ATP/ADP switches the higher-order structure of DNA in the presence of spermidine

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Abstract In the living cellular environment, DNAs exist in a compact state in the presence of a polyamine, such as spermidine. We found that the hydrolysis of ATP into ADP induces the folding of elongated DNAs, by the single-chain observation of individual T4 DNA molecules. This result is discussed in relation to the possible role of ATP as a regulatory factor in genetic activity, in addition to its well-established role as an energy source.

Key words: Unfolding transition; Coil-globule transition; Single-molecule observation; Hydrolysis of ATP; Spermidine

1. Introduction

Modern biology has established that DNA is the key compound in genetics while ATP is the key compound in energetics. There is considerable evidence that genetic activity, i.e. replication, recombination and gene expression, is closely associated with the energy state of the cell. ATP supplies energy for most biological processes, including duplication and transcription. In DNA viruses, it has been shown that ATP is necessary for the packing of DNA chains into the head of bacteriophages [1]. In cell division during the process from interphase to mitosis, ATP is found to induce marked morphological change in chromosome DNAs from a diffuse state into a compact form [2,3]. It has also been reported that the rate of protein synthesis strongly depends on the ATP (and GTP) concentration and that the concentration of ATP regulates DNA transcription [4,5]. These studies have indicated that ATP participates in the regulatory mechanism through its function as an energy source. On the other hand, ATP is also a multivalent anion in the cytoplasmic environment. Thus, it is possible that ATP may not only act as an energy source, but may also change the chemical environment in the intracellular or intranuclear space.

In living cells, DNA is often found in a very compact state. As models for the compaction of DNAs, there have been many studies on DNA condensation induced by polyamines such as spermidine and spermine [6–8], neutral polymers [9], and metal cations such as Co^{3+} [10]. However, the term condensation seems to have been mainly applied to the aggregation or precipitation of multiple DNA molecules [11]. Recently, using the method of single-chain observation by fluorescence microscopy, it has become clear that the folding transition from the elongated to the compact state is a switching event, or first-order phase transition, at the level of individual DNA molecules [12–14]. On the other hand, for the ensemble of DNA molecules, the transition seems sharp but not discrete; i.e. there is no switching of the physico-chemical properties in the DNA ensemble. In the present study, we observed the change in the conformation of individual DNA molecules at different concentrations of ATP and ADP in the presence of a fixed concentration of spermidine, indicating that ATP/ADP causes a marked change in the higher-order structure of DNA.

2. Materials and methods

2.1. Materials

Bacteriophage T4 dC DNA (166 kbp) was purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). The fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI), sodium dihydrogenphosphate, magnesium chloride, and glucose were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Spermidine trihydrochloride (SPD) and 2-mercaptoethanol (2-ME) were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Disodium salt of adenosine-5′-triphosphate (ATP), disodium salt of adenosine-5′-diphosphate (ADP), and hexokinase were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan).

2.2. Preparation of DNA solution

T4 DNA was dissolved in TE buffer solution (10 mM Tris, 1 mM EDTA, pH 7.3). To avoid the intermolecular aggregation of DNA molecules induced by the addition of spermidine, we prepared a dilute solution: the final DNA concentration was taken to be 0.1 μM in nucleotide units. To visualize individual DNA molecules by fluorescence microscopy, DNA was stained with 0.1 μM DAPI. For the sample solution, 4% (v/v) 2-ME was added to prevent fading of the fluorescent dye during the observation. It has been previously confirmed that the persistence and contour lengths of DNA remain essentially constant at the concentration of DAPI used in the present study [15].

2.3. Fluorescence microscopic measurement

Fluorescence images of DNA molecules were observed at room temperature, using a Carl Zeiss Axiovert 135 TV microscope equipped with a 100× oil-immersed objective lens. They were recorded on videotape through a high-sensitivity Hamamatsu SIT TV camera. The video images were analyzed with an image processor (Argus, Hamamatsu Photonics). To characterize the size of DNA, we measured the long-axis length, L, which was defined as the longest distance in the outline of the DNA image. Due to the blurring effect of fluorescent light, the apparent size L in a DNA image is about 0.3 μm larger than the actual DNA coil [12].

3. Results

Fig. 1 (left) shows fluorescence images of T4 DNA molecules in (A) the fully unfolded state, (B) the partially folded state, and (C) the fully folded state; where (A) is in pH 7.3 buffer solution with 10 mM Tris, 1 mM EDTA, 0.1 μM DAPI and 4% (v/v) 2-ME, (B) is in buffer solution after the hydrolysis of ATP by hexokinase, and (C) is in buffer solution with

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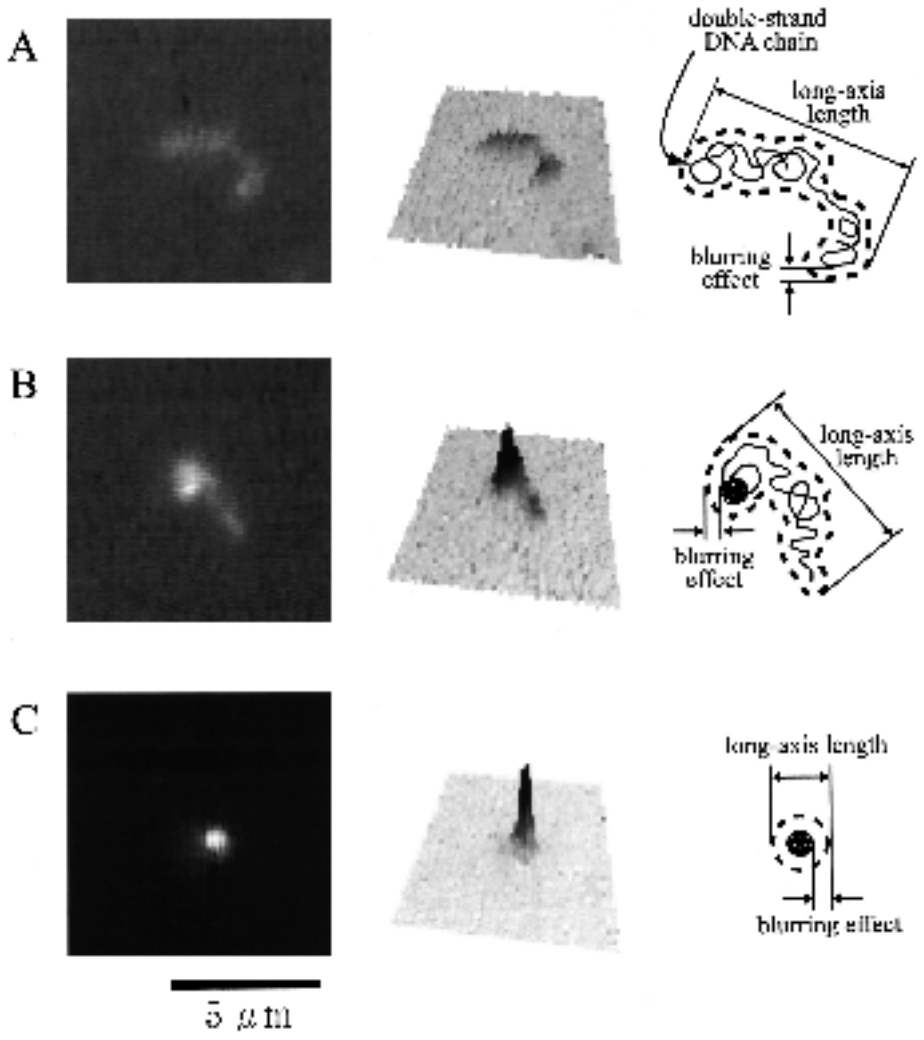

Fig. 1. Images of single T4 DNAs in (A) the elongated coil state in TE buffer, (B) the partially folded state after the hydrolysis of ATP in the medium with hexokinase (see Fig. 5), and (C) the fully folded compact state in TE buffer containing 0.55 mM spermidine (see Fig. 2). Left: Fluorescence microscopic image of T4 DNA. The fully stretched length, or contour length, of T4 DNA is 57 μm. Middle: Fluorescence light intensity distributions for the images on the left. The integrated fluorescence intensity was essentially the same for DNAs with different morphologies. Right: Schematic representation of fluorescence images and the corresponding conformations in single double-stranded T4 DNA molecules. The apparent size in the fluorescence image is larger than the actual size by about 0.3 μm due to the blurring effect.

0.55 mM spermidine. As shown in the middle column of Fig. 1, the spatial intensity distribution of fluorescence is markedly different between the elongated state (A) and the folded compact state (C). In the actual measurement, we observed large thermal fluctuations in the conformation in the unfolded state. In contrast, for fully folded DNA molecules, significant translational motion, as in Brownian motion, was observed with no apparent intrachain fluctuation. Based on quantitative measurements of Brownian motion by fluorescence microscopy, it has been previously shown that the hydrodynamic radius in the fully folded state is 0.063 ± 0.014 μm, while that in the unfolded state is 0.62 ± 0.17 μm [16]. Thus, the effective volume in the folded state is 1/1000–1/10 000 of that in the unfolded state. With the current studies on the folded state of DNAs, we have already confirmed that toroidal structures with a diameter of 88 ± 16 nm (0.6 mM spermidine) are generated as the typical morphology under essentially the same conditions as in the fluorescence microscopy measurement [16]. With careful observation, partially folded DNA can be identified, as shown in Fig. 1B. Based on successive observation of partially folded chains, it has become clear that the partially folded state, where folded and unfolded regions coexist in a single DNA molecule, persists throughout the period for the practical observation of individual molecules, i.e. the partially folded conformation appears to be a thermal equilibrium state or at least a metastable state. When we analyze the conformation of the individual DNA chains by measuring the long-axis length L, the partially folded conformation is classified as an elongated chain, as shown in the histograms in Figs. 2 and 3.

In the present study, we examined the effect of ATP on compact DNA chains in a model intracellular or intraviral environment, using spermidine. Spermidine is a polyamine that is found throughout the cytoplasm in both prokaryotic and eukaryotic cells [17]. It has been previously reported that spermidine causes a discrete change in the conformation of individual giant DNAs from an elongated state into a compact state [13]. Since the effect of spermidine on the higher-

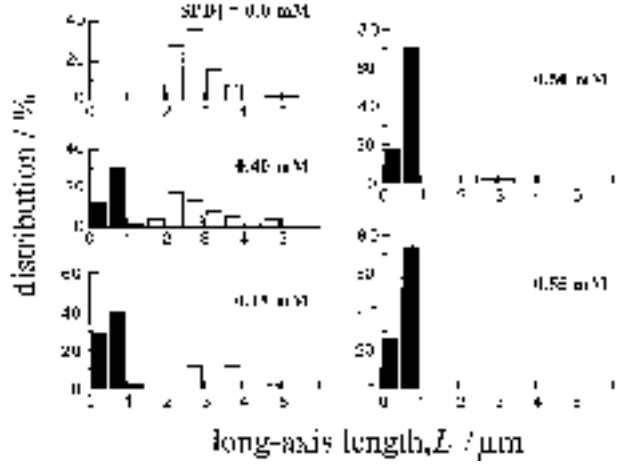

Fig. 2. Histogram of the long-axis length, L, of T4 DNA molecules at various concentrations of spermidine. The area of each histogram is normalized to be equal, where at least 50 molecules were counted at each concentration of spermidine. The open and closed columns correspond to the elongated state with the fully unfolded and partially folded conformation (see Fig. 1A,B), and the tightly folded state (see Fig. 1C).

order structure of DNAs is markedly dependent on the ionic environment of the solution, we re-examined the effect of spermidine on the conformation of T4 DNA in the buffer solution used in the present study (Fig. 2). Based on this result, we used a spermidine concentration of 0.55 mM in

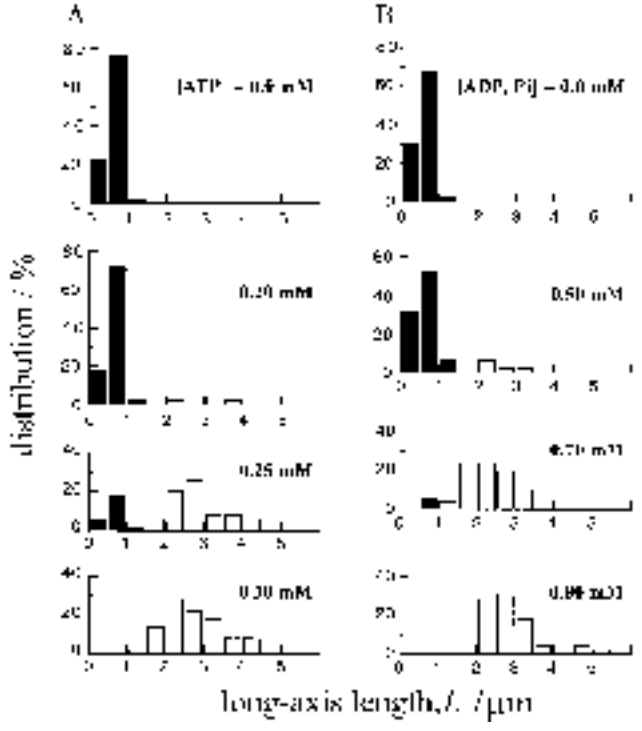

Fig. 3. Change in the size of T4 DNAs depending on the concentration of (A) ATP and (B) ADP and inorganic phosphate, Pi, in the presence of 0.55 mM spermidine. At least 50 DNAs were counted for each distribution. In B, the concentrations of ADP and Pi are the same. The open and closed columns correspond to the elongated state with the full unfolded and partially folded conformations, and the tightly folded state, respectively.

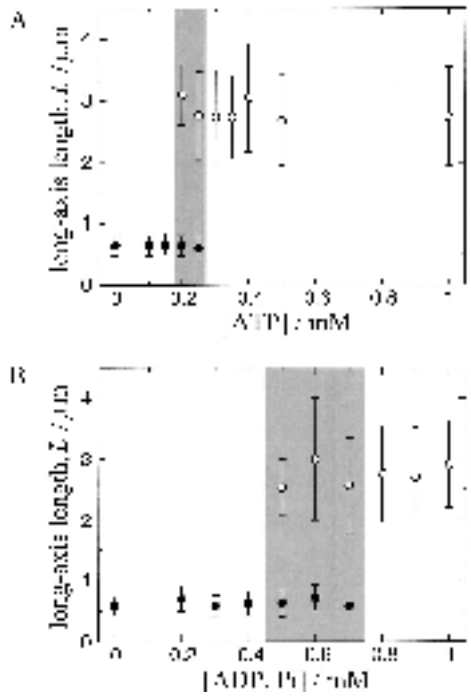

Fig. 4. Long-axis length, L, of T4 DNA molecules vs. the concentration of (A) ATP and (B) ADP and Pi, where in the latter the concentrations of ADP and Pi are the same. The open and closed circles indicate the mean value of L in the elongated (fully unfolded and partially unfolded) and folded state, respectively. The vertical bars show the standard deviation. The shaded area shows the region where the elongated and folded states coexist. The transition is almost all-or-none at the level of individual DNA molecules.

the following experiments to examine the effect of ATP/ADP on the DNA conformation.

Fig. 3 shows the change in the length distribution of T4 DNAs at different concentrations of either (A) ATP or (B) ADP and Pi, where [ADP] = [Pi], in the presence of a fixed concentration of spermidine (0.55 mM). A bimodal distribution is noted at intermediate concentrations of ATP and ADP, where the intermediate region is found at lower concentrations of ATP. Fig. 4 shows the average values of the long-axis length distribution together with the standard deviation of the individual peaks. Fig. 4 indicates that the region of the coexistence of the elongated and folded states appears at an ATP concentration much lower than that of ADP. Thus, Fig. 4 suggests that when [ATP] is between 0.25 and 0.40 mM, DNAs exhibit an elongated conformation, and that when ATP is completely hydrolyzed into ADP and Pi by an enzymatic reaction, DNAs fold to a compact state. To better examine this possible effect of ATP hydrolysis, we measured the change in the conformation of T4 DNA in a medium of 0.40 mM ATP with 1.0 U/ml hexokinase and 22 mM glucose. The results (Fig. 5) indicate that ATP hydrolysis strongly affects the conformation of DNA molecules. We confirmed that 85% of ATP is hydrolyzed under the conditions in Fig. 5Ab, based on measurement of the absorbance at 340 nm as NADPH, using a two-step coupled reaction [18]. In this experiment, we did not try to identify the conditions that would yield the most significant change in the DNA conformation. Instead, we tentatively selected the experimental conditions based on the data in Fig. 4. Although the percentage of perfectly folded

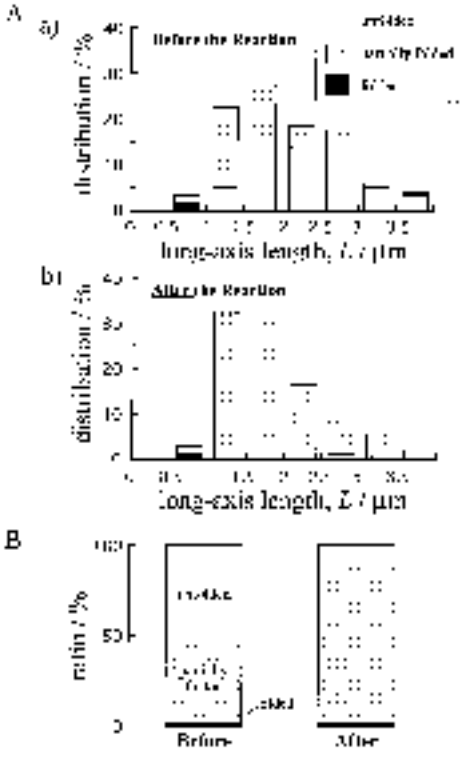

Fig. 5. Change in the distribution of the long-axis length, L, with the conversion of ATP into ADP by hexokinase. A: a: Distribution of L for 0.1 μm T4 DNA solution with 0.35 mM spermidine, 0.4 mM ATP, 22 mM glucose, and 0.2 mM $MgCl_2$. b: Distribution of L after incubation for 30 min at 37°C after the addition of hexokinase (final concentration is 1.0 U/ml) for the solution in a). B: Ratio of unfolded (see Fig. 1A), partially unfolded (Fig. 1B) and folded (Fig. 1C) DNA molecules, before and after the hydrolysis of ATP.

DNAs did not significantly increase in this experiment, that of completely unfolded DNAs markedly decreased, and therefore the percentage of partially folded DNAs increased. Thus, it is clear that the ATP/ADP ratio can affect the switching of the higher-order structure of DNAs.

4. Discussion

It has recently been established that individual giant DNA chains exhibit a large discrete transition, or switching, between an elongated unfolded state and a compact folded state, depending on the concentration of various kinds of condensation agents, such as polyamines, polyvalent metal cations, hydrophilic neutral polymers, alcohols, and surfactants [12–14,19]. In the present study, we found that the ATP/ADP ratio affects the switching of the higher-order structure of DNA. We noted a marked increase in the fraction of partially folded DNA with the progression of hydrolysis. Based on a theoretical consideration of the correlation length in the discrete transition of string molecules, the coexistence of the partially folded conformation together with the remaining folded part along a single macromolecule is expected in the case of a double minimum free energy profile with a relatively low energy barrier for relatively long DNA chains [20]. Thus, from a thermodynamic point of view, both the all-or-none change in the conformation of individual molecules and the coexistence of compact and elongated parts in a chain molecule can be explained by considering a system with a double minimum free energy. When we denote the relative statistical weight of the unfolded and folded states as u and f ($u+f=1$), respectively, the difference in free energy between the two states is given as

$$\Delta G = kT \ln (f/u) \tag{1}$$

It has been previously shown [13] that the folding transition of giant DNA induced by a polyvalent cation is mostly driven through the process of ion exchange between monovalent and polyvalent cations, as the counter-ions for the negatively charged phosphate groups along double-stranded DNA. Thus, the relative stability of the unfolded and folded states is mostly dependent on the chemical potential of the free polyvalent cation, which is spermidine in the present study. Thus, as a first approximation [21–23], the relative fraction of the DNA conformations can be expressed as

$$\ln (f/u) = \alpha \ln [\mathrm{Sper}]_\mathrm{f} + \beta \tag{2}$$

where $[\mathrm{Sper}]_\mathrm{f}$ is the concentration of free spermidine in the solution, and α and β are parameters. α reflects the physicochemical significance of the degree of ion exchange in counter-ion-condensed DNA. Since ATP and ADP are multivalent anions and spermidine is a multivalent cation, one can expect binding equilibria in a bulk aqueous solution,

$$(\mathrm{Sper})_\mathrm{f} + (\mathrm{ATP})_\mathrm{f} \overset{K_1}{\rightleftarrows} (\mathrm{Sper{-}ATP})_\mathrm{b} \tag{3}$$

$$(\mathrm{Sper})_\mathrm{f} + (\mathrm{ADP})_\mathrm{f} \overset{K_2}{\rightleftarrows} (\mathrm{Sper{-}ADP})_\mathrm{b} \tag{4}$$

where the suffixes f and b denote the free and bound states. K_1 and K_2 are the apparent binding equilibrium constants. It is reasonable to suppose that K_1 is much larger than K_2, and we can speculate on the plausible effect of ATP/ADP on the switching of the higher-order structure of DNA molecules. Before the hydrolysis of ATP, as in Fig. 5Aa, since a significant amount of spermidine is bound to ATP, the concentration of free spermidine is not enough to fold DNA chains. Thus, ATP behaves as an antagonist to spermidine with regard to its effect on the folding of DNA molecules. As the hydrolysis of ATP progresses, the concentration of free spermidine in the bulk solution gradually increases. This increase in free spermidine causes the folding of DNA chains.

In conclusion, we have found that ATP/ADP can contribute to the folding transition of DNA chains, through 'cross-talk' with spermidine. Since spermidine and related polyamines are generally found in the cytoplasmic environment, the present results suggest that gene expression may be regulated by the energy state in the cytoplasmic fluid. Further experiments are needed to verify this working hypothesis. Although the present experiment examined the folding/unfolding transition of 'naked DNAs', we can expect that ATP/ADP may have a similar effect on nucleosomal DNAs. In this regard, it is interesting to note that Tsukiyama et al. recently reported that nucleosome disruption is suppressed by the depletion of ATP using hexokinase and glucose [24]. Furthermore, ATP disrupts chromatin and facilitates the binding of transcription factor to nucleosome core particles [25–31]. In these studies, ATP has been generally regarded as playing a

role solely as an energy source. As a future subject to be solved, it may be of value to examine the other possible effect of ATP as a mediator to change the cytoplasmic environment. Related to this, Nilsson et al. found that the interaction between DNA and histone H1 is modulated by nucleotides [32]. Unfortunately, they did not notice the switching behavior on the higher-order structure of DNAs, most probably due to the limitation of the experimental methodology.

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