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Direct observation of the biphasic conformational change of DNA induced by cationic polymers

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The interaction between T4 DNA and basic polypeptides was observed using fluorescence microscopy. Free DNA molecules exhibited random Brownian motion accompanying the conformational change. With the addition of polycation, such as histone and polyarginine, DNA molecules tended to shrink to become spherical shapes. The persistent lengths and the distributions of long axis lengths of DNA-polyarginine complexes were determined from the video images at various polyarginine concentrations. It is demonstrated that the conformation of DNA changes in a biphasic manner in the presence of polyarginine.

Fluorescence microscopy; DNA-polypeptide complex; Direct observation; biphasic conformational change

1. INTRODUCTION

Interactions between DNA and basic proteins play a key role in the regulation of various fundamental functions in living systems. In general, DNA molecules in eukaryotic chromosomes form complexes with basic, or cationic, proteins such as histones and protamines. As these interactions are regarded to play a major role in gene expression, it is important to obtain insight into the dynamic structure of the DNA complexes.

The interactions between DNA and cationic homopolypeptides such as poly-L-lysine (p-Lys) and poly-L-arginine (p-Arg) have been studied as a model system of DNA–protein interactions [1–5]. These cationic peptides have been shown to bind to DNA by electrostatic interaction between positively charged amino acid residues and negatively charged phosphate groups. The interactions between DNA and these cationic polypeptides have been analyzed by means of spectral and melting profile measurements [4]. The binding of cationic polypeptides to DNA has been speculated to be cooperative. In relation to this, it has been reported that the mixture of DNA–polypeptide complexes can be separated by centrifugation to give totally complexed and 'naked' DNA molecules [1].

From the study using sedimentation and light-scattering, Shapiro et al. estimated the DNA complexes to be roughly spherical [6]. Direct observation of such DNA–polypeptide interactions has been performed with electron microscopy by Inoue et al. [7], but such observation provides only static information on the structures of pretreated, dry DNA. Thus, information on the dynamics of DNA complexes has been missed in the past.

Fluorescence microscopy provides useful information about the conformation of large DNA molecules in solution [8,9]. Individual DNA molecules stained with fluorescent dyes can be observed as moving images under appropriate excitation. We have previously reported that dynamic change of the conformation of DNA can be quantitatively analyzed by this technique [9]. In the present paper, we report the direct observation of DNA–polypeptide complexes exhibiting Brownian motions, and the results of quantitative analysis of the dynamic interaction.

2. MATERIALS AND METHODS

Bacteriophage T4dC DNA was purchased from Nippon Gene. Histone (from calf thymus, Type II-A) and poly-L-arginine (DP 120) were purchased from Sigma. The DNA was diluted with 0.5× TBE buffer solution (45 mM Tris, 45 mM borate, 1 mM EDTA) containing 4% (v/v) 2-mercaptoethanol as an anti-oxidant, and DAPI (4′,6-diamidino-2-phenylindole) as a fluorescent probe. The final concentrations of DNA in nucleotide and DAPI were 0.24 μM and 0.20 μM, respectively. The polypeptides were added to the above mixture at various concentrations. It was ascertained that the effect of DAPI on the radius of gyration or persistent length of DNA was negligibly small under the experimental conditions in this study.

5 μl of the solution was put on a glass slide and covered with a cover-glass. The surrounding of the cover-glass was sealed by fingernail polish to prevent thermal convection and evaporation. The distance between the two plates was ca. 20 μm.

An inverted microscope (Nikon) equipped with a 100× oil-immersed objective lens and a high sensitive SIT TV camera with an image processor, Argus 10 (Hamamatsu Photonics), were used with 365 nm UV excitation. Two-dimensional real-time image data were recorded

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on video tapes, and afterwards the data were analyzed with a personal computer.

3. RESULTS AND DISCUSSION

Individual DNA molecules were observed using fluorescence microscopy and recorded on video tapes as moving images. Fig. 1 (a–c) demonstrates the successive movement of a T4 DNA molecule in an aqueous solution.

We have applied this technique to study the interaction between DNA and histone. Calf thymus histone was added to the T4 DNA solution and the resulting complex of DNA and histone was observed. It was found that DNA molecules exhibit shrunken spherical shapes in the presence of histone. The complex between DNA and histone showed Brownian fluctuational movement and kept the spherical shape during the observation. This suggests that the stretching motion is restrained by the binding of histone due to the effect of cross- linking between the phosphate groups outside the double-helix. In order to shed light on the interaction, poly- L-arginine (p-Arg) was used as a simple model for the basic protein. Microscopic observation was performed in the presence of p-Arg. When p-Arg was added to the T4 DNA solution, DNA exhibited spherical shapes similar to those observed in the presence of histone (Fig. 1d–f).

The higher-order structure of DNA complexed with p-Arg was quantitatively analyzed by means of the following procedure. The conformation of DNA was observed under various concentrations of p-Arg. The mole ratios of amino acid residues of p-Arg and phosphate groups of DNA were Arg/DNA = 0.1, 0.2, 0.5 and 1.0. The radius of gyration, R_g, and persistent length, λ, were estimated for each p-Arg concentration as follows:

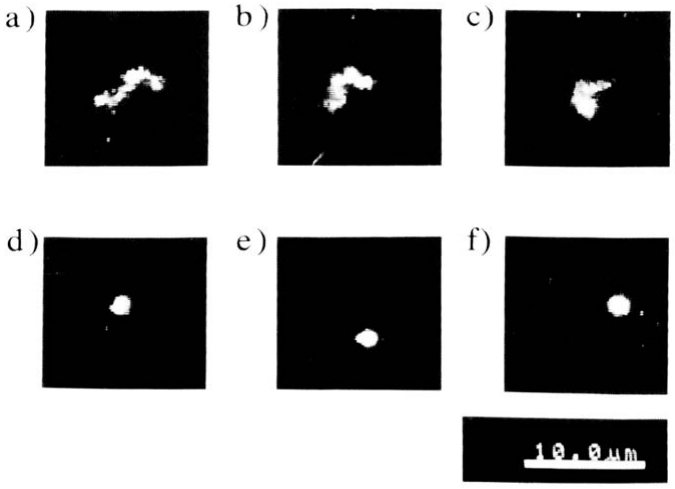

Fig. 1. Micrographs of T4 DNA molecules showing Brownian motion in aqueous solutions. (a–c) T4 DNA in TBE buffer solution. (d–f) T4 DNA complexed with p-Arg. Arg/DNA = 1.0 (mole ratio of monomer units).

Table 1

The length of long axis, l, the radius of gyration, R_g, and persistent length, λ, of T4 DNA complexed with p-Arg

Arg/DNA[a]	l[b] (μm)	R_g (μm)	λ (μm)
0	3.47 ± 0.51	1.22 ± 0.42	0.081
0.1	3.35 ± 0.92	1.10 ± 0.14	0.066
0.2	3.13 ± 1.07	1.03 ± 0.09	0.058
0.5	1.77 ± 0.33	0.43 ± 0.01	0.010
1.0	1.80 ± 0.27	0.44 ± 0.02	0.011

[a]Mole ratio of monomer units of p-Arg and DNA.
[b]The length of long axis, l, is the maximum distance between two points on the profiles of the DNA image. The statistical error is given as the standard deviation.

$$R_g = \left\{\tfrac{1}{2}(R_l^2 + R_s^2)\right\}^{\frac{1}{2}} \text{ and } \lambda = \frac{3R_g^2}{L}$$

where R_l and R_s are the larger and smaller values, respectively, of the two principal values of the radius of gyration tensor, and L is the contour length of the T4 DNA, 55 μm, which was estimated from the distance between the adjacent base pairs, 3.3 Å [10].

The R_g and λ values are given in Table I, which also lists the lengths of long axis, l, determined as the longest distance between the points on the outline in the two-dimensional image of DNA. It was found that both l and λ decrease with the increase of p-Arg. This result indicates that the binding of p-Arg decreases the persistent length of the DNA molecule, due to the electrostatic

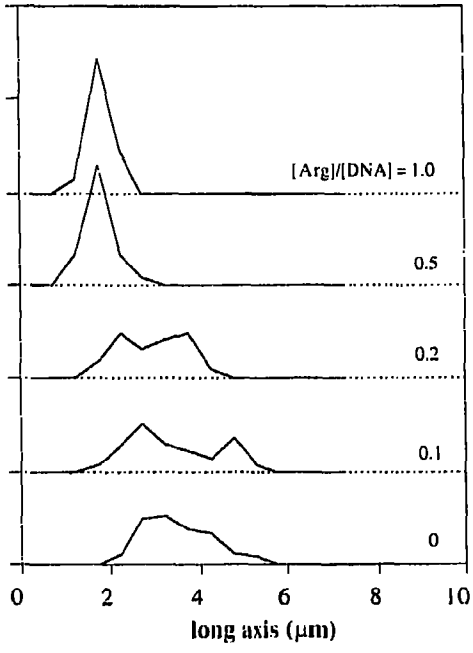

Fig. 2. Distribution of the lengths of long axis of T4 DNA complexed with p-Arg; the mole ratios of p-Arg and DNA in monomer units were Arg/DNA = 0, 0.1, 0.2, 0.5 and 1.0, as given in the figure. The numbers of molecules analyzed were 100, 27, 34, 25 and 14, respectively. Each area of the distribution function was normalized to be equal.

interaction between cationic arginine residues and anionic phosphate groups of DNA. It is also noted that the lengths change abruptly between Arg/DNA = 0.2 and 0.5, suggesting a cooperative behavior of p-Arg binding to the DNA molecules.

Fig. 2 shows the distributions of the long axis lengths of T4 DNA at Arg/DNA = 0, 0.1, 0.2, 0.5 and 1.0. It should be noted that there are two peaks in the distributions of the lengths in the case of Arg/DNA = 0.1 and 0.2. It is obvious that DNA molecules simultaneously exhibit two distinct conformations, i.e. elongated and shrunken spherical conformations. At the higher concentrations of p-Arg, however, there is a single peak in the distribution. This result demonstrates that the p-Arg molecules bind to DNA in a cooperative manner. The initial addition of p-Arg causes the conformational change of some DNA molecules, whilst the others remain almost free from p-Arg. When more p-Arg is added to the mixture of 'partly complexed' and 'free' DNA, p-Arg prefers to bind to the former. Thus, at the intermediate concentration of p-Arg, the DNA molecules exhibit two distinguishable conformations, namely, the totally complexed (spherical) and 'naked' (elongated) forms. Such a cooperative phenomenon in binding of polypeptides has been proposed based on the separation of the totally complexed DNA from the mixture by centrifugation [1]. In the present study, we have obtained clear evidence of the coexistence of the two conformations, suggesting the cooperative binding of p-Arg to DNA.

It is not strange to speculate that the cooperative interaction between DNA and basic proteins concerns the mechanism of the control of gene expression in living cells.

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