DNA Compaction in a Crowded Environment with Negatively Charged Proteins

M. K. Krotova,[1] V. V. Vasilevskaya,[2] N. Makita,[3] K. Yoshikawa,[4] and A. R. Khokhlov[1,2]

[1]*Physics Department, M. V. Lomonosov Moscow State University, Moscow 119992, Russia*
[2]*Nesmeyanov Institute of Organoelemental Compounds (INEOS), Russian Academy of Sciences, Vavilova ul. 28 Moscow 119991, Russia*
[3]*Faculty of Environmental and Information Sciences, Yokkaichi University, Yokkaichi 512-8512, Japan*
[4]*Department of Physics, Kyoto University, Kyoto 606-8502, Japan*

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We studied the conformational properties of DNA in a salt solution of the strongly charged protein bovine serum albumin. DNA is compacted when a suitable amount of bovine serum albumin is added to the solution due to a crowding effect and strong electrostatic repulsion between DNA and bovine serum albumin, both of which carry negative charges. However, DNA undergoes an unfolding transition with an increase in the salt concentration. This observation contradicts the current understanding of polymer- and salt-induced condensation, ψ condensation. We propose a simple theoretical model by taking into account the competition between the translational entropy of ions and electrostatic interaction.

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Living cells maintain their lives by using a closed medium that contains a rich variety of biomacromolecules under crowded conditions. In general, as the actual crowding inside living cells, macromolecules occupy a significant fraction of the total volume, on the order of 20%–30% (w/v) [1]. This molecular crowding significantly affects the structure and function of biomacromolecules by changing the thermodynamic conditions [1–7]. To mimic molecular crowding, *in vitro* studies have been performed by adopting various molecules as cosolutes. Commonly used cosolutes include polyethylene glycol (PEG), dextran, proteins, alcohol, glycol, and amino acids [6]. The addition of aqueous polymers to the solution of DNA macromolecules leads to a sharp decrease in DNA size, which is referred to as the ψ condensation of DNA. The ψ condensation of DNA [ψ (psi): polymer- and salt-induced] was discovered almost four decades ago by Lerman [8], who found that DNA coils shrink abruptly when PEG above a certain critical concentration is added to the solution with a low-molecular-weight salt. Since that time, this effect has attracted considerable attention from both experimenters and theoreticians because of its fundamental significance in polymer physics and important applicability in a wide range of research fields such as molecular biology, pharmaceuticals, medicine, and chemical and material sciences [9]. In the ψ condensation of DNA in PEG, the introduction of salt leads to a shift of the coil-to-globule transition to a lower PEG concentration; i.e., low-molecular-weight salt promotes condensation [10–12]. Currently, DNA condensation is considered to combine single-molecule compaction, multimolecular aggregation, and precipitation. Recently, it has been confirmed that DNA molecules undergo a large discrete transition into a tightly compact state by use of the experimental technique of single-molecule observation by fluorescence microscopy (FM) [10].

In this study, we performed single-molecule DNA observation to study the compaction of individual DNA molecules in a crowded environment with negatively charged proteins. Interestingly, the result indicates that a low-molecular-weight salt prevents the compaction of DNA, which contradicts the current understanding of ψ condensation. The fact that negatively charged proteins fold DNA into a compact state is of particular biological significance, since they exist in large amounts in living cells.

Figure 1 summarizes the change in DNA conformation upon the addition of different amounts of bovine serum albumin (BSA) and NaCl. At 100 mM NaCl and 1% (w/v) BSA, all of the DNA molecules assume an elongated conformation with a mean long-axis length L of ca. 3 μm. The rather broad distribution of L is due to the large thermal fluctuation of elongated DNA. The coexistence of elongated and compact conformations is noted at 10% (w/v) BSA. Above 15% (w/v) BSA, all of the DNA molecules show the folded compact state. The coexistence of the coil and globule states around 10% (w/v) BSA indicates that the folding transition of DNA is a first-order phase transition [12,13]. The effect of salt is seen for DNA compaction at 15% (w/v) BSA, where DNA returns back to the coil state with an increase in NaCl up to 200 mM. Thus, in contrast to the usual scenario of ψ condensation, an increase in the salt content here leads to the unfolding of compact DNA.

BSA is a plasma globular protein [13]. Its charge and structure depend on the ionic strength of the solution. At neutral pH, BSA is a compacted particle ($40 \times 40 \times 140$ Å) with a negative charge (approximately $-18e$) distributed homogeneously on its surface [13]. The size of BSA has been shown to be comparable to the diameter d of the double helix of DNA ($d = 20$ Å). It is expected that the compaction of DNA in a crowded environment with such protein proceeds due to two main factors: depletion

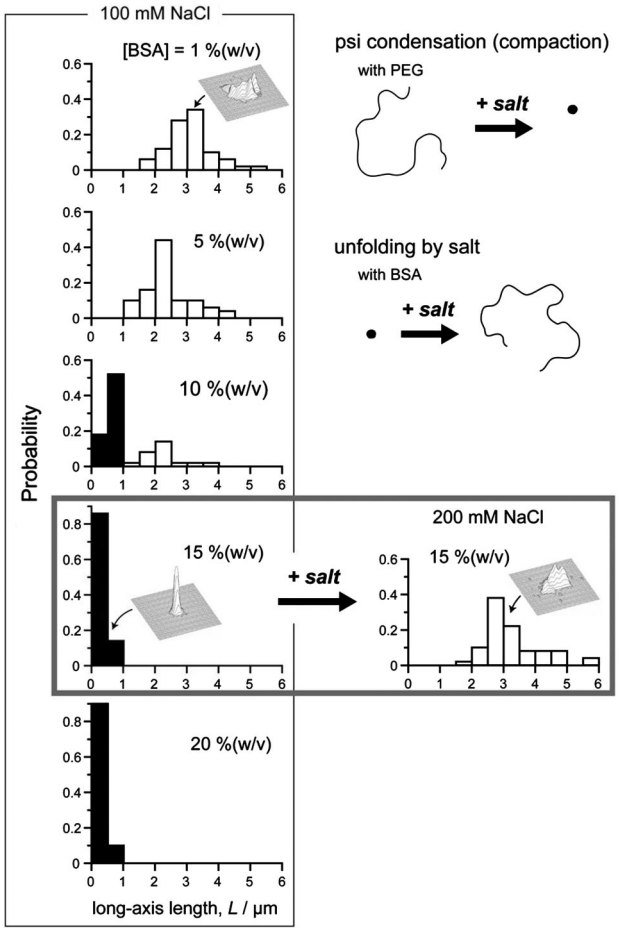

FIG. 1. Conformational transition of DNA induced in a crowding environment with a negatively charged protein. Left: Distribution of the long-axis length of T4 DNA (165.6 kbp, where bp denotes base pairs) at different concentrations of BSA, deduced from single-molecule observation with FM. Right top: Schematic representation of the effect of salt on the folding-unfolding transition of DNA. The insets show pseudo-three-dimensional representations of the fluorescence intensity of DNA images obtained by FM observation.

interaction and electrostatic repulsion [13–16]. Under the current understanding of DNA compaction or condensation by a crowding effect, the promotion of condensation by salt has been interpreted as lowering of the free energy penalty to fully neutralize DNA due to screening of the translational entropy of the DNA counterions entrapped in its vicinity. However, it has been shown that even in salt solution, the counterions of a strongly charged macromolecule are mainly distributed not only in the vicinity of the macroion but over the whole volume, and the macroion as a whole has a nonzero charge [17]. This factor, which is especially important for DNA condensation in the crowding environment with charged species, has to be taken into account.

In order to develop the corresponding theory, we divide the total volume by the effective volume of DNA $V_{\rm eff}$ and the external solution $V_{\rm ext}$ (see Fig. 2).

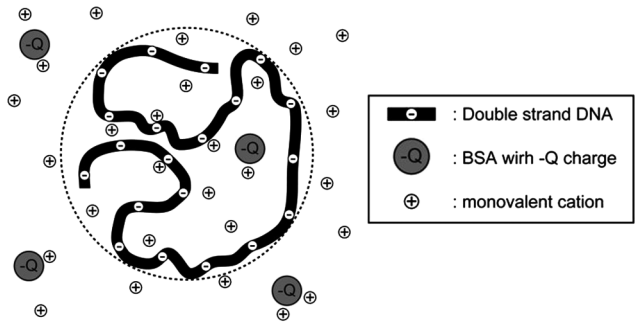

FIG. 2. Schematic representation of DNA in a BSA solution. The effective volume of DNA is shown by the dotted line.

We also propose that a DNA macroion (N, degree of polymerization; f, degree of ionization; d and l, diameter and length, respectively, of the Kuhn segment; e, elementary charge) in a salt solution of protein BSA (rd, radius; Qe, charge) loses a fraction β of positively charged counterions. As a result, the macroion as a whole possesses an effective negative charge $Q_{\rm eff} = \beta N f e$ [17]. The negatively charged protein molecules are repelled by negatively charged DNA. The parameter $\chi_{\rm PN}$ of this repulsion is estimated as a ratio of the electrostatic energy of the interaction of protein molecule and part of the chain with size rd in close contact to the thermal energy kT: $\chi_{\rm PN} \sim \frac{e^2}{\varepsilon d k T} f Q \sim u f Q$.

The free energy is written separately for both phases, DNA and external solution, under the assumption that the volume fraction of BSA $\phi_P^{\rm in}$ and low-molecular-weight salt $n_S^{\rm in}$ inside the effective volume of DNA differs from these values (ϕ_P and n_S) in the external solution. The volume fractions $\phi_P^{\rm in}$ and $n_S^{\rm in}$, as well as the equilibrium size $V_{\rm eff}$ of DNA and its charge $\beta N f e$, are determined by the equality of osmotic pressures and chemical potentials of BSA and low-molecular salt in these two regions (two phases) and by the condition that the derivative of the total free energy $F_{\rm tot}$ with respect to β is equal to zero.

The total free energy $F_{\rm tot}$ of a system is presented as the sum of five contributions:

$$F = F_{\rm el} + F_{\rm mix}^{\rm in} + F_{\rm tr} + F_{\rm el\text{-}stat} + F_{\rm ext}, \qquad (1)$$

where the first three terms refer to the effective DNA volume, $F_{\rm el\text{-}stat}$ describes electrostatic interaction of the DNA macroion with the external solution, and $F_{\rm ext}$ is the free energy of the external solution.

$F_{\rm el}$ is the free energy of the elastic deformation of DNA with respect to the so-called ideal conformation:

$$\frac{F_{\rm el}}{kT} = \frac{3}{2}\left[\left(\frac{V_{\rm eff}}{V_0}\right)^{2/3} + \left(\frac{V_0}{V_{\rm eff}}\right)^{2/3}\right], \qquad (2)$$

where V_0 is the effective volume of DNA in the ideal conformation: $V_0 \sim N^{3/2} l^3$. The contribution $F_{\rm mix}^{\rm in}$ describes the DNA and BSA mixing energy in the Flory-Huggins approximation:

$$\frac{F_{\text{mix}}^{\text{in}}}{kT} = \frac{V_{\text{eff}}}{d^3}\left(\frac{\phi_P^{\text{in}}}{r^3}\ln\phi_P^{\text{in}} + (1-\phi-\phi_P^{\text{in}}) \times \ln(1-\phi-\phi_P^{\text{in}}) + \chi_{\text{NP}}\phi\phi_P^{\text{in}}\right), \quad (3)$$

where d is the elementary spacing in the Flory-Huggins model and ϕ is the volume fraction of DNA: $\phi = Nld^2/V_{\text{eff}}$.

$F_{\text{tr}}^{\text{in}}$ describes the contribution due to the translational entropy of low-molecular-weight ions (counterions of DNA and protein, and ions of the low-molecular-weight salt):

$$\frac{F_{\text{tr}}^{\text{in}}}{kT} = \frac{V_{\text{eff}}}{d^3}\left[[(1-\beta)f\phi + n_s^{\text{in}}]\ln[(1-\beta)f\phi + n_s^{\text{in}}] + Q\frac{\phi_p^{\text{in}}}{r^3}\ln\left(Q\frac{\phi_p^{\text{in}}}{r^3}\right) + n_s^{\text{in}}\ln n_s^{\text{in}}\right]. \quad (4)$$

$F_{\text{el-stat}}$ is the electrostatic interaction of the DNA macroion with an excess of positive charges in the external solution:

$$\frac{F_{\text{el-stat}}}{kT} = \left(\frac{4\pi}{3}\right)^{1/3}(\beta Nf)^2 u\frac{1}{V_{\text{eff}}^{1/3}}\exp\left(-\frac{V_{\text{eff}}^{1/3}}{r_D}\right), \quad (5)$$

$$r_D \cong \sqrt{\frac{u}{Id}}, \quad (6)$$

where r_D is the Debye-Hückel radius and I is the ionic strength determined by the total concentration of counterions and salt ions (all of which have elementary charge e) and also by the concentration of protein molecules with total charge Q and volume $(\text{rd})^3$ [18,19] in the external solution:

$$I = \frac{\beta Nfd}{V_{\text{ext}}} + 2n_s^{\text{out}} + Q\frac{\phi_p}{r^3} + \left(\frac{Q}{r^3}\right)^2\phi_p. \quad (7)$$

The free energy of the external solution F^{ext} in correspondence to the above-described theoretical model was written as a sum of mixing energy and energy due to the translational entropy of low-molecular ions [see Eqs. (3) and (4)]:

$$\frac{F^{\text{ext}}}{kT} = \frac{V_{\text{ext}}}{d^3}\left[\frac{\phi_P}{r^3}\ln\phi_P + (1-\phi_P)\ln(1-\phi_P) + Q\frac{\phi_P}{r^3} \times \ln\left(Q\frac{\phi_P}{r^3}\right) + \left(\frac{\beta Nfd^3}{V_{\text{ext}}} + n_s\right) \times \ln\left(\frac{\beta Nfd^3}{V_{\text{ext}}} + n_s\right) + n_s\ln n_s\right]. \quad (8)$$

The results of the calculation for $Q = -10$, $N = 100$, $l/d = 36$, $u = 1$, $f = 0.1$, and $r = 2$ and for different values of concentration n_s are presented in Fig. 3, which shows the dependency of the DNA swelling ratio $\alpha = (V_{\text{eff}}/V_0)^{2/3}$ on ϕ_P. The addition of BSA leads to the compaction of DNA. At a relatively low concentration ϕ_P, the decrease in DNA size proceeds smoothly (curves a–c). At some critical values of $\phi_P = \phi_i$ $(i = a, b, c)$, DNA undergoes a discrete transition into a compact state.

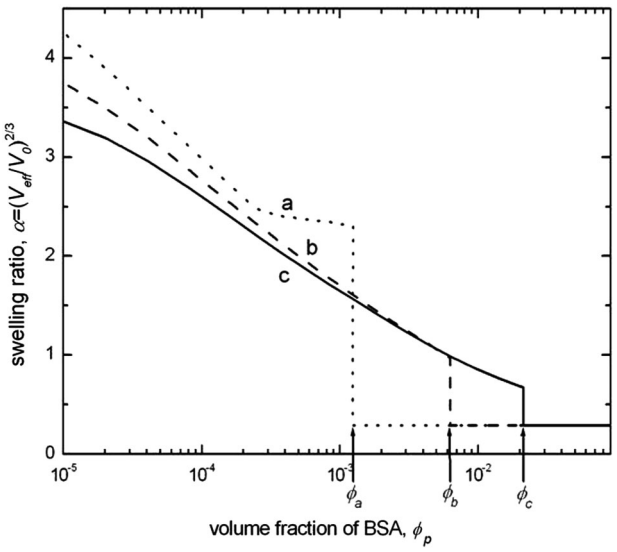

FIG. 3. Dependence of the swelling ratio α on the protein volume fraction ϕ_P for different salt concentrations $n_s = 10^{-6}$ (a), 5×10^{-6} (b), and 10^{-5} (c). ϕ_i is the critical protein concentration for inducing the folding transition into a compact state.

It is clear that an increase in the salt concentration n_s leads to a decrease in DNA size at a low concentration of protein. Also, with an increase in the salt concentration, the point of the sharp coil-to-globule transition shifts to a higher protein concentration, and the amplitude of the change in the swelling ratio at the coil-to-globule transition becomes smaller.

The first-order collapse transition of DNA proceeds when the effect of repulsive BSA-DNA interaction exceeds the effect of osmotic pressure of counterions exerted from DNA. On the other hand, at low salt concentration n_s, the fraction β of counterions that escape from the interior of the DNA coil decreases with an increase of low-molecular salt n_s. The main physical reason of this phenomenon is easily understood. Indeed, the introduction of low-molecular salt leads to the appearance of compressive osmotic pressure and, thus, to the contraction of DNA size R. At constant values of β it could lead to a significant growth of the electrostatic free energy [see Eq. (5)]. In order to prevent this growth and to keep the value of the electrostatic free energy relatively low in this region, some fraction of counterions returns back to the DNA interior and the compressive osmotic pressure of salt is equilibrated by the exerting osmotic pressure of counterions absorbed by DNA (the fact that the fraction β at low salt concentration is a nonmonotonic function of salt concentration has been addressed in detail in Ref. [17]).

As a result, with an increase of n_s the osmotic pressure of counterions kept within the DNA coil increases and many more BSA molecules should be added to induce the DNA collapse transition. Thus, the higher salt concentration n_s is, the higher BSA concentration is, when the DNA collapse transition becomes favorable; i.e., the

addition of salt results in a decrease of the ability of proteins to act as condensing agents.

We also found that the plateau region can be observed right before DNA collapse for low salt concentration (Fig. 3, curve *a*). It happens due to the complex interplay of the translational entropy contribution and electrostatic interaction leading to the appearance of a region where the fraction of counterions β that escape DNA is kept as a constant.

The fact that with an increase in the salt concentration n_s the point of the sharp transition shifts to a higher content ϕ_P of BSA means that an increase in the low-molecular-weight salt (in the above case, for example, in the region between concentration ϕ_a and ϕ_c starting from $n_s = 10^{-6}$) could lead to the unfolding of DNA. This theoretical conclusion corresponds to the experimental data. Also in correspondence with experiment, we obtained that the introduction of charged protein first calls the smooth decrease in DNA size, whereas a sharp first-order transition into a collapsed state is observed only after the significant decrease of DNA size [compare averaged DNA coil size for [BSA] = 1% (w/v) and 10% (w/v), Fig. 1].

In this Letter, we considered a rather simple situation, a DNA molecule in a crowding environment of one type of strongly charged proteins. We have found that in this case the introduction of low-molecular salt could lead to counterintuitive DNA behavior, namely, to DNA decompactization. The simple theoretical analysis shows that this effect occurs due to the complex interplay of electrostatic interaction and translational entropy of counterions. We would like to mention that the effect found and described here can be observed for any charged macromolecule in the presence of strongly charged species immersed in salt solution. We do not perform a direct comparison of theoretical and experimental results since, in the particular case of DNA compressed by BSA, for detailed comparison additional factors should be taken into account such as counterion condensation on DNA, dependence of BSA charge and size on salt concentration, etc. Say, in the case of the double stranded DNA, only 24% of base pairs are charged, while the remaining 76% are neutralized due to counterion condensation, and these numbers can be varied with the addition of charged species [20]. In the experiment, we have shown that BSA at a concentration of 15% induces the compaction of DNA. By considering that the actual concentration of biomacromolecules is 20%–30% in cellular interiors [1], the experimental concentration of 15% BSA suggests the actual significance of the crowding effect in living cells. Based on the results in the present study, we may expect that macromolecular crowding and depletion effects are major factors in the packing of DNA *in vivo*, stimulating further experimental work toward the understanding of higher-order structural change of DNA and chromatin in relation to the self-regulatory mechanism of genetic activity.

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Appendix.—The single-molecule DNA observation was performed as in the previous studies [10–12]. Bacteriophage T4 DNA (165.6 kbp) stained with the fluorescent dye YOYO-1 (quinolinium,1, 1′-[1,3-propanediyl-bis[(dimethylimino)-3,1-propanediyl]]bis[4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]]-tetraiodide) was dissolved in aqueous BSA solution (T4 DNA, 250 n*M* in base pairs; YOYO-1, 25 n*M*) and observed by FM using a Nikon Eclipse TE2000-U microscope equipped with a 100× oil-immersed objective. Real-time fluorescence images were recorded on DVD through a highly sensitive Hamamatsu EB-CCD camera and a DVS-20 image processor. FM observations of free DNA in solution were carried out at ca. 24 °C. The long-axis length L, defined as the longest distance in the outline of a DNA image, was measured for 50 randomly chosen DNA molecules under fixed conditions.

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