Anomalous Salt Effects on DNA Conformation: Experiment and Theory

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ABSTRACT: The condensation of a single DNA can be induced by multivalent counterions and is thought to be due to ion−ion correlations. Both Monte Carlo simulations and fluorescence microscopy experiments confirm this picture. The effect is less pronounced for chain counterions (polyamines) than for simple spherical counterions with the same charge. When simple salt is added to the system, the polyelectrolyte unfolds. MC simulations show that this is due to a competition between the different small ion species. The multivalent ions in the vicinity of the macroion are exchanged for univalent ones, decreasing the effect of ion−ion correlations. It is also found that the temperature effect on the macroion conformation is quite insignificant in the studied interval (5.0−65.0 °C).

1. Introduction

Charged polyions are an important subset of polymers comprising biopolymers as DNA, RNA, and proteins and also synthetic polyelectrolytes, which are important in many practical applications. The electrostatic forces influencing the behavior of such macromolecules are still not fully understood and need to be further characterized. One of the intriguing features of polyelectrolytes is that under certain circumstances the macromolecule can condense into a compact structure. One example is DNA, which in a living cell has a compact structure but when placed in a test tube, containing aqueous buffer solution, takes on a random coiled conformation. Conventional wisdom (simple mean-field theories) tells us that this expansion can be seen as an effect of the repulsion between the negatively charged phosphate groups of the molecule. In a living cell, though, other species affect the DNA molecule, and it has been shown that the multivalent, naturally occurring, polyamines can induce condensation of DNA molecules.[1−3] In later studies various condensing agents have been discovered.[4] Among others, these include simple multivalent ions[5,6] and solvents with low dielectric permittivity.[7,8] In more concentrated solutions DNA molecules tend to aggregate instead of condensate.[9]

The cause of attraction is apparently to be sought for in the electrostatic interactions, and indeed several theoretical studies have confirmed that like-charged aggregates can attract each other under certain circumstances.[10−14] This counterion mediated attraction is thought to be due to the old idea of ion−ion correlation,[15−17] which simply can be said to be a classical analogy to the quantum mechanical dispersion interaction.[18] Recently, the condensation of a single polyelectrolyte has been observed in molecular dynamics[19,20] and Monte Carlo (MC)[21] simulations when the systems were highly charged and correlation effects became important. Here we expand the MC results to include simple monovalent salt, and moreover we confirm the theoretical results by DNA fluorescence experiments. In order to compare the simulated system with DNA−polyamine systems, we provoke the macroion condensation with flexible multivalent counterions, which could be thought of as polyamines. The temperature dependence is also addressed. Some interest is also directed toward the discrete nature of the coil−globule transition, and when this intriguing effect is discussed, a square-well potential is added between the monomers.

2. Simulation Details

Monte Carlo simulations were performed for a single flexible polyelectrolyte in a cell model[22] and are described in further detail in a previous report.[8] The polyelectrolyte was made up of negatively charged discrete monomers which were connected by freely jointed rigid bonds. The cell also contained positive counterions and both negative and positive salt particles. The counterions were both of simple hard sphere type and of a flexible type, where three or four monomers were connected as a small positive polyelectrolyte (with monomer−monomer distance r_{ion}). While the middle monomer is kept fixed at the center of the spherical cell, all other particles are allowed to move in the cell. The total charge of the cell was always equal to zero, and the interactions between species belonging to different cells were neglected; i.e., there are no interactions ranging outside the cell. The polyelectrolyte concentration (or monomer concentration c_m) determines the cell volume V_c and hence the radius R_c. For the polyelectrolytes used, 24 monomers were connected with bonds of the size 6 Å; the cell radius was $R_c = 60$ Å corresponding to a monomer concentration of 44 mM. For this cell size the polymer never touches the cell boundary. The size of the cell can be compared with the Debye screening length κ^{-1}. If 24 monomers with a charge of −1 are neutralized by monovalent counterions, $\kappa^{-1} = 20$ Å; divalent counterion, $\kappa^{-1} = 10$ Å; trivalent counterions, $\kappa^{-1} = 7$ Å; and tetravalent counterion, $\kappa^{-1} = 5$ Å. Only the counterions have been used in the computation of κ^{-1}, which thus is a conservative estimation.

The solvent was treated as a dielectric continuum with a relative permittivity equal to that of water at room temperature (78.7). The interactions between the

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particles were of Coulomb and hard sphere type, and the interaction potential $u_{ij}(r_{ij})$ between two species was

$$u_{ij}(r_{ij}) = \begin{cases} z_i z_j e^2/4\pi\epsilon_\epsilon\epsilon_0 r_{ij}, & r_{ij} > r_i + r_j \\ \infty, & r_{ij} \le r_i + r_j \end{cases} \quad (1)$$

where z_i was the charge of particle i, r_i was the radius of particle i, and r_{ij} was the distance separating particles i and j. The Monte Carlo simulations were performed with the traditional Metropolis algorithm[23,24] in a canonical ensemble. Two different types of moves were performed: the chains were pivoted,[25,26] and the small ions, including the flexible counterions, were translated.[27] The total number of pivot moves were around 10^7-10^8. Every run was preceded by an equilibration run of about 10^7 moves. For every pivot move half of the small ions were moved. As a measure of the conformational changes of the polyelectrolyte the mean-square end-to-end distance was used.

2.1. Square-Well Potential. Most simulations were carried out with only electrostatic and hard core interactions, as described above. In a few simulations, however, a square-well potential was also included

$$u_{ij}(r_{ij}) = \begin{cases} -\epsilon_{sq}, & r_i + r_j < r_{ij} < \lambda(r_i + r_j) \\ 0, & r_{ij} \ge \lambda(r_i + r_j) \end{cases} \quad (2)$$

where ϵ_{sq} is the square-well depth and $\lambda(r_i + r_j)$ is the square-well width. The square-well potential was included in order to investigate the effect of additional short-range attractive interactions on the coil−globule transition. This simple square-well model is a reasonably realistic model which has been used successfully to reproduce thermodynamic properties of alkanes ranging from methane to eicosane.[28] In this work $\epsilon_{sq} = 0.4$ and $\lambda = 3$. In these simulations chains with 60 monomers are used since the discrete coil−globule transition is more pronounced for longer chains.[28] The cell radius was $R_c = 150$ Å, corresponding to a monomer concentration of 7 mM and a Debye screening length $\kappa^{-1} = 50$ Å for monovalent counterions.

3. Experimental Details

3.1. Materials. Coliphage T4 DNA ($M = 1.1 \times 10^8$ D, ca. 167 kilobase pairs, contour length ca. 57 μm [29]) was supplied by Sigma. The DNA concentration was determined spectrophotometrically, considering the molar extinction coefficient of DNA bases to be equal to 6600 M^{-1} cm^{-1};[30] the ratio of absorbency of DNA stock solution at 260 nm to that at 280 nm was found to be 1.8. Fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), and antioxidant, 2-mercaptoethanol (ME), were obtained from Sigma. Spermine tetrahydrochloride (SPM; 98%, Aldrich) and spermidine trihydrochloride (SPD; 99%, Aldrich) were thoroughly desiccated before the preparation of the stock solutions. Sodium chloride (Sigma) was dried before use. Bidistilled water used for the preparation of samples was filtered through 0.22 μm Millipore filters.

3.2. Sample Preparation. For the preparation of the sample solutions DNA molecules were diluted with the distilled water containing NaCl, 4% (v/v) ME, and a fluorescent dye. The resulting solution was gently mixed with the aqueous SPM or SPD solution to prevent mechanical damage of large DNA macromolecules and then was kept for 24 h before observation. The final concentrations were as follows: DNA in nucleotide units; 0.5 μM, DAPI; 0.5 μM. In these conditions, the binding number of DAPI per one DNA base pair in an aqueous buffer solution is estimated to be equal to 0.05[31] and the persistence length of a DNA chain is expected to remain nearly the same as that in the absence of DAPI.[32]

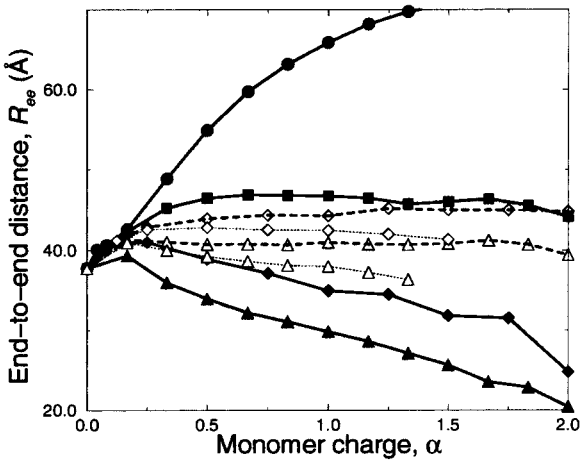

Figure 1. End-to-end distance of a 24 monomer polyelectrolyte as a function of monomer charge. In the MC simulations different counterions have been used. Solid lines are for simple ions, dashed lines are for chain ions (connected monovalent ions) with $r_{ion} = 6.0$ Å, and the dotted lines are for chain ions with $r_{ion} = 4.05$ Å. The valencies of the counterions are as follows: circles, monovalent; squares, divalent; diamonds, trivalent; triangles, tetravalent.

3.3. Microscope Glasses. Special care was taken to clean the microscope glasses (No. 0, Chance Propper, England) thoroughly before observation to prevent DNA degradation as well as precipitation to the glass surface. Glasses were soaked in conventional detergent solution for at least 1 day, washed with distilled water, immersed into concentrated hydrogen peroxide for several hours, washed repeatedly with distilled water, and then immersed in ethanol for at least 1 h. Finally they were washed with Millipore water and dried at 50 °C.

3.4. Methods. A fluorescence microscopy study was performed as follows: the samples were illuminated with a UV−mercury lamp; the fluorescence images of single DNA molecules were observed using a Zeiss Axioplan microscope, equipped with a 100× oil-immersed objective lens, and digitized on a personal computer through a high-sensitive video camera MTI VE1000 (DAGE-MTI, USA). The observations were carried out at 5.0 ± 0.1, 25.0 ± 0.1, 45.0 ± 0.1, and 65.0 ± 0.1 °C; the temperature was kept constant with a water-jacket microscopic cell connected to a TS-4 ER thermocontroller (Physitemp Instruments).

4. Results and Discussion

In line with our previous study[21] the effect of increasing the electrostatic interactions in a polyelectrolyte−counterion system was investigated. The difference between simple hard sphere counterions and chain ions, mimicking polyamines, was of interest due to the comparison with experimental DNA−polyamine systems. Bearing in mind that salt is to be added later, the system was kept small. A polyelectrolyte with 24 monomers was simulated, although the general trends were the same for systems with longer polyelectrolytes.[21] Figure 1 confirms the picture that in a system containing monovalent counterions the chain expanded when the monomers were charged up, in a way predicted by mean-field theory. When multivalent counterions were present, the polyelectrolyte initially expanded with growing monomer charge but for high monomer charges an attractive force between the monomers appeared and the chain dimensions started to decrease. It is interesting to note that for high charges the contraction continued below the size of a neutral chain, which indicates a net attractive force that goes beyond the normal mean-field screening. This attractive force

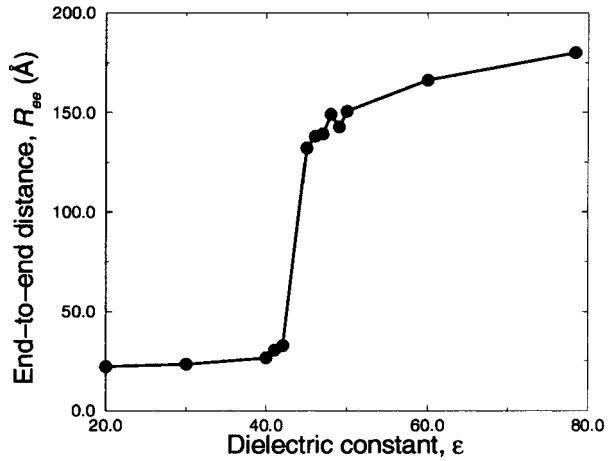

Figure 2. End-to-end distance of a 60 monomer polyelectrolyte as a function of the dielectric constant. Here the monomer charge is fixed to −1, and simple monovalent counterions are used. All particles have a hard core radius of 4 Å, and the bond length is 6 Å.

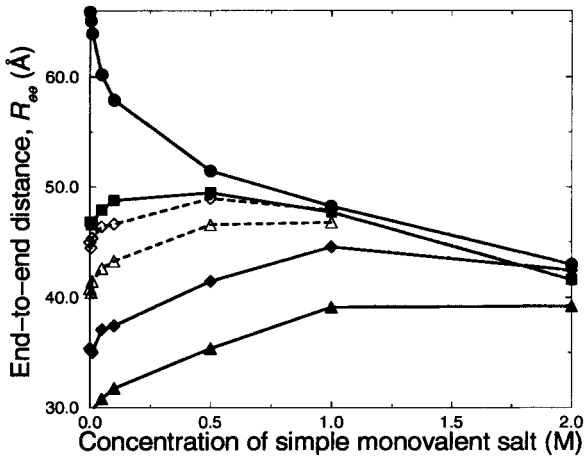

Figure 3. End-to-end distance of a 24 monomer polyelectrolyte as a function of salt concentration. Here the monomer charge is fixed to −1. The same symbols are used as in Figure 1.

is believed to be due to ion−ion correlations, as mentioned above.

The change of hard sphere counterions to flexible counterions decreases the attractive interactions between polyelectrolyte segments. In studies of attraction between different rodlike polyelectrolyte chains it was found that the effect of trivalent chain−counterions was approximately the same as for divalent simple ions,[14] a conclusion also supported by Figure 1. The decrease in interaction is of course affected by how flexible the counterions are. When the monomer−monomer separation is decreased, the interaction increases, which leads to smaller polyelectrolyte dimensions, as illustrated in Figure 1. A general conclusion is that for every polyelectrolyte it should be possible to find a parameter regime, for both flexible and hard sphere counterions, where attractive interactions dominate, and the polyelectrolyte condenses.

The condensation of a polyelectrolyte can be readily understood by considering the balance between energy and entropy for a system which consists of only the polyelectrolyte chain and counterions. The ground state ($T = 0$ K) will be a condensed state, in close analogy to an ionic crystal like NaCl, since this is the energetically favored state. However, at finite T, it is entropically unfavorable since the counterions are spatially confined to the large molecule and have hence no translational entropy. Upon increasing the temperature, the entropy becomes more and more important, such that finally the counterions are released. This results in a stretching of the chain. The higher the counterion valancy, the smaller is the translational entropy per counterion; hence, multivalent ions favors energy and the collapsed state. The same arguments hold for the flexible counterions. The smaller they are, the stronger is the reduction in entropy.

A well-known feature of DNA condensation is that the transition from an elongated coil to a compact globule occurs as a discrete (phase) transition.[4] In our previous study this transition was induced by lowering the dielectric constant ϵ and observed experimentally with fluorescence microscopy.[8] With only electrostatic and hard core interactions Monte Carlo simulations predict a gradual transition between the coil and globular state.[8] However, a distinct change in chain size can be seen in simulations if an attractive square-well potential is added between the monomers (see eq 2). Here a chain with 60 monomers, with each having the charge −1, was studied. The counterions were monovalent, and no salt was present in the system. When the strength of the electrostatic interactions was increased by decreasing ϵ (see eq 1), the chain started to decrease in size. At $\epsilon = 44$ the chain shrank by a factor of 6, and it is clear from Figure 2 that the condensation process indeed is discrete. This simulation was done for a fully flexible and rather short chain. If the simulated chain is made more DNA-like, i.e., longer and stiffer, the coil−globule transition would probably be even steeper.[28,33−36]

The effect of adding simple monovalent salt, e.g. NaCl, to the polyelectrolyte−counterion system is depicted in Figure 3. The system containing monovalent counterions again behaved in a way predicted by simple mean-field theory; i.e., when salt was added, the electrostatic repulsion between the monomers was screened and the polyelectrolyte shrank. The systems which included multivalent counterions, and thus were compacted to some extent, behaved in a totally different way. On the addition of salt the dimensions of the polyelectrolyte increased in a manner which cannot be accounted for by mean-field theory.

Figure 4 shows the radial distribution functions for tetravalent counterions. The counterion concentration near the monomers was much lower for systems with a high salt concentration. Some of the multivalent ions in the vicinity of the polyelectrolyte were replaced by monovalent salt. This competition effect decreased the electrostatic interactions near the polyelectrolyte, and the correlation effect became less important, which explains why the attractive forces diminished and the chain dimensions increased.

In order to experimentally verify the effect of monovalent salt on the multivalent ion-induced DNA condensation, a series of measurements on the T4 DNA conformational behavior were performed at various NaCl concentrations. First, the effect of a tetravalent cation, SPM, was studied in aqueous DNA solutions. It was found that DNA exhibits a discrete coil-to-globule transition at all studied NaCl concentrations. Firstly, at low SPM concentrations all DNA molecules exhibited relatively slow Brownian motion, without any precipitation to the microscope glass surface. Later on, with the

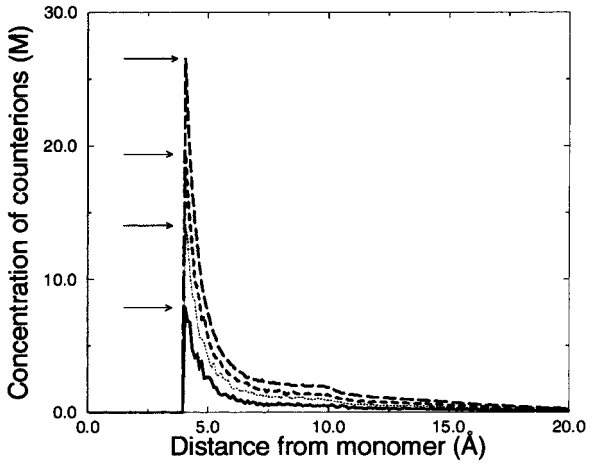

Figure 4. Distribution functions for the tetravalent counterions at different salt concentrations: long-dashed, 0 M; dashed, 0.5 M; dotted, 1.0 M; solid, 2.0 M. The arrows indicate the counterion concentration at the closest possible distance to the monomer.

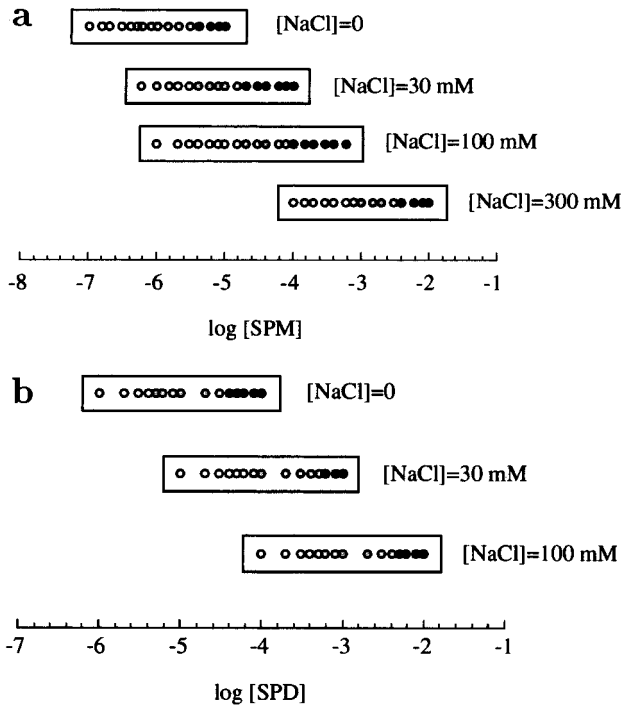

Figure 5. (a) Dependence of the conformational behavior of single T4 DNA chains in aqueous solution on the SPM concentration. Open circles indicate the coiled state of single T4 DNAs, and shadowed circles correspond to the existence of both coils and globules in the solution, while filled circles indicate the globular state of single DNA chains. (b) Dependence of the conformational behavior of single T4 DNA chains in aqueous solution on the SPD concentration. The symbols are used are the same as those in a.

increase of SPM concentration, both compacted globular and elongated coiled DNA chains were observed. A further increase in the SPM concentration led to complete DNA collapse in the sample. Under those conditions, DNA globules partly exhibited fast translational motion in the sample and a significant fraction of globules was precipitated on the glass surface. Since the glass surface is slightly negatively charged, the precipitation of DNA might be another manifestation of the importance of correlation attraction in the system. The results of the fluorescence microscopy observations are

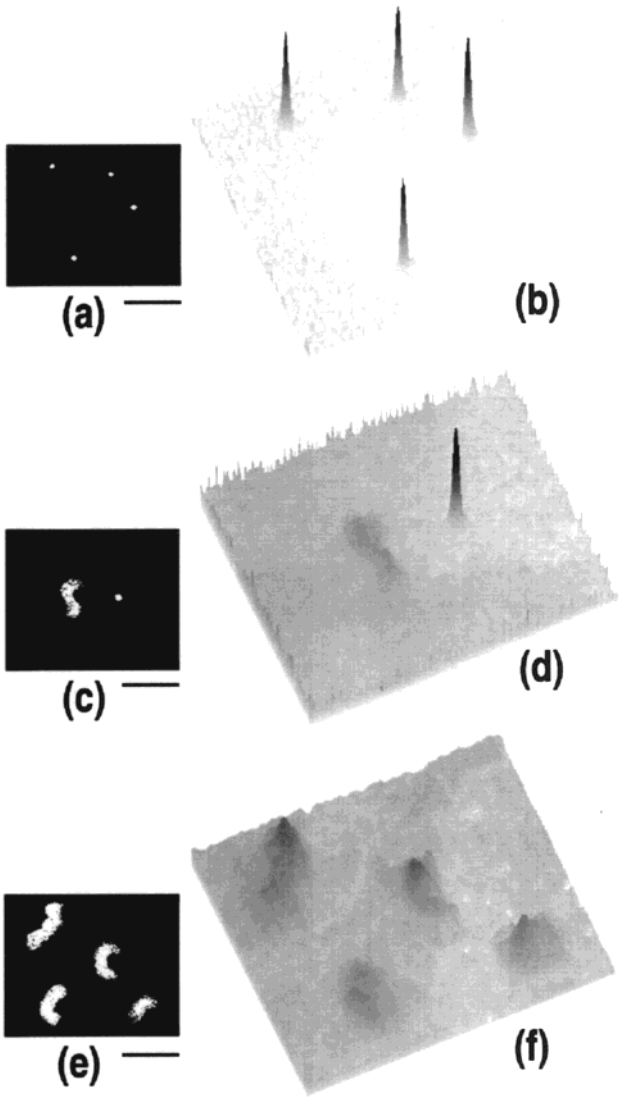

Figure 6. Experimental evidence that simple salt unfolds DNA compacted by SPM. Parts a, c, and e are video frames from the fluorescence microscopy image of single T4 DNA molecules at [SPM] = 2.0×10^{-6} M. The scale bar represents 5 μm. Parts b, d, and f are the corresponding quasi-three-dimensional representations of the fluorescence intensity. Parts a and b show the salt-free case, [NaCl] = 0, where the DNA molecules exhibit a globular conformation. In c and d, [NaCl] = 30 mM, the DNA molecules coexist in both elongated coiled and compacted globular structures. For high salt concentrations, [NaCl] = 300 mM, all DNA molecules have a coiled structure.

summarized in Figure 5a and show that the more NaCl is present, the more SPM is needed to condense the DNA molecules.[2]

The same trend was detected for the DNA solutions in the presence of NaCl and a trivalent polyamine, SPD. Depending on the monovalent salt concentration, individual DNA molecules undergo a first-order phase transition from an elongated coil to a compact globular state. Obviously, the transition to the globular state occurs at higher concentrations of trivalent SPD, compared to the tetravalent analogue, SPM. Quantitative characteristics of the DNA coil−globule transition in the presence of SPD and NaCl are summarized in Figure 5b.

Figure 5 indicates that the variation of monovalent salt concentration, at constant SPM concentration, can

induce a conformational transition in a single DNA chain. To further visualize this result, T4 DNA was observed at fixed SPM concentration, equal to 2.0×10^{-6} M, while the NaCl concentration was varied. For the salt-free case as well as for low NaCl concentrations all DNA molecules were found in a compact globular conformation (Figure 6a,b). The introduction of higher concentrations of NaCl led to the unfolding of globules and appearance of coiled DNA chains together with globules, as is shown in Figure 6c,d. The further increase of monovalent salt concentration in the solution induced the complete unfolding of DNA macromolecules (Figure 6e,f). The same sort of effects have also been seen at high concentrations of DNA, where aggregation induced by multivalent cations can be resolubilized when simple salt is added.[9]

As a next step of our research, the effect of temperature on the DNA−SPM interaction in the presence of the monovalent salt was examined. Previously there has been a report by Widom and Baldwin on the temperature dependence of DNA condensation induced by Co^{3+} ions.[37] It has been noted that the midpoint of the DNA transition into condensed structure occurs at a lower Co^{3+} concentration at higher temperature values. Under the experimental conditions of that study the formation of multimolecular DNA aggregates took place, which prevented an unambiguous interpretation of the observed phenomenon.

We performed the single-molecule T4 DNA observation at [NaCl] = 30 mM while varying the concentration of SPM at four different temperatures: 5.0, 25.0, 45.0, and 65.0 °C. The main observations were that both the SPM concentration corresponding to the appearance of globules in the DNA solution and the width of the coexistence region do not depend on the temperature of the solution within the examined interval.

It is difficult to make an exact statement on the difference of the globule to coil ratios at various temperatures, because of the significant effect of precipitation of globular DNA−SPM complexes onto the microscope glass surface. At the least, fluorescence microscopy observations allow us to draw the conclusion that the temperature effect on the multivalent-ion-induced DNA compaction is quite insignificant for the temperatures examined here.

The same picture is reflected in MC simulations. The temperature does not affect the conformations of the polyelectrolyte in the given interval (5−65 °C). This is true for both compact and stretched polyelectrolytes.

5. Conclusions

Although the present simple polyelectrolyte model cannot be used to quantitatively describe real DNA, the qualitative results are confirmed by fluorescence microscopy experiments. Both simulation and experiment show that it is possible to find regimes where electrostatic interactions are strong and in a nonintuitive way lead to attractive forces between like-charged monomers in a polyelectrolyte. This correlation attraction can be induced with multivalent counterions and is counteracted when simple salt is added to the solution. Due to the competition between the simple salt and the multivalent condensing agents, the latter are forced out from the neighborhood of the polyelectrolyte and the correlation effect becomes less important, leading to an expansion of the chain.

Furthermore, it was found that the effect of temperature on the macroion conformations is insignificant in the studied temperature interval.

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