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Folding and unfolding of a giant duplex-DNA in a mixed solution with polycations, polyanions and crowding neutral polymers

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Abstract

To understand the conformational behavior of a giant duplex-DNA chain in a mixed solution with various biopolymers with different state of ionization, the higher-order structure of the DNA chain was analyzed with a fluorescence microscope in the presence of polycations (poly-arginine), polyanions (poly-glutamic acid), and neutral polymers (poly-ethylene glycol) as a model for cellular environment. Concentrated medium with neutral polymer induced the discrete folding transition of the DNA. At the threshold condition for the transition, addition of small amounts of either the polycation or the polyanion caused marked structural changes in the folded DNAs. Based on thermodynamic considerations on the experimental results, profile of free energy of a single giant DNA chain was depicted with respect to the size, or the expansion factor α, in the three-dimensional structure of the DNA. The effect of the neural crowding polymer on the degree of folding of a single giant DNA chain is discussed in a semi-quantitative manner.

Keywords: Fluorescence microscopy; DNA condensation; Macromolecular crowding effect; Polyion-complex; Single molecular observation; Higher-order structure

1. Introduction

In general, giant DNA molecules in vivo such as genomic DNAs are highly folded, and packed into a very narrow region inside cells. For example, while the full-stretch length, or the contour length, of an individual genomic DNA with on the order of mega-base pairs reaches on the order of centimeter, the actual size of the folded DNA is around several micrometers [1,2]. Although the giant DNAs in cellular environment retain the compact structure as a whole, selective structural

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changes such as the local unfolding in a particular region would be induced at the proper moment of the expression of the desired genes.

Concerning the folded state of DNA, DNA condensation in vitro has been actively studied as a model for DNA compaction in vivo [3–8]. DNA condensation is induced in vitro by the various kinds of condensation agents; e.g., a hydrophilic neutral polymer such as poly-ethylene glycol [3,4,9], a low molecular-weight multivalent cation such as polyamine and hexaamine cobalt (III) [10,11], a basic polypeptide [9,12], a cationic and neutral surfactant [13–15], and so on. From these studies, it has become clear that a rich variety of chemical species in the cellular environment exhibit potential to condense DNA molecule.

Among the above-mentioned condensation agents, main agents for the DNA compaction in vivo are attributed not only to the binding basic-proteins such as histone, but also to the presence of highly concentrated environments with cytoplasmic proteins. For examples, in the case of prokaryote, the typical concentration of cytoplasmic proteins is considerably high in the order of hundreds of milligrams per milliliter [16–18]. Although the cytoplasmic proteins have no direct interaction with the genomic DNAs (in this sense, the proteins are often referred to as 'inert' polymers for genomic DNAs), such a concentrated medium with cytoplasmic proteins has been shown to play an important role in functions and structures of the DNAs (referred to as the macromolecular crowding effect [19–23]). In addition, it should be noted that there also exists polyanions such as transcribed RNAs and newly replicated DNAs in nuclear regions both in prokaryote and eukaryotes. The chemical surroundings of genomic DNAs is thus generally characterized by the presence of polycations and polyanions in the nuclear region and of highly concentrated medium with various cytoplasmic proteins. It is therefore important to understand how the conformational behavior of a single giant duplex-DNA chain is affected by the environmental changes in a mixed solution with various polymers with different state of ionization.

To better understand the conformational behavior of a single giant DNA in such a mixed solution, we adapted the model system composed of poly-ethylene glycol, poly-arginine, and poly-glutamic acid, and observed the manner of folding of single giant DNAs by means of fluorescence microscopy. It is to be noted here that we will investigate the characteristics of chain-folding in isolated giant DNAs. Although DNA condensation has been actively studied during the past several decades, most of the researches have examined on the condensation of a plural number of DNA chains [3–8]. The traditional experimental techniques such as light scattering, sedimentation, circular dichroism, etc., usually afford information only on an ensemble average of many DNA molecules. Observation with fluorescence microscopy has been shown to be useful for investigating the characteristics of the chain-folding of single giant DNAs. Using this method, we have tried to unveil the hidden properties on the chain-folding of single giant DNA induced by various condensation agents [24–29].

In the present study, we applied the technique of fluorescence microscopy to analyze the structural behavior of folded single giant DNAs in the above-mentioned mixed solution, by avoiding the interaction between different DNA molecules in a very dilute condition. It was found that the manner of chain-folding in single giant DNA sensitively depends on the compositional balance of coexisting polycations and polyanions in the crowded medium with the inert polymers.

2. Experimental

2.1. Sample preparation

Bacteriophage T4 DNA (166 kb pairs) was purchased from Nippon Gene (Tokyo, Japan). Poly-L-arginine (poly-Arg; mol wt., 10 800; DP, 56), and poly-L-glutamic acid (poly-Glu; mol wt., 14 300; DP, 95) were purchased from Sigma Chemicals (St Louis, MO). Poly-ethylene glycol (PEG; mol wt., 10 000; DP, 227) was available from MERCK-Schuchardt (Schuchardt, German).

Sample solutions were prepared according to the following mixing procedure. First, stock solutions of DNA, poly-Arg, poly-Glu, and PEG were made at the following concentrations in distilled

and sterilized pure water; [DNA] = 30 μM in phosphate, corresponding to 9.75 μg/ml; [poly-Arg] and [poly-Glu] = 3 μM in residue; [PEG] = 10 M in monomer unit. A stock solution of Tris–Borate buffer (10 × TB; 900 mM Tris and 900 mM Borate, pH = 8.16) and a fluorescent dye solution (4′,6-diamidino-2-phenylindole, DAPI, obtained from Wako Chemicals, Osaka, Japan; 30 μM in pure water) were also prepared. To obtain the desired composition of each substance in the fixed volume of sample solutions (500 μl), an appropriate volume of each stock solution was added to a suitable volume of pure water by the following procedure. (1) Addition of 10 × TB, 50 μl (final concentration, 90 mM); (2) addition of PEG; (3) addition of poly-Arg; (4) addition of DAPI, 5 μl (final concentration, 0.3 μM); (5) addition of 2-mercaptoethanol (2-ME; purchased from Wako Chemicals), 20 μl [final concentration, 4% (v/v)]; (6) vortexing; (7) addition of DNA, 5 μl (final concentration, 0.3 μM in phosphate); (8) standing still at 55°C for 15 min; (9) incubation at 20°C for 30 min; (10) addition of poly-Glu; (11) standing still at 55°C for 15 min; (12) incubation at 20°C for 30 min before observation. Finally, observation was done at 20°C.

Here, the ascending heat to 55°C was carried out to complete mixing DNAs with concentrated PEG solution, instead of mixing by strong vortexing with avoiding a cleavage of the giant T4 DNA. It has already been confirmed that the presence of DAPI at this concentration has no significant effect on the persistence length and on the contour length of DNA [30]. 2-ME was added as an antioxidant reagent [31].

2.2. Single molecular observation of giant DNAs in solution

Fluorescence images of the DNA/poly-Arg complex in PEG and poly-Glu solution were obtained using a fluorescence microscope Axiovert 135TV (Carl Zeiss, German), recorded on videotape, and processed using the image-processor Argus 50 (Hamamatsu Photonics, Japan).

Sample solutions were situated between two thin glass plates (Matsunami No.1, thickness: 120–170 μm) at a depth of approximately 150 μm using spacer glass plates. When the sample depth was set as in the usual observation with the fluorescence microscope (~ 5 μm), almost all the complex became attached to the glass surface. Related to this, it is noted that the mean size of T4 DNA molecules in pure water is approximately 3–5 μm. By avoiding the surface effect with the relatively large sample depth, we succeeded in conformational observation of the DNAs in the bulk solution. Since the DNA concentration (0.3 μM in phosphate, or 0.1 μg/ml) was chosen to be very low so as to minimize the inter-chain interaction between DNAs, it has become possible in the present study to analyze the properties of a *single giant* duplex-DNA chain *in solution*.

3. Results

3.1. Evaluation of the manner of folding in a single giant DNA

Fig. 1 shows typical fluorescence images of single duplex-T4 DNAs with the corresponding profiles of fluorescence intensity and schematic representations of the conformation of the DNA chains.

As shown in Fig. 1a, T4 DNAs in TB buffer solution exhibit an elongated random coiled state with a long-axis length of approximately 3–5 μm. While the contour length, or the full-stretch length, is 57 μm in T4 DNA [30], the random coiled state of the DNA chain in solution is naturally shrunken to the small size due to its entropic nature. The coiled state is defined by the character of a marked intra-chain thermal fluctuation [32]. When the condensation agents such as PEG and poly-Arg are added to the solution, the DNA molecule is folded as shown in Fig. 1b–d.

Fig. 1b shows tightly collapsed globular state of T4 DNAs in 8 M PEG solution with the single steep maximum in fluorescence intensity, which exhibits an enhanced translational Brownian motion in keeping the tight conformation.

Fig. 1c shows a loosely folded state of the DNAs in 0.12 μM poly-Arg solution (in residue; [Arg]/[phosphate] = 0.4) with a lower peak-height

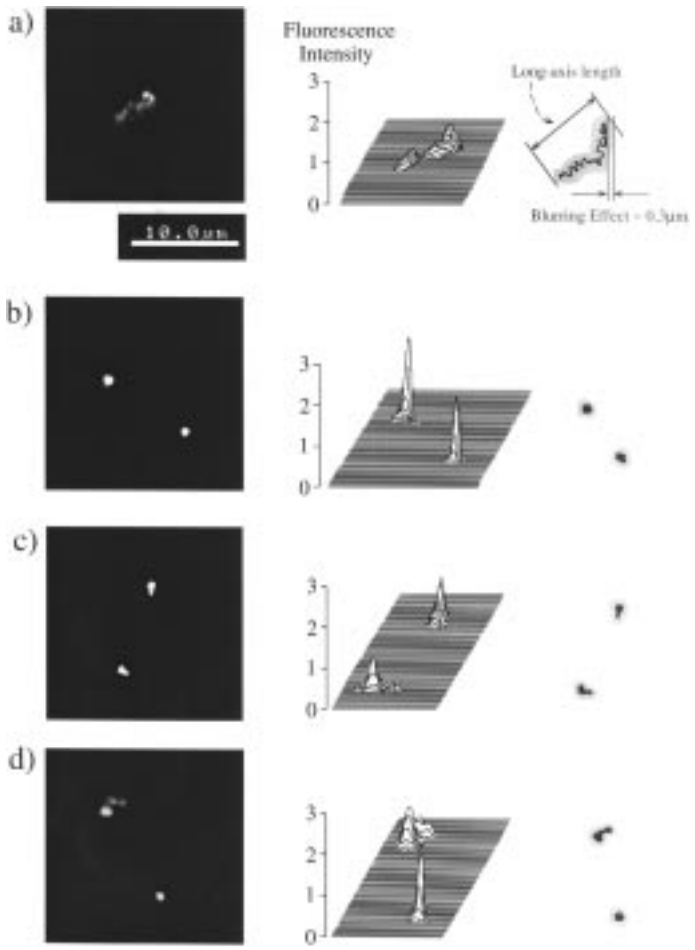

Fig. 1. The left: fluorescence images of the single T4 DNA molecules. (a) Random coiled state in 90 mM TB buffer; (b) tightly collapsed globular state in [PEG] = 8 M solution; (c) loosely condensed globular state in [Arg]/[nucleotide] = 0.4 solution; (d) the tight globule and the loose globule in the mixed system of [PEG] = 4 M, [Arg]/[nucleotide] = 0.4 and [Glu]/[nucleotide] = 2.0. The middle: quasi three-dimensional profiles of fluorescence-intensity distribution for the corresponding left-hand-side images. The noise in the fluorescence images has been smoothed out with image processing. The right: schematic representation of the correspondence between the conformation of DNAs and the fluorescence images.

than those observed in the tightly collapsed state as seen in Fig. 1b. The fluorescence intensity distribution is somewhat diffuse. Here, we refer to the b-type of folded DNA as a 'tight globule' and to the c-type as a 'loose globule'. In the present article, we will use the term 'globule' with the following definition; i.e. the state of the chain that is compressed to smaller size than that in its 'θ-state' conformation, in which the correlation radius of link concentration fluctuations is much smaller than the size of the DNA [32] (definition A; tight globule), *or* the state that has a markedly condensed core and a fluctuating short tail within a single chain (definition B; loose globule). The size of θ-state in T4 DNA chain is deduced to approximately be 1.2 μm from Khun length; 120 nm and the contour length; 57 μm [33].

Fig. 1d shows a coexistence of the tight globule and the loose globule in the mixed system of 4 M PEG, 0.12 μM poly-Arg, and 0.6 μM poly-Glu (in residue; [Glu]/[phosphate] = 2.0). The tight globule and the loose globule were discriminated

by their fluctuating character; i.e. tight globule exhibits no significant time-dependent fluctuation of the folded morphology within a period of observation more than several minutes, and retain the compact bead-like structure. On the other hand, loose globule simultaneously has both the fluctuating tail and the condensed core within a single chain as seen in Fig. 1c,d. It is noted that since the coiled state does not have such condensed core, the discrimination between coil and loose globule is also well-defined. In the present study, to evaluate the degree of folding of giant DNAs in a semi-quantitative manner, we measured the populations of the tight globules, loose globules, and random coils in the ensemble containing more than 50 DNA molecules at each fixed condition. It will become clear in the next section that the relative population of the tight globules, loose globules, and random coils changes sensitively depending on the chemical composition of the solution.

3.2. PEG-induced chain-folding in a single giant DNA

An inert polymer such as PEG has been found to induce large discrete transitions in the higher-order structures of individual giant DNA chains [24]. With the addition of an inert polymer, the conformation of the DNA chain is altered from an elongated random coiled state to the collapsed globular state; i.e. the coil–globule transition. The critical concentration and width of the coexistence region depends on the ionic strength of the solution [34]. To characterize the conditions for the folding transition of the DNA chain in the 90 mM TB buffer solution used throughout the present study, we examined the dependence of the long-axis length of the DNAs on the PEG concentration.

Fig. 2a shows the distributions of long-axis length in T4 DNAs induced at different PEG concentrations. Here, the long-axis length is defined as the longest distance within the fluorescence image (see the schematic representation in Fig. 1a). At PEG concentrations of < 4 M, all

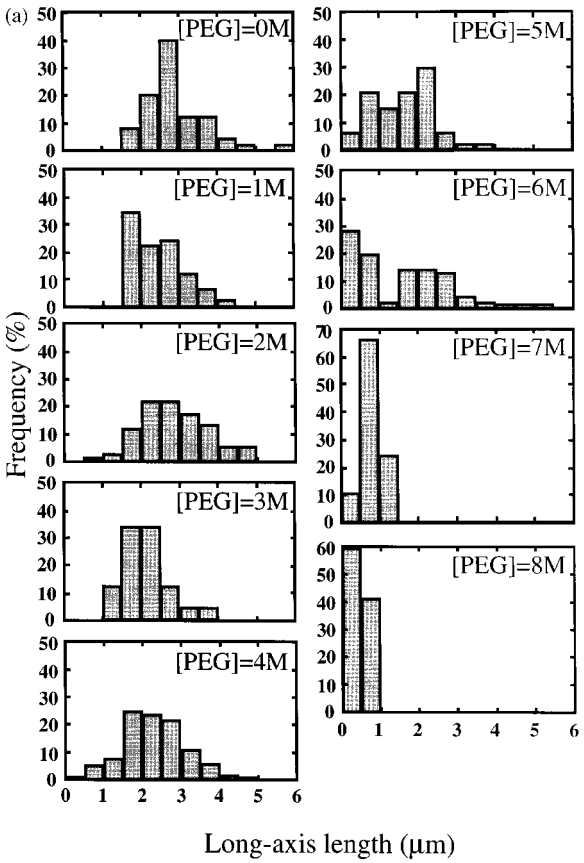

Fig. 2. (a) Distributions of the long-axis length of the T4 DNA molecules at various PEG concentrations. Fifty DNAs have been analyzed at each concentration. Fig. 2. (b) The fraction of tight globules dependent on the PEG concentration. Error bars show the standard error in three experimental runs, each with 50 images.

DNA chains were found to be in the elongated coiled state with an average size of approximately 3 μm. When the PEG concentration became higher than 7 M, however, all the DNAs were folded into the globular state with a size < 1 μm. It is to be noted that all the globules were tightly collapsed without the appearance of loose globule. In other words, the transition is all-or-none. In the region of [PEG] = 4–6 M, the elongated coil and tight globule coexisted at thermal equilibrium, as is shown in the bimodal distributions in Fig. 2a. As previously reported [24,27,28,34], this bimodality characterizes the PEG-induced folding of a single giant DNA as a first-order phase

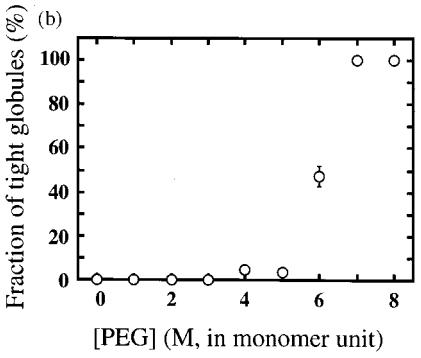

Fig. 2. (*Continued*)

transition between a random coil and a tight globule. Especially, the fraction of tight globules increased along with an increase in the PEG concentration as shown in Fig. 2b.

3.3. DNA / poly-Arg complex in PEG-crowded medium

Fig. 3a shows the dependence of the fraction of tight globules on the poly-Arg concentration at different concentrations of PEG. Open squares, open circles, and closed circles indicate the results with PEG concentrations of 0, 2 and 4 M, respectively. Under these conditions, the fraction of tight globules increased with an increase in poly-Arg concentration. It is apparent from the result in the absence of PEG (open squares), all DNAs were collapsed into tight globules when the ratio of [Arg]/[phosphate] reached 1.0. This implies that the collapse is induced at conditions where the poly-Arg concentration is nearly equimolar to DNA [35]. On the other hand, in the presence of PEG, DNA is collapsed into tight globules even in conditions where [Arg]/[phosphate] < 1, indicating the presence of a cooperative effect between poly-Arg and PEG on the folding transitions. It should be noted that at the 4-M PEG conditions, more than half of the DNA chains transformed into tight globules, even when [Arg] is only 30% of [phosphate]. The minimum concentration to generate the tight globule in the coexistence region is 4 M of PEG. The fractions of loose globule and random coil are also given in Fig. 3b,c, respectively. It is noted that only a small amount of polycation is necessary to induce the

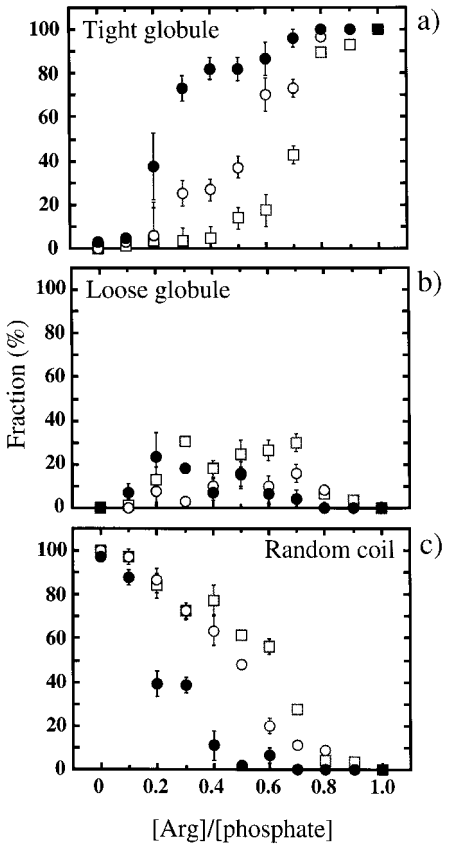

Fig. 3. Dependence of the conformational fraction of T4 DNAs on the poly-Arg concentration in the PEG medium with different concentrations. (a) tight globule, (b) loose globule, and (c) random coil. Each state is defined in the text. Open squares, open circles, and closed circles correspond to [PEG] = 0, 2, 4 M in monomer units, respectively. Error bars show the standard error in 4–6 experimental runs, each with 50 images.

folding transition of giant DNA in the crowded situation, as shown in Fig. 3a.

3.4. Interaction between the DNA / poly-Arg complex and poly-GLU in PEG-crowded medium

Next, we investigated the effect of the polyanion, poly-Glu, on the folding transition of DNA in a PEG-crowded medium containing the polycation, poly-Arg. As the system is complicated, in Fig. 4 we show only the essential portion of the results which indicate the particular effect of poly-Glu on the structural regulation of the com-

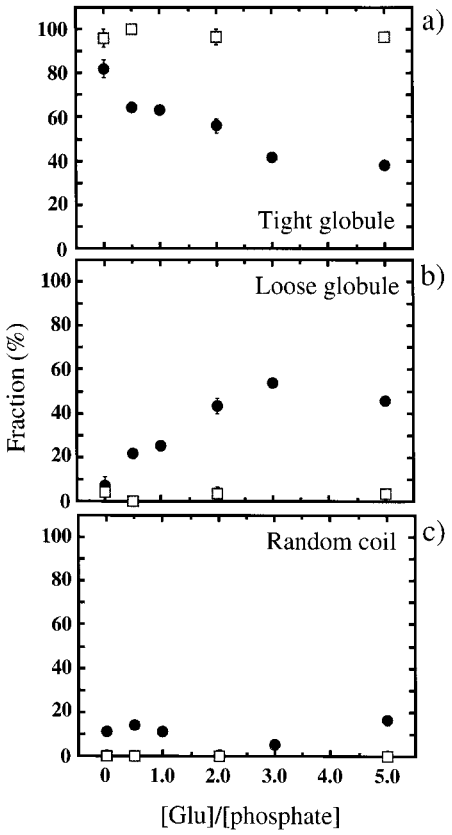

Fig. 4. Change of the conformational fraction in T4 DNAs with poly-Glu concentration in the solution containing 4 M PEG and poly-Arg. [Arg]/[phosphate] = 0.7 for the open squares and [Arg]/[phosphate] = 0.4 for the closed circles. (a) Tight globule; (b) loose globule; and (c) random coil. Error bars show the standard error in 4–6 experimental runs, each with 50 images.

plex in the PEG medium, i.e. polyanion-induced unfolding of the collapsed DNA with different concentrations of polycation in the fixed concentration of inert crowding polymer. Similar to the result of Fig. 3, fraction of each conformational state [(a) tight globule, (b) loose globule, (c) random coil] are shown in Fig. 4.

When [Arg]/[phosphate] = 0.7 in 4 M PEG, there is almost no effect from the addition of poly-Glu up to the ratio of [Glu]/[phosphate] = 5.0 as shown in Fig. 4a, open squares. In other words, essentially all the DNAs remain in the tight globule, even with a large excess of the polyanion. In contrast, when [Arg]/[phosphate] = 0.4, the addition of the poly-Glu induces a rather remarkable effect, with tight globules becoming unfolded as shown in Fig. 4a, closed circles. According to control results on giant-DNA/poly-Glu interaction appearing in [36], poly-Glu does not affect the conformation of giant T4 DNA in solution until the concentration reaches 0.8 M in residues; i.e. [Glu]/[phosphate] = 2.7×10^6. Thus, it is noted that poly-Glu is completely inert for the structural change of giant DNAs in the present range of the concentration of [Glu]/[phosphate] = 0–5.0.

Such a result indicates that the manner of folding of a single giant DNA present in a crowded medium with inert neutral polymer is affected by a delicate compositional balance between the existing polycations and polyanions.

4. Discussion

4.1. Energy character of the macromolecular crowding effect on the folding-unfolding of a single giant DNA chain

In this study, isolated single giant T4 DNA molecules (test macromolecules) were situated in a concentrated solution environment of hydrophilic neutral polymer, PEG. The complex formation between the single DNA and other polycations, poly-Arg, has been examined in the concentrated PEG medium. Particular effects on the properties of the test macromolecules which are created by such concentrated situations have often been referred to as the macromolecular crowding effect, indicating (a) a favoring effect for the formation of a compact conformation of the test macromolecule and (b) an enhancement of the binding between the test macromolecule and other macromolecules, etc. [19]. It is noted that effect (a) can be seen in Fig. 3a from the vertical section with the fixed concentration of poly-Arg. The present findings visually confirm that the macromolecular crowding medium affects on the regulation of higher-order structure of the complex between the single giant DNA and polycation.

Here, we would like to clarify the physicochemical meaning of the crowding potential to

the structural regulation of giant DNA affected by the addition of poly-Arg and poly-Glu from a thermodynamic viewpoint.

In the present system, tight globules, loose globules, and random coils were found to coexist during the period of observation (~several tens of minutes) with equilibrium manner, as is exemplified in Fig. 1d, and is shown quantitatively in Figs. 3 and 4. This means that profile of free energy of the DNA chain, ΔG vs. expansion factor of chain α, exhibits multi local-minima. Thus we estimate the profile of free energy of the DNA chain as in Figs. 5 and 6, based on the relative populations.

To illustrate the profile, we first calculate the difference of free energy between tight globule and random coil, and between tight globule and loose globule in terms of the relationship of the Boltzmann distribution, i.e.

$$\Delta G_{\mathrm{tg}\leftarrow\mathrm{c}} = -kT\ln\frac{P_{\mathrm{tg}}}{P_{\mathrm{c}}} \tag{1}$$

$$\Delta G_{\mathrm{tg}\leftarrow\mathrm{lg}} = -kT\ln\frac{P_{\mathrm{tg}}}{P_{\mathrm{lg}}} \tag{2}$$

where $\Delta G_{\mathrm{tg}\leftarrow\mathrm{c}}$, $\Delta G_{\mathrm{tg}\leftarrow\mathrm{lg}}$ are the free energy differences between tight globule (tg) and random coil (c), and between tight globule (tg) and loose globule (lg), respectively. P is the fraction of each conformational state indicated by its subscript, k is Boltzmann constant, and T is absolute temperature. Based on the calculated values of the free energy difference, relative stability of the three different states (random coil, loose globule, and tight globule) has been deduced. Next, barriers among the minima corresponding to tight globule, loose globule and random coil are assumed to be larger than energy of thermal fluctuation of the solvent molecules, 1.5 kT, in a tentative manner, based on the observation that the three kinds of conformational state could coexist. In addition, though long-axis length of random coil and loose globule diffusely decrease with the increase of poly-Arg concentration in fact, the shift of α-

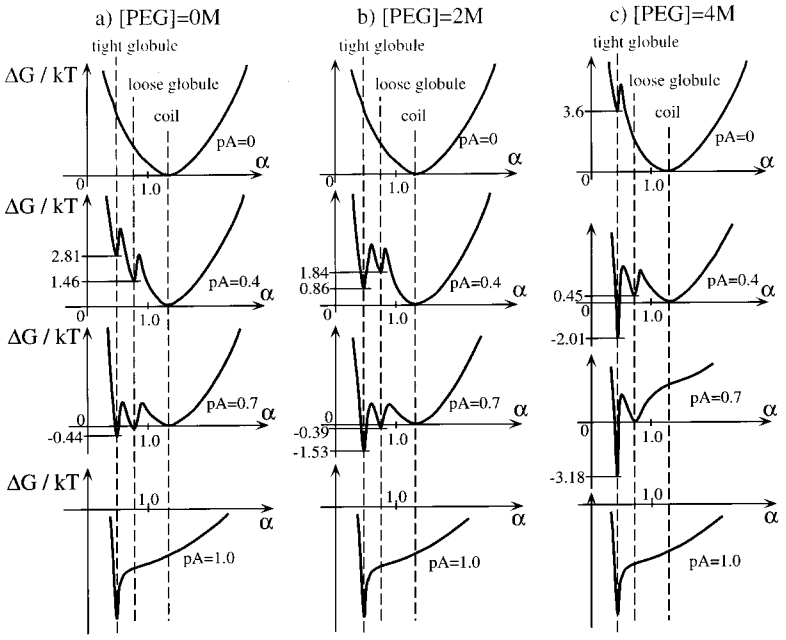

Fig. 5. Schematic representation on the free energy profile of single giant DNA chain in the mixed system of PEG and poly-Arg, as have been evaluated from the relative populations. Relative differences of free energy (ordinate, $\Delta G/kT$) among different conformational state (abscissa, expansion factor α; $\alpha = 1.0$ corresponds to the random coiled state of ideal chain) are given, referring the theoretical curve in the free energy as have been reported in Yoshikawa and co-workers [27,28]. pA denotes [Arg]/[phosphate]. (a) [PEG] = 0 M, pA = 0, 0.4, 0.7, 1.0. (b) [PEG] = 2 M, pA = 0, 0.4, 0.7, 1.0. (c) [PEG] = 4 M, pA = 0, 0.4, 0.7, 1.0.

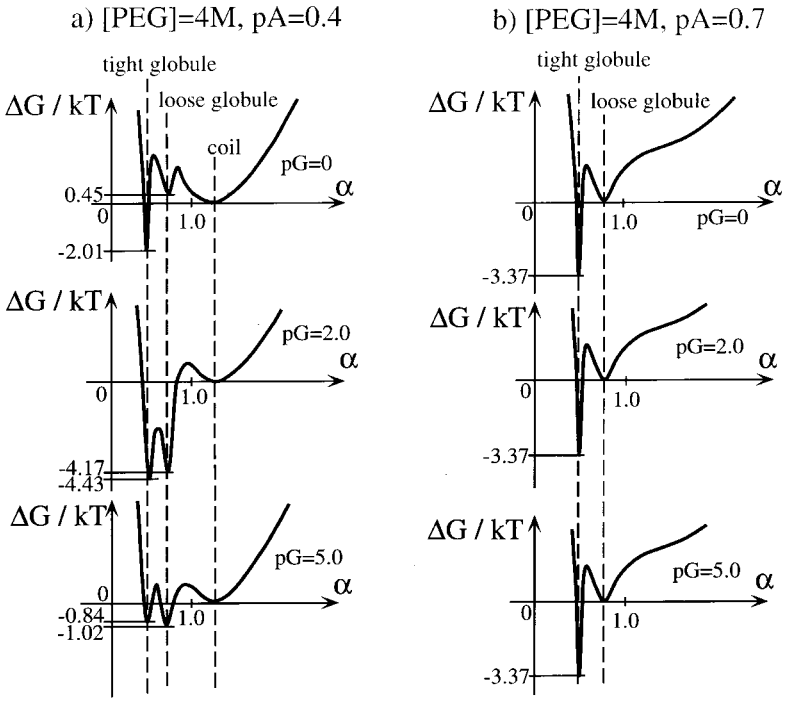

Fig. 6. Schematic representation on the free energy profile of single giant DNA chain in the mixed system of PEG, poly-Arg, and poly-Glu. pG denotes [Glu]/[phosphate]. (a) [PEG] = 4 M, pA = 0.4, pG = 0, 2.0, 5.0. (b) [PEG] = 4 M, pA = 0.7, pG = 0, 2.0, 5.0.

position of coil and loose globule is not depicted for simplicity in the illustrations.

Obtained profiles in case of mixed system with PEG-crowding medium and poly-Arg are shown in Fig. 5, especially for the condition with [Arg]/[phosphate] = 0, 0.4, 0.7, 1.0 in [PEG] = (a) 0, (b) 2, (c) 4 M. Zero point of ΔG is expediently fixed at the free energy of random coiled state except for the condition that coiled state disappear (Fig. 5c; [Arg]/[phosphate] = 0.7: the state of $\Delta G = 0$ is decided as loose globule state) and that only the tight globule is formed (Fig. 5a–c; [Arg]/[phosphate] = 1.0: the state of $\Delta G = 0$ is not decided).

It should be again noticed that crowding background generates double-minima character of free energy in the absence of poly-Arg (Fig. 5c; pA = 0). Since free energy of DNA chain gets such a bimodal character in the crowded situation, tight globule initially obtains the stabilizing effect on the conformation. Thus, minimum value of free energy which corresponds to tight globular state is markedly lowered by the addition of less amount of poly-Arg (Fig. 5c; pA = 0.4, 0.7) in such a crowded medium. From the consideration on the profile of free energy, crowding background was found to provide potential to fold DNA chain through the bimodal character of its free energy. Similar estimation has also been done for the results of Fig. 4. Fig. 6 shows the resulted profiles. While the minimum value corresponding to tight globule was lowered with the addition of poly-Glu ([Glu]/[phosphate] = 5.0) in [Arg]/[phosphate] = 0.4 (Fig. 6a), that did not change in [Arg]/[phosphate] = 0.7 (Fig. 6b). These illustrations show that a little difference of added amount of poly-Arg contributed to regulate the relative stability between tight globule and random coil.

Summarizing such analysis, folding and unfolding of single giant DNA chain in the mixed system of crowding neutral polymer, polycation, and polyanion are found to have the following two characters: (1) crowding background of inert polymer initially introduces the profile of the dou-

ble-minima to the free energy of the DNA chain; (2) compositional balance of polycation and polyanion changes the relative stability in the local-minima values of the free energy, in the state of tight globule, loose globule, random coil.

4.2. Biological implications of the structural regulation of the folded giant DNAs observed in the present mixed medium

The biological significance of the macromolecular crowding effect has been investigated in many studies (see reviews [21–23]), because the cellular environment is highly concentrated with various kinds of biopolymers in general, and the biopolymers originally function under the physiologically crowded situation. It has well been characterized that the macromolecular crowding causes in vitro DNA condensation, which has been known as Ψ-condensation [3,4]. Recently, it is advocated that the macromolecular crowding yields a mandatory condensation of DNA in vivo [37]. The effect is becoming one of the essential issues in the biological research on the DNA-condensation phenomena [8,23].

Our present results indicate that the structural changes in the folded state of single giant duplex-DNA sensitively depends on the compositional balance of coexisting polycations and polyanions, especially in the crowded medium with inert polymers. Whether retaining or local unfolding the tightly collapsed structure of the DNA-polycation complex is determined by the concentration both of the binding polycations and of the surrounding polyanions in the crowded medium.

The higher-order structure of genomic DNAs in vivo is known to be regulated by the interplay between cationic and anionic proteins. An example of an in vivo system is seen in an interaction between sperm chromatin and nucleoplasmin in fertilized eggs. Sperm chromatin is tightly compacted by binding of sperm-specific protein, protamine, that is highly basic with arginine residues over 80% in its amino acid sequences [38]. On the other hand, nucleoplasmin is a thermostable acidic protein [39] and has clusters of charged residues including a long poly-glutamic acid tract [40,41]. Nucleoplasmin decondenses sperm nuclei of *Xenopus*, *Mytilus*, salmon, human, etc., due to the removal of protamines from the sperm chromatin [42–44]. In sperm nuclei, the interplay between polycations (protamines) and polyanions (nucleoplasmins) plays an essential role for the structural regulation of folded state of genomic DNAs. It is noted here that the interplay should naturally be affected by the presence of other proteins inside nucleus such as nuclear matrix proteins, and by the compressive potential imposed by the crowded medium with inert proteins in cytoplasm. The present results suggest physico-chemical aspect of how the interplay affecting for the structural regulation of genomic DNAs is influenced by the cellular crowded surroundings.

5. Conclusion

In the present study, it has been experimentally clarified that in the crowded medium with inert neutral polymer, relative stability in local-minima of free energy among different conformational states of single giant DNA chain is dependent in a delicate manner on the compositional balance of polycation and polyanion. Due to the double-minima character of the free energy generated by the crowding background, especially at the threshold conditions in the folding transition of DNA induced by the crowding polymer, a small change in the concentration of either of the polymers can induce a large change in the higher-order structure of giant DNA. Since the solution environment in the living cell physiologically establishes such a polymer situation, the present findings can be expected to provide insight into the regulation of folding-unfolding of genomic DNA in vivo.

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