Visualization of Different Pathways of DNA Release from Interpolyelectrolyte Complex†

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The release of double-stranded DNA from its interpolyelectrolyte complex with positively charged poly(allylamine hydrochloride) via exchange reaction with added polyanion, poly(sodium styrenesulfonate), is directly observed by fluorescence microscopy. It is shown that the pathways of DNA release depend essentially on the amount of added low-molecular-weight salt. At low salt content, the DNA release proceeds via the formation of an intermediate "beads-on-string" structure, whereas at high salt content the release goes directly from globule to coil states without any intermediate structures. The reasons for different character of DNA release are discussed.

Introduction

Diversity of conformational states assumed by DNA in biological processes together with the transitions between these states has currently attracted a great interest because of their fundamental significance and useful practical applications. The concept of gene therapy can be considered as a particular example, where the conformational transitions of DNA are of primary importance for providing the required functioning of the system. On the one hand, DNA molecule for transfection should be condensed into a compact state to ensure its protection against enzymatic digestion and its safe trafficking through the diverse barriers toward the nucleus of the target cell where the gene will be expressed. On the other hand, once inside the nucleus, the DNA should become unfolded (at least partially) to be accessible to transcription factors, which are responsible for decoding genetic information from the base sequencing of DNA genome.[1] While for the compaction of DNA there is a vast set of synthetic gene delivery vehicles[2] (i.e., polycations, cationic lipids, etc.), the choices for DNA release are limited to a few number of strategies. Among them, one of the most promising methodologies is the release of DNA from its interpolyelectrolyte complexes (IPECs) with polycations via an exchange reaction with added anionic polymers.[3−10] It is suggested that this type of exchange reaction may take place in the release of DNA from its complex with polycations and cationic polypeptides in intracellular environment because various types of negatively charged macromolecules, including mRNA and sulfated sugars, exist as essential cellular components.[3,5]

Although the release of DNA was investigated both in vivo and in vitro,[3−7] the peculiarities of the conformational changes of DNA during its replacement in IPEC by another polyanion are still poorly understood. At the same time, this point is of key importance for an appropriate design of gene delivery systems, because the transcriptional activity of DNA is strongly affected by the degree of compaction of DNA macromolecule in its vector.[11]

Thus, the main aim of the present paper is to visualize the conformational changes of single DNA macromolecules at collapse/decollapse transitions taking place at the formation of IPEC with polycation (collapse) and further release of DNA from the IPEC (decollapse) induced by added polyanion. In this study, poly(allylamine hydrochloride) (PAH) was used as polycation triggering DNA collapse. As a competitive polyanion inducing the decollapse, poly(sodium styrenesulfonate) (PSS) was chosen. Among the limited number of polyanions capable of displacing DNA in its IPEC with polycation, PSS is characterized by its almost complete competitiveness with DNA in a broad range of pH[12] and ionic strength.[13,14] Besides, PSS forms strong IPEC with the polycation under study, PAH, which is stable even at high ionic strength.[15] To visualize the conformational transitions in DNA, fluorescence microscopy technique was applied.[16−18]

The paper is organized as follows. First, the coil−globule transition (collapse) of DNA in the presence of a polycation PAH is discussed and the morphology of the DNA during its collapse is elucidated. Second, the globule−coil transition (decollapse) of the DNA macromolecules in the presence of an added polyanion PSS is examined and the effect of salt on the morphology of DNA chains during the release process is discussed.

Experimental Section

Materials. Bacteriophage T4dc DNA (166 000 base pairs) from Nippon Gene (Japan) as well as PSS (MW ∼ 1 000 000 g/mol, 25 wt % solution in water) and PAH (MW ∼ 70 000 g/mol) from Sigma-Aldrich were used without further purification. A fluorescence dye 4,6′-diamidino-2-phenylindole (DAPI) intercalating in DNA duplex and an antioxidant reagent 2-dithiothreitol were obtained from Wako Pure Chemicals (Japan) and were used as received. Water was purified with a Milli-Q system (Millipore).

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Sample Preparation. Stock solutions of 0.1 M Tris·HCl buffer (pH 5.75), 0.1 M 2-dithiothreitol, 10 μM DAPI, 1 μM PAH, and 10 μM PSS were prepared, and sample solutions were made by appropriate addition of them to bidistilled water. In the final solutions, the concentration of DNA (0.1 μM in phosphate groups) as well as the number of DAPI molecules per base pair (ca. 0.05) was always kept constant. Under these conditions, the persistence length of DNA remains almost the same as in the absence of DAPI.[19] In collapse experiments, the PAH/DNA monomole ratio q was varied from 0.01 to 10. For decollapse study, the PAH/DNA ratio was fixed, while the PSS/PAH monomole ratio p was gradually increased from 0.1 to 1.1. In contrast to polyanions (DNA and PSS) that are always fully charged in water, the degree of charging of polycation PAH is pH-dependent. In this study, the pH value was kept at low enough level (5.75) to ensure a complete protonation of PAH.[18]

The DNA release in low salt content (0.01 M) was carried out by the addition of PSS to PAH/DNA solution and in the case of high salt content (1 M), PSS and NaCl were mixed separately and then were added to the PAH/DNA solution.

Fluorescence Microscopy. For preparing the samples for fluorescence microscopy, the solution was placed between two cover glasses and then was sealed with nail enamel to prevent both convection and evaporation. Glasses were preliminarily cleaned in an oven at 500 °C for 1 h. The solution was illuminated by a high-pressure mercury lamp (365 nm), and fluorescence images of DNA molecules at wavelengths longer than 420 nm were observed using a Carl Zeiss Axiovert 135 TV (Germany) microscope equipped with a 100× oil-immersed objective and a high-sensitivity Hamamutsu SIT TV camera (Japan). Images were recorded on videotape and were analyzed using the Hamamutsu Argus-20 image processor (Japan). From the simple estimation with the DNA concentration, the number of DNA chains that should be observed on the monitor (43 × 43 μm^2) is approximately five.[20] To characterize the conformation of DNA, the mean long axis length L (which is defined as the apparent longest distance in the outline of the DNA fluorescence image, including the blurring effect) was measured.

In the collapse experiments, the fluorescence microscopy measurements were performed in 15 min after the mixing of components, whereas in the decollapse experiments the measurements were made after 1 h of incubation to ensure the accomplishment of interpolyelectrolyte exchange reaction.

Results and Discussion

Coil−Globule Transition. First, the ability of PAH polycation as a DNA compacting agent to induce coil−globule transition was studied. In fluorescence microscopy images, coils and globules are easily distinguished from each other: coils are rather long (3−4 μm) hazy objects that show only internal movement with no translational displacement, whereas globules are small (<0.5 μm) bright spots with quick translational Brownian motion (Figure 1).

To characterize the conformation of DNA quantitatively, the long axis length L of the macromolecules was determined directly from the fluorescence microscopy image. At each content of added polycation, more than 100 images were treated, and on the basis of these data, the histograms of the distribution of long axis lengths of DNA chains L were constructed. Typical histograms thus obtained are shown in Figure 2. It is seen that all of them are monomodal. The average values of long axis length $\langle L \rangle$ determined from each histogram are plotted as a function of polycation/DNA monomolar ratios (q) in Figure 3.

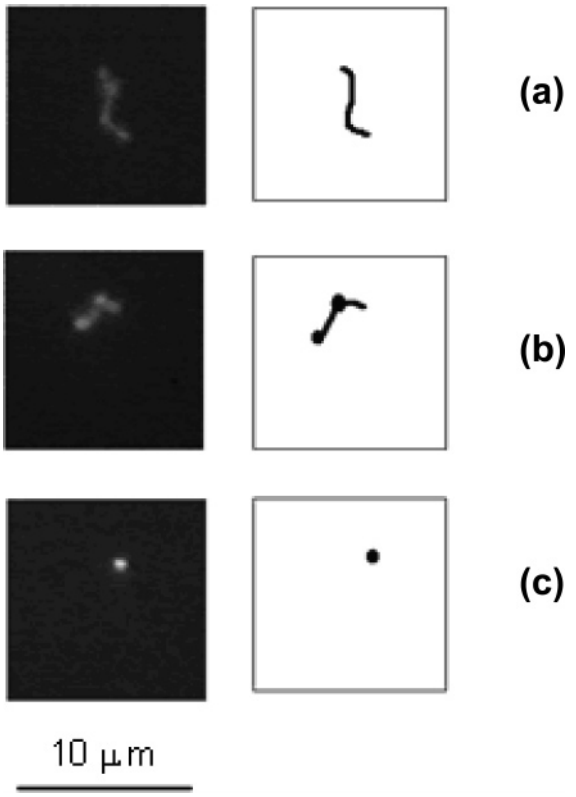

Figure 1. (left) Typical fluorescence images of DNA in coiled (a), intrachain segregated (b), and globular (c) states. (right) Schematic representations of the corresponding fluorescence microscopy images.

It is seen that at low content of polycation ($q \leq 0.1$), the average long axis length $\langle L \rangle$ is around 3−4 μm, which is typical for elongated DNA coils in the absence of polycation. At high content of polycation ($q \geq 0.8$), the DNA chains acquire a long axis length of ca. 0.5 μm that is the characteristic size of globular state by taking into account the blurring effect. Thus, PAH polycation induces the change of conformation of the DNA chain from an elongated coil into a compact globule. This is obviously due to the formation of IPEC, which leads to the screening of electrostatic repulsion between similarly charged segments of DNA and the abundant release of condensed counterions into the bulk of solution.

At the stoichiometric ratio of DNA and polycation repeat units, the formed globules are rather stable and do not aggregate with each other (Figure 2f). Such behavior was already observed for other DNA/polycation complexes.[21] It may be due to the charged surface of the globules. Indeed, it was demonstrated[22] that at stoichiometric ratio of cationic and anionic units DNA globules carry a small negative net charge, which is attributed to the noncompensated phosphate groups at the surface of the globules. Such a slight negative net charge (10%)[23] is enough to keep the globules colloidally stable and to preserve them dispersed in the solution, not tending to make aggregates at such an extremely low concentration of DNA. Further addition of polycation will compensate the negative charge of the surface, but this DNA/polycation was not explored in this paper. At higher excess of added polycation ($q = 5, 10$), the surface of the globules becomes positively charged maintaining the dispersion of the globules in the solution. In this way, because of electrostatic repulsion between the globules, the system always preserves its colloidal stability either at stoichiometric conditions or at high excess of polycation.

The sharp drop of the average values of long axis length $\langle L \rangle$ of DNA indicating the transition from coils to globules proceeds

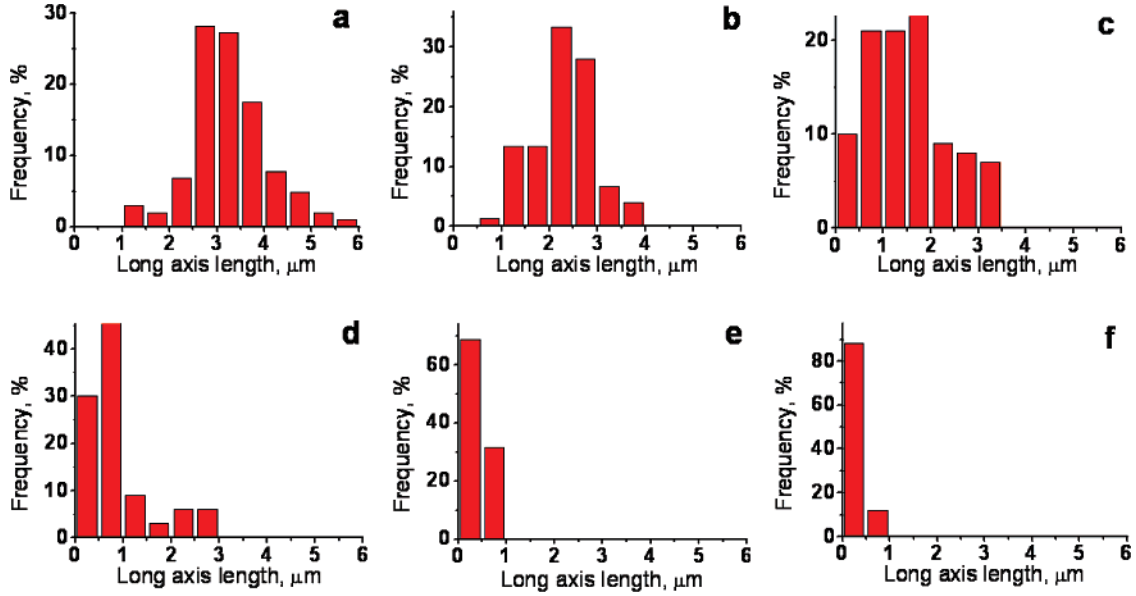

Figure 2. Histograms of the distribution of long axis lengths of DNA molecules at different PAH/DNA monomolar ratios: 0 (a), 0.15 (b), 0.3 (c), 0.5 (d), 0.7 (e), and 1 (f).

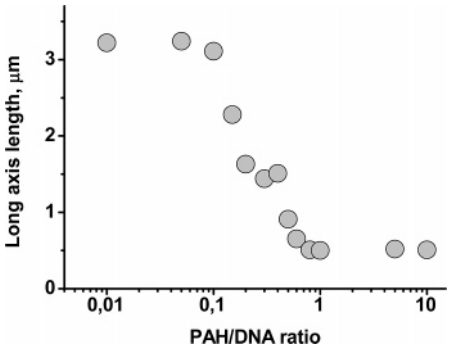

Figure 3. Dependence of the average long axis length of DNA molecules ⟨*L*⟩ on the PAH/DNA monomolar ratio.

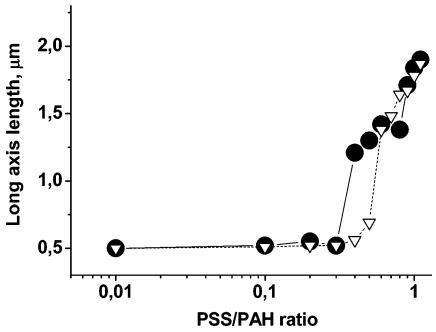

Figure 4. Dependence of the average long axis length of DNA molecules ⟨*L*⟩ on the PSS/PAH monomolar ratio at the release of DNA from its interpolyelectrolyte complex induced by exchange reaction with added PSS polyanion at different salt concentrations: 0.01 M (●) and 1 M (▽).

in a wide range of *q* values from 0.1 to 0.8 (Figure 3). At these conditions, we observed intrachain segregated DNA structures with folded (beads) and unfolded (string) parts coexisting along single DNA duplex. A typical fluorescence image of such a chain is shown in Figure 1b. The study of coil−globule transition here was performed at low salt content (0.01 M). At low ionic strength, the appearance of the "beads-on-string" structures was also observed for many other nonstoichiometric DNA/polycation complexes.[24−26] According to theoretical considerations,[27,28] the beads-on-string structures can be formed in salt-free solutions of hydrophobically modified polyelectrolyte as a result of the interplay of two counteracting tendencies: hydrophobic association of nonpolar units and electrostatic repulsion of similarly charged units. A nonstoichiometric IPEC may be considered as a hydrophobically modified polyelectrolyte, in which the hydrophobic sites are DNA segments complexed with polycation, whereas the hydrophilic sites are charged "naked" DNA units. When forming the beads-on-string structures, the system gets a compromise: attracting hydrophobic units associate with each other into the beads, whereas repelling charged units constitute long strings, allowing them to come farther apart.

Thus, at low salt content, the coil−globule transition proceeds via the formation of an "intermediate" conformational state, beads-on-string structure, in which coil and globular states coexist within the same DNA chain. At high salt content, the coil−globule transition always goes[21] directly from coils to globules without the intermediate intrachain segregated structures, which is due to the screening of the repulsion of similarly charged units responsible for the appearance of strings and the enhancement of hydrophobic attractions of the complexed parts (beads).

Globule−Coil Transition. In the next step, we tried to release DNA from its complex with polycation via an interpolyelectrolyte exchange reaction with added PSS polyanion:

$$\text{IPEC(DNA/}n\text{PAH)} + x\text{PSS} \rightarrow \text{IPEC(}x\text{PSS/}n\text{PAH)} + \text{DNA}$$

For this purpose, stoichiometric DNA/PAH complexes were used, each of them being based on a single DNA duplex. Then, different amounts of PSS polyanion were added. Figure 4 shows the variation of the average long axis length ⟨*L*⟩ of DNA chains upon addition of polyanion PSS. In the initial state (before the addition of PSS), all DNA chains are fully collapsed with ⟨*L*⟩ of ca. 0.5 μm, which is typical for the globular state of DNA. No visible effect was noticed up to a certain amount of added polyanion PSS, and then the sharp increase of ⟨*L*⟩ is observed indicating the unfolding of DNA. It means that PSS replaced DNA in its complex with PAH, and this interpolyelectrolyte exchange reaction resulted in the release of DNA chains in the coil state.

The total free-energy change (ΔG_{total}) for the interpolyelectrolyte exchange reaction may be written[10] as the sum of free-

energy changes of polymer chains (ΔG_{pol}) and of counterions (ΔG_{count}):

$$\Delta G_{total} = \Delta G_{pol} + \Delta G_{count}$$

Let us assign to each of these free-energy terms the appropriate entropic and enthalpic components. As for the polymer species (ΔG_{pol}), it is obvious that there is no change in their translational entropy during exchange reaction, because the total number of them remains the same. As to the enthalpic component of ΔG_{pol}, it should depend on the energy of interactions between polyions in IPEC, in particular, on the number of ionic links formed between them. It is reasonable to suggest that the total number of ionic links formed by PAH in the final IPECs (*x*PSS/*n*PAH) is much higher than in the initial IPECs (DNA/*n*PAH) because of the perfect complementarity of PSS and PAH chains possessing the same intercharge spacing d (2.5 Å). By contrast, in the components of the initial IPEC (DNA and PAH), the intercharge spacings *d* differ significantly (3.4 vs 2.5 Å) that inevitably leads to some mismatched ionic links and therefore to smaller gain in enthalpy.

Now let us consider the role of counterions (ΔG_{count}). All polyelectrolytes under study are highly charged, and therefore, in a noncomplexed state some of the counterions should be condensed, according to Manning−Oosawa theory.[29,30] Formation of IPEC leads to the release of these counterions into the bulk of solution: the higher the fraction of condensed counterions, the larger the gain in their translational entropy upon their release. The intercharge spacing in DNA chain ($d = 3.4$ Å) is higher than in PSS chain ($d = 2.5$ Å), which means that the fraction of condensed counterions in DNA is somewhat lower. This is consistent with the experimental data, which show that about 70% of monovalent counterions are condensed on DNA chain,[31,32] while ca. 80%[33] of monovalent counterions are condensed on PSS chains. So, the replacement of DNA by PSS in IPEC should give a gain in the translational entropy of counterions. An additional gain may be provided by the fact that in the initial IPEC (DNA/PAH) mismatched ionic links may trap some counterions inside IPEC to provide its electroneutrality. As a result, the "defected" IPECs are less favorable because of loosing the translational entropy of some counterions.

Thus, we can conclude that the exchange reaction under study is favorable for two reasons: gain in enthalpy of polyions interactions in the resulting IPEC and gain in entropy of the released counterions.

Now let us consider the effect of salt on this process. From Figure 4 it is seen that the higher is the salt content, the larger the amount of added polyanion is required to induce the release of DNA. This is because higher salt content decreases the gain in entropy of counterions and makes the exchange reaction less favorable.

At first glance on the basis of averaging over the ensemble of chains, the interpolyelectrolyte exchange reaction seems to be qualitatively identical at low and high salt concentrations (Figure 4). However, the histograms of the distribution of long axis lengths *L* giving the picture at the level of single chains (Figures 5 and 6) show significant difference: at low salt content, they are always monomodal, whereas at high salt content an obvious bimodality appears indicating the first-order cooperative character of the transition.[34] Thus, an increase in ionic strength of the solution (from 0.01 M to 1 M) changes the character of globule−coil transition from a continuous to a discrete one.

Moreover, a closer look over the morphology of individual chains reveals that the pathways of the DNA release are quite

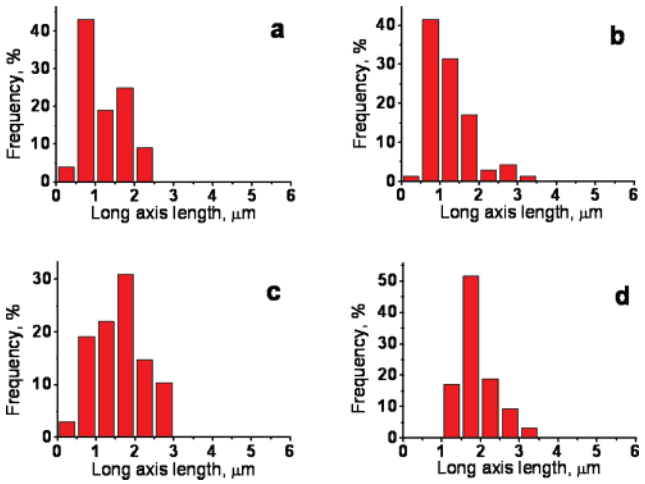

Figure 5. Histograms of the distribution of the long axis lengths of DNA molecules during their release from IPEC by exchange reaction with PSS at different PSS/PAH monomolar ratios: 0.4 (a), 0.7 (b), 0.9 (c), and 1.1 (d) at low (0.01 M) salt content.

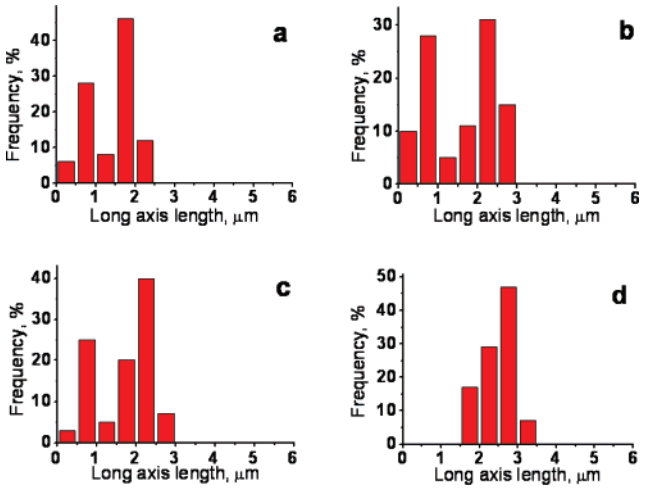

Figure 6. Histograms of the distribution of the long axis length of DNA molecules during their release from IPEC by exchange reaction with PSS at different PSS/PAH monomolar ratios: 0.6 (a), 0.8 (b), 0.9 (c), and 1.1 (d) at high (1 M) salt content.

different for different salt contents (Figure 7). Whereas at low salt concentrations the globule−coil transition proceeds via the formation of beads-on-string structures, at high salt concentrations it follows an "all-or-none" scenario, that is, it proceeds directly from globules to coils without any intermediate structures. This means that there are two pathways of DNA release: (1) by removing some polycations from most DNAs or (2) by removing all polycations from a few DNAs leaving other IPECs intact. In the first case, the system consists of nonstoichiometric IPECs, whereas in the second case the mixture of stoichiometric IPEC and released naked DNA is formed. The first pathway is observed at low salt content, and the second one is observed at high salt content.

Let us consider the possible reasons for the different pathways of DNA release. Initial stoichiometric IPEC is in the collapsed state, which indicates that segments of DNA complexed with polycation attract each other and try to avoid contact with water that points to hydrophobicity of these sequences. The most efficient attraction of such hydrophobic sequences with each other can be realized in the globular state of DNA. When added polyanion removes some polycationic chains from DNA, charged noncomplexed sequences on DNA appear together with

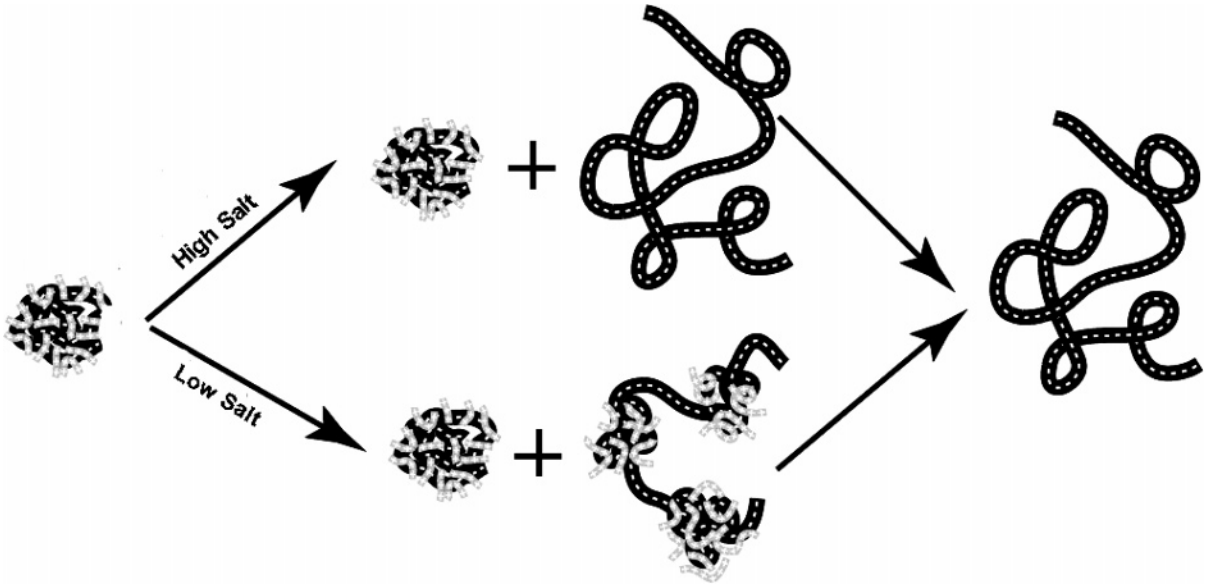

Figure 7. Schematic representation of two different pathways of DNA release from its interpolyelectrolyte complex induced by interpolyelectrolyte exchange reaction with added polyanion.

counterions. The electrostatic repulsion of similarly charged DNA units in these sequences and the osmotic pressure exerted by counterions induce the unfolding of the nonstoichiometric IPEC that inevitably leads to the unfavorable contact of complexed DNA sequences with water. Further exchange reaction is more likely to proceed with partially unfolded DNA, that is, with nonstoichiometric IPEC, as a "cooperative decompaction" process until the full removal of polycation (rather than disrupting stoichiometric globules from their stable, water-avoiding state), and this indeed happens at high salt content.

By contrast, at low salt content this pathway of DNA release is too much energy consuming because the exchange reaction of negatively charged nonstoichiometric IPEC with added polyanion is known to proceed[35] via the formation of a united coil between the macroions followed by a redistribution of salt bonds between them. As both components are similarly charged, the formation of united coil is hindered because of the electrostatic repulsion, the effect being more pronounced at higher content of naked DNA in nonstoichiometric IPEC. Therefore, at low salt content further removing of polycation from nonstoichiometric IPEC is hindered resulting in the preferential polycation exchange reaction from all of the IPECs present in the solution rather than from a few of them.

So, different pathways of DNA release from its IPEC come from the competition between hydrophobic attraction between complexed DNA sequences and electrostatic repulsion (together with osmotic pressure of counterions) arising from charged sequences of naked DNA. At low salt content, the input of the repulsion is significant and does not allow to remove all polycations from one DNA chain at once. At high salt content, the hydrophobic attraction prevails, which forces the exchange reaction to follow "all-or-none" scenario allowing gain more in hydrophobic attraction. Up to now, only the "all-or-none" decollapse was observed for the release of DNA from its IPEC by another polyanion.[20] The same character of decollapse was also found for the release of DNA by low molecular weight anions[36] and anionic surfactants.[37] To the best of our knowledge, the present paper is one of the first, where the DNA decollapse was found to proceed via an intermediate intrachain segregated state, in which that coiled and globular states coexist within the single DNA duplex.

Conclusions

Addition of salt switches the pathway of DNA release from a continuous progressive mode with intramolecular segregation into a discrete all-or-none scenario with intermolecular segregation between coils and globules. This is due to the screening of electrostatic repulsion in charged sequences of naked DNA and the decrease of the osmotic pressure of counterions.

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