig. 3, elongated and compact DNAs coexist. Based on the results of these measurements, we selected the concentrations of SPD in the present study to examine the effect of the addition of RNA.

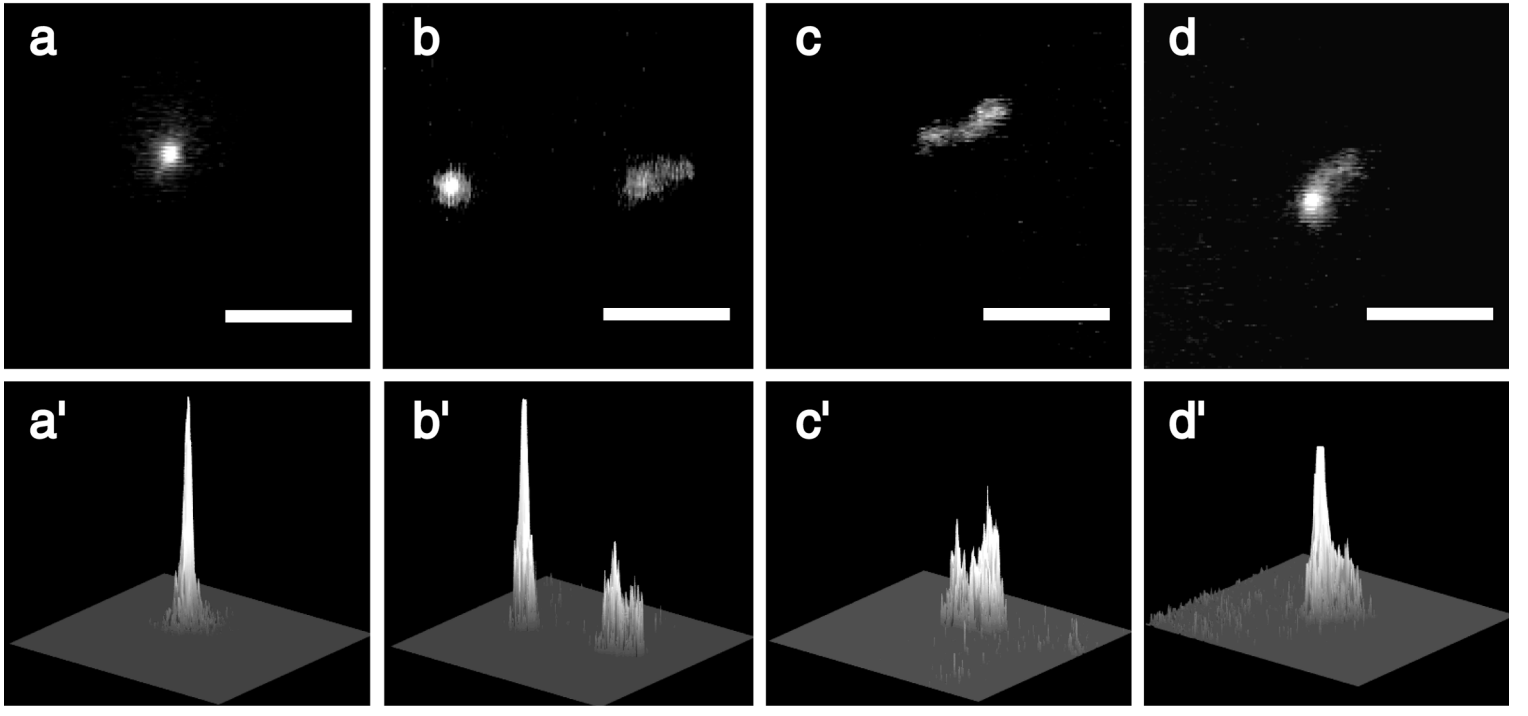

Fig. 1. Fluorescence images of single T4 DNA molecules in aqueous solution at various RNA concentrations and the corresponding profiles of fluorescence intensity distributions. (a,a′) Compact folded state without RNA molecules; (b,b′) coexistence of the compact and elongated states, [RNA] = 200 μM in phosphate; (c,c′) elongated unfolded state, [RNA] = 400 μM; (d,d′) partially compact state, [RNA] = 200 μm. 'Partially compact' refers to the DNA conformation for the coexistence of compact and elongated parts in a single DNA chain. The scale bar is 5 μm in length.

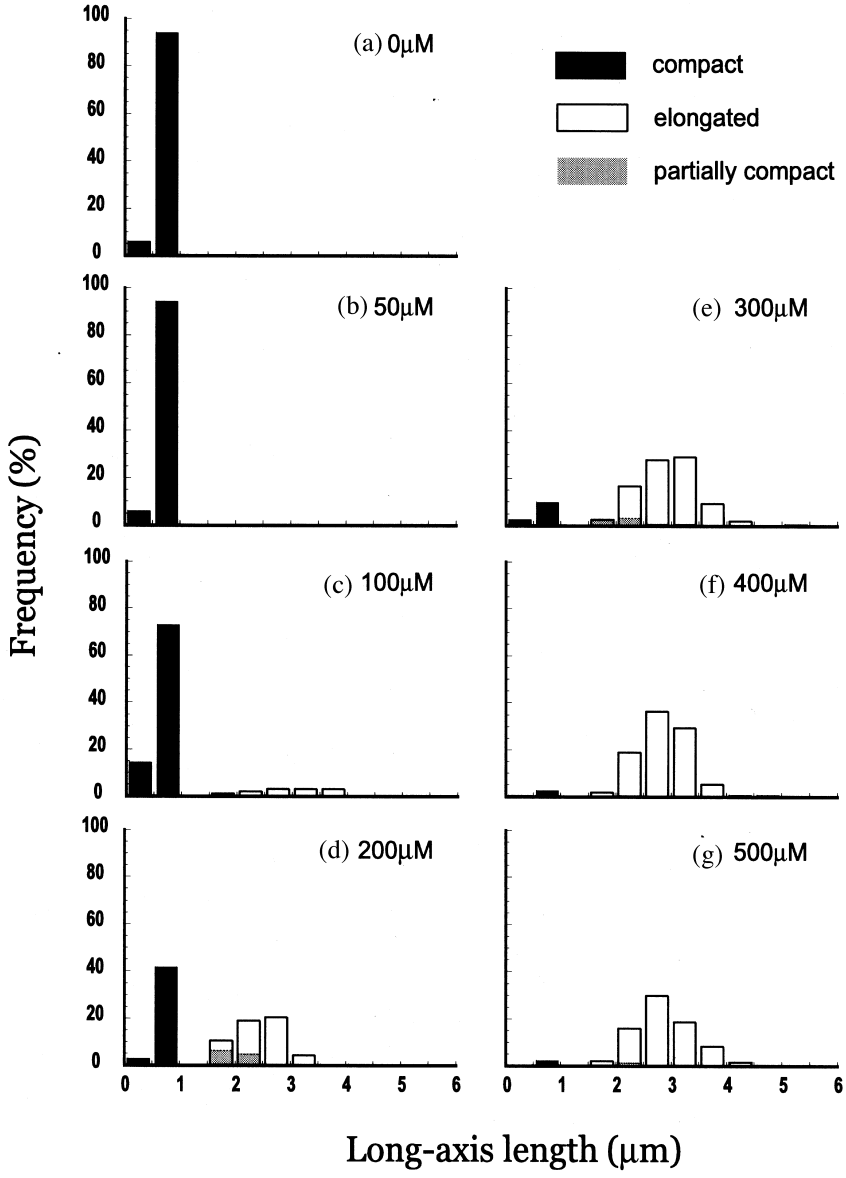

Fig. 2. Histograms of the long-axis length of T4 DNA molecules at the various RNA concentrations, as deduced from fluorescence microscopic measurement of the conformation of individual DNA chains. Before adding RNA, DNA molecules were collapsed at [SPD] = 500 μM. At least 100 molecules were analyzed for each concentration: (a) [RNA] = 0 μM in phosphate; (b) 50 μM; (c) 100 μM; (d) 200 μM; (e) 300 μM; (f) 400 μM; (g) 500 μM. For classification of the morphology, see Fig. 1.

3.2. Analysis of unfolding a circular DNA chain

From the measurement by fluorescence microscopy, it is difficult to evaluate conformational changes of DNAs several tens of kilobase pair in size, since the blurring effect is on the order of 0.3 μm [23]. Thus, to evaluate the conformational changes of cosmid vector DNA, we traced the Brownian motion of individual DNA chains [24].

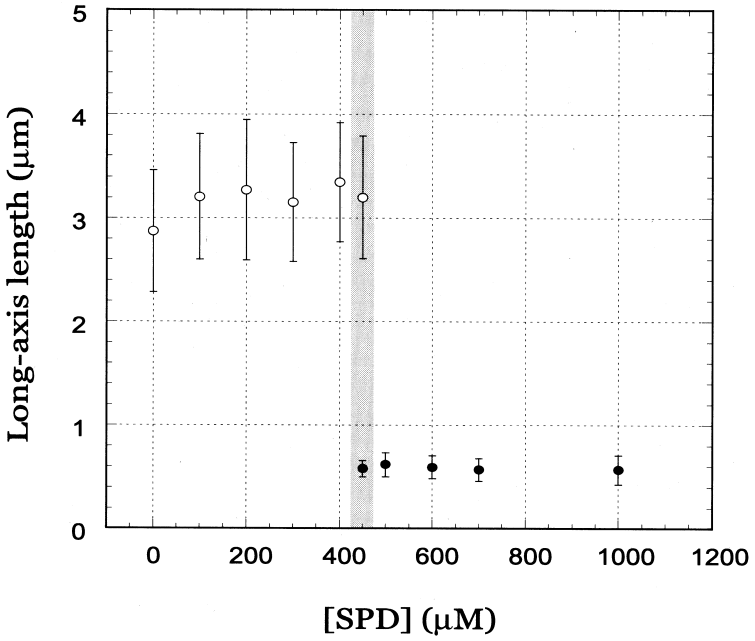

Fig. 3. Distribution of the long-axis length of T4 DNA molecules at various SPD concentrations. Open and closed circles indicate the elongated and compact states, respectively. At least 50 DNA molecules were analyzed for each SPD concentration. Error bars indicate the S.D. in the distribution. The shaded region indicates the coexistence of elongated and compact DNA chains.

Using the following relationship in Eq. (1), one can deduce the diffusion constant of individual DNA chains, by eliminating the effect of convection in the sample fluid.

$$\langle (\mathbf{R}(t) - \mathbf{R}(0))^2 \rangle = 4Dt + At^2 \quad (1)$$

where $\mathbf{R}(t) = (R_x, R_y)$ is the position of the center of mass for DNA at time t, $\langle (\mathbf{R}(t) - \mathbf{R}(0))^2 \rangle$ is the mean square displacement, and A is a numerical constant related to the convection. The hydrodynamic radius $\mathbf{R}_H$ of a single DNA molecule was evaluated from the D value based on the Stokes–Einstein equation given in Eq. (2).

$$\mathbf{R}_H = k_B T / 6\pi\eta D \quad (2)$$

where k_B is the Boltzmann constant and η is the viscosity of the solvent. Each η at $T = 298$ K is approximately equal to 1.0 mPa·s, which is essentially the same as that of pure water, based on the viscometric measurement of the viscosity of the solvents used in the experiment (data not shown).

Fig. 4 shows the $\mathbf{R}_H$ of a circular DNA molecule under three different conditions. All of the molecules are in the elongated state without SPD or RNA, and in the compact state at [SPD] = 400 μM without RNA. However, the addition of RNA molecules to the solution with SPD induces the unfolding of a circular DNA molecule from the compact to the elongated state. Therefore, most of the DNAs were unfolded. Fig. 4 also indicates that the ratio of the effective volume in the compact state to that in the elongated state is on the order of 1:1000, based on the above result that the ratio of $\mathbf{R}_H$ is approximately 1:10.

3.3. Reason why DNA chains are unfolded abruptly

An RNA molecule is a polyanion that has an approximately 10-fold greater affinity for SPD, considering the binding ratio, than a DNA molecule [25]. With an increase in the RNA concentration, SPD molecules would tend to prefer to bind to the phosphate groups of an RNA molecule, which would decrease the free SPD

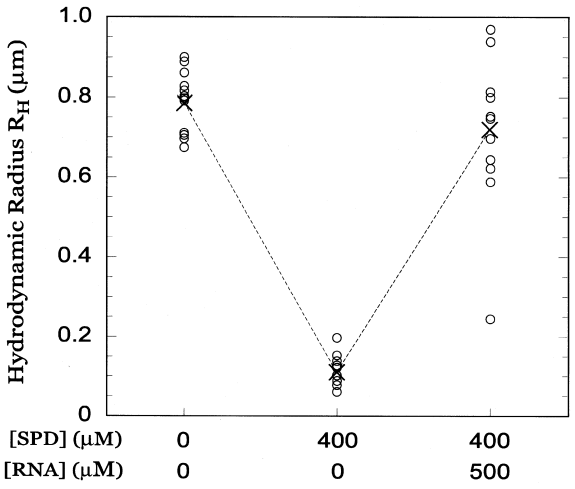

Fig. 4. Diagram of the hydrodynamic radius $\mathbf{R}_H$ of a single circular DNA (cosmid vector; 42.2 kbp). $\mathbf{R}_H$ was evaluated based on an analysis of Brownian motion from the fluorescence microscopic measurement of DNA chains. Open circles indicate $\mathbf{R}_H$ values of individual DNAs. Crosses indicate the mean value. The RNA concentration is represented by that of the phosphate group.

concentration. When the free SPD concentration decreases below a critical concentration, compact DNA chains exhibit an unfolding transition, as depicted schematically in Fig. 5.

3.4. Does this effect work in vivo?

Do considerable amounts of polyanionic chains like RNA actually exist around DNA in vivo? As has been observed in the puff or the lampbrush chromosome [17,18], chromatins are considered to be decondensed in the transcriptionally active region on a chromosome, where abundant polymers, such as newly synthesized RNA chains, are present, some of which are anchored to the chromosomal DNA due to ongoing synthesis. DNA molecules in this region are expected to experience a rather high anionic polymer concentration. Some studies dispute the relationship between the structure of chromatins and ADP-ribosylation [26], which produces RNA-like polyanionic chains, i.e. poly(ADP-ribose), on histones, suggesting the probability of a locally high concentration of polyanionic chains around DNA.

From this in vitro model system containing DNA, SPD, and RNA, we found that RNA molecules have a significant effect on the determination of the higher-order structure of single DNA molecules, through the effect of SPD as an intermediator. Thus, we may speculate that, in addition to the function of regulatory proteins around chromosomal DNAs, the interaction, or cross-talk, between DNA and RNA in such a

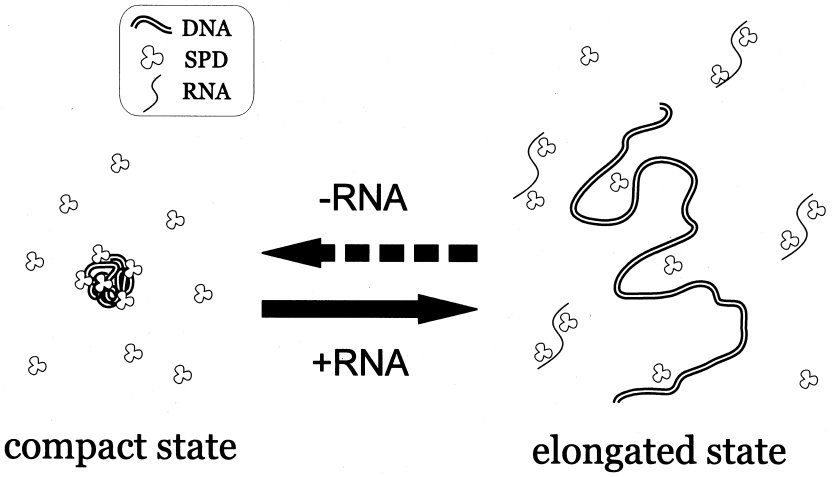

Fig. 5. Schematic representation of the structural change in a giant DNA molecule induced by the addition of RNA molecules. Since SPD binds more strongly to RNA than to DNA, individual compact DNA unfolds abruptly with an increase in the RNA concentration.

manner, is concerned with the dynamics and function of chromosomal DNAs. It would be worthwhile to examine this hypothesis in vivo.

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