Salt-Controlled Intrachain/Interchain Segregation in DNA Complexed with Polycation of Natural Origin

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Salt-Controlled Intrachain/Interchain Segregation in DNA Complexed with Polycation of Natural Origin

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ABSTRACT: A coil−globule transition in individual double-stranded DNA triggered by chitosan polycations was directly observed by fluorescence microscopy. It was shown that the character of the conformational transition depends essentially on the amount of added low-molecular-weight salt. At low salt content the collapse proceeds via the formation of an intermediate necklace structure, which is stabilized by Coulomb repulsion between uncompensated charges of DNA chains. By contrast, at high salt content, when the electrostatic repulsion is sufficiently screened, the collapse proceeds directly between coil and globule states without intermediate structures. Also, salt affects the collapsed state of chitosan/DNA complexes: it decreases the size of single globules and favors the interglobular aggregation.

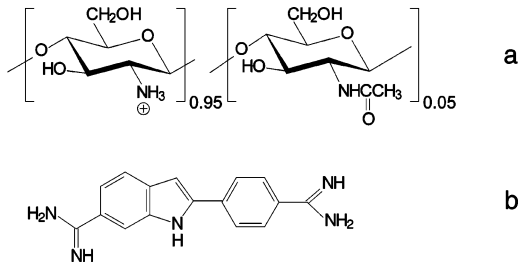
Introduction

Extremely long DNA chains exist as very compact globules in both prokaryotic and eukaryotic living cells. For example, in human cells, DNA chains with a total length of ca. 5 cm are contained in a small space of the size of 1 μm.[1] At the same time, the effective transcription of the base-sequence information stored in DNA into RNA should be accompanied by a transition from a tightly packed collapsed to a coiled state of DNA; i.e., DNA should be, at least partially, unfolded. Thus, DNA conformational transitions are vital for storage, transcription, and replication of genetic information.

Apart from their fundamental significance, the conformational transitions in DNA are also quite important for biomedical applications, especially for the development of effective gene transfer into living cells in gene therapy.[2] Cationic polymers are recognized to be of particular interest to condense and deliver DNA into living cells.[3−10] They form interpolyelectrolyte complexes (IPECs) via multiple ionic links with oppositely charged DNA chains, thus reducing their net charge. Simultaneously, a number of small counterions replaced by polycationic chain are released to the external solution, which substantially decreases the intrachain exerting osmotic pressure. Both effects favor the DNA contraction. Moreover, when the backbone of the polycation chain is rather hydrophobic, its "sticking" to the nucleic acid leads to the formation of a hydrophobic uncharged site that attracts other similar hydrophobic sites, further promoting the collapse transition.[7]

Various synthetic linear polycations were used to condense DNA: polyvinylpyridinium salts,[7] polyethyleneimine,[11,12] and polypeptides (e.g., poly-L-lysine (PLL)).[12,13] In contrast to the abundance of different synthetic polycations, there are only a small number of polycations of natural origin available, with chitosan being the most widespread among them. This polymer may present a number of characteristics beneficial to gene delivery due to its biocompatibility, low immunogenicity, and minimal cytotoxicity.[14,15]

Chart 1. Chemical Structure of Chitosan (a) and of Fluorescent Dye 4,6′-Diamidino-2-phenylindole (b)



It is known that chitosan can induce DNA condensation.[16−20] However, most of the studies were devoted to the collapsed state of DNA, while little is known about the change of morphology of DNA chains in the process of the coil−globule transition induced by chitosan. At the same time, the partially collapsed states of DNA may be of primary importance for the transcription of genetic information.[21]

The aim of the present paper is to monitor the changes of the shape of individual double-stranded DNA macromolecules in dilute aqueous solution at the collapse transition triggered by oppositely charged chitosan chains by means of fluorescence microscopy.

Experimental Section

Materials. Bacteriophage T4dC DNA (166 000 base pairs) was purchased from Nippon Gene (Japan) and used without further purification. It has a contour length of 57 μm and a persistence length of 65 nm (ca. 150 base pairs).[22] The mean distance between charged phosphate units is 3.4 Å.[1] A fluorescent dye 4,6′-diamidino-2-phenylindole (DAPI) intercalating in DNA duplex (Chart 1) and an antioxidant reagent 2-dithiothreitol were obtained from Wako Pure Chemicals (Japan) and used as received. Water was purified with a Milli-Q system (Millipore).

Chitosan (Chart 1) with molecular weight (MW) of 50 000 was obtained by alkaline N-deacetylation of chitin and purified

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from metal salts by repeated precipitation from 1 M HCl solution to 2 M NaOH and washing of the precipitate by distilled water. The degree of acetylation of chitosan samples was determined by ^{1}H NMR as described elsewhere[23] and found to be equal to 0.05. The MW value was estimated from intrinsic viscosity data obtained in aqueous solution containing 0.3 M CH_3COOH and 0.2 M CH_3COONa at 25 °C by using the Mark−Houwink−Kuhn−Sakurada equation: $[\eta] = (7.6 \times 10^{-2})M^{0.76}$ (dL/g).[24] Chitosan has a contour length of ca. 150 nm and a persistence length of 7.5 nm.[25] Solutions of chitosan for the study of interactions with DNA were prepared in 0.3 M CH_3COOH, where the amino groups of polymers are fully protonated.[26] Under these conditions the mean distance between charged units is ca. 5 Å.[27]

Sample Preparation. Sample solutions for fluorescence microscopy were prepared by mixing appropriate amounts of bidistilled water, 0.1 M Tris·HCl buffer solution (pH 5.2), 0.1 M 2-dithiothreitol, 10 μM DAPI solution, and 10 μM T4 DNA solution. Then a calculated volume of 10 μM initial solution of chitosan in 0.3 M acetic acid was added, and the sample was gently mixed. In the prepared solutions, the chitosan/DNA ratio is expressed as the ratio of the repeat units of both polymers.

In the final solution the concentration of Tris·HCl buffer was equal to 10 mM. In all the experiments the concentration of DNA was kept constant (10^{-7} M in base pairs). Such an extremely dilute solution of DNA is necessary to avoid the interactions between different DNA chains. The number of DAPI molecules per base pair was ca. 0.05; under these conditions the persistence length of DNA remains almost the same as in the absence of DAPI.[28]

Fluorescence Microscopy Measurements. To perform the fluorescence microscopy measurements, a drop of the sample solution was poured between glass slips and sealed with nail enamel to prevent both convection and evaporation. The glass slips were preliminarily cleaned in an oven at 500 °C for 1 h. The solution was illuminated with 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 Hamamatsu SIT TV camera (Japan). Images were recorded on videotape and analyzed using the Hamamatsu 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.[29] To characterize the conformation of DNA, the length of the long axis L (which is defined as the apparent longest distance in the outline of the DNA image including the blurring effect) was measured.[28]

The hydrodynamic radii R_H of single globules were calculated from their translational diffusion constants D obtained from the mean-square displacement of their centers of mass as described elsewhere.[30] For this purpose the Stokes−Einstein relation was used:

$$R_H = \frac{k_B T}{6\pi\eta_s D}$$

where k_B is the Boltzmann constant and η_s is the viscosity of the solvent.

Results and Discussion

Coil−Globule Transition. Fluorescence microscopy was shown to be a powerful technique to study the coil−globule transitions in single DNA molecules.[9,22,28−36] Typical pictures of coil and globular states of DNA as seen by this technique are presented in Figure 1a,b. It is evident that it is easy to clearly distinguish both states of DNA. The globule is a bright small sphere with the diameter on the order of 1/10 μm, while the coil looks like a thread with a mean long-axis length L of several micrometers; it exhibits incessant snakelike intramolecular and translational Brownian motion and it is much less luminous than the globule.

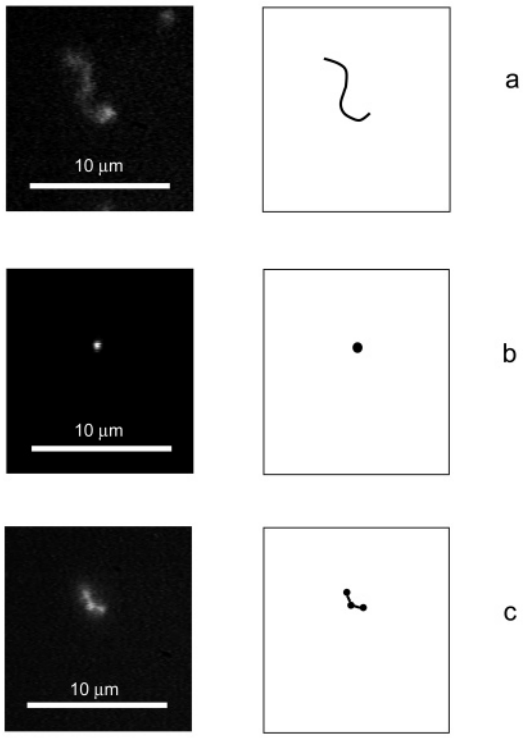

Figure 1. (left) Typical fluorescence images of T4 DNA in coiled (a), globular (b), and intrachain segregated (c) states obtained at chitosan/DNA monomolar ratios of 0.05 (a), 1.00 (b), and 0.45 (c). (right) Schematic representations of the pictures seen in the corresponding fluorescence images.

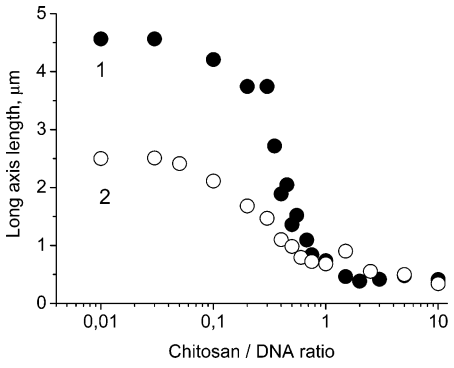

Figure 2. Dependence of the average long-axis length of DNA molecules on the chitosan/DNA monomolar ratio in the absence (1) and presence (2) of 0.1 M NaCl.

By averaging over the ensemble of several hundreds of individual molecules, the mean long-axis lengths $\langle L \rangle$ of DNA at given conditions were determined. Figure 2 shows the dependence of thus-obtained $\langle L \rangle$ values on the amount of added chitosan (expressed as chitosan/DNA monomolar ratio q). Because the behavior of polyelectrolytes and of their complexes with oppositely charged polymer chains is quite sensitive to added low-molecular-weight salt, the experiments were performed at different salt concentrations.

Low Salt Content. Let us first consider the case of low salt content (0.01 M) (Figure 2, curve 1). It is seen that in the presence of small amounts of chitosan DNA molecules assume the elongated random coil state with the mean long-axis length $\langle L \rangle$ of ca. 4.5 μm. When the chitosan/DNA ratio q becomes as high as 0.3, a sharp decrease of the average size of DNA molecules is

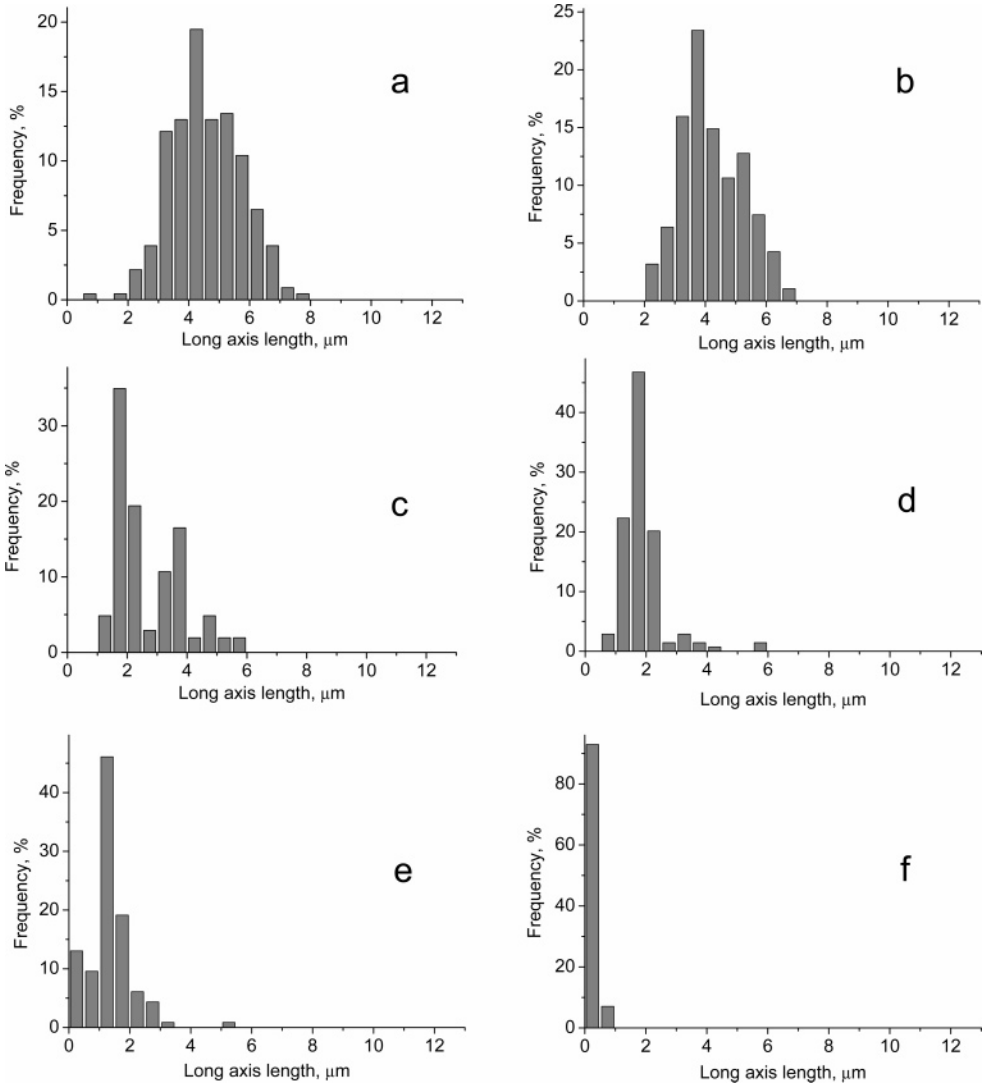

Figure 3. Histograms of the distribution of long-axis lengths of DNA molecules at different chitosan/DNA monomolar ratios without added NaCl: 0 (a); 0.10 (b); 0.35 (c); 0.40 (d); 0.50 (e); 2.5 (f). The number of analyzed DNA molecules is ca. 200. The area is normalized to be equal for each histogram.

observed, leading finally (at $q > 1$) to low $\langle L \rangle$ values characteristic of the globular state of DNA. Thus, chitosan induces the coil−globule transition of DNA. It is obviously due to the formation of IPEC between DNA polyanions and chitosan polycations that is driven by electrostatic attraction between them and by the gain in translational entropy of many small counterions released to the solution. In addition, the hydrophobic interactions between parts of DNA chains that are already coupled with chitosan molecules may contribute to the collapse of IPEC. It should be noted that stoichiometric IPEC with equal amounts of charged groups of both signs should contain ca. 1000 chitosan chains per 1 DNA duplex.

Further increase in the concentration of polycation leads to recharging of the complex.[37] Under these conditions, the IPEC particles are stabilized in solution by the positively charged polycation loops bound to DNA. Such recharging may induce the reswelling of polymer chains.[37] In this study we used the excess of chitosan (in monomoles) as high as 100-fold with respect to DNA and no sign of reentrant globule−coil transition was observed. At the same time, the chitosan/DNA globules do not demonstrate any tendency to aggregation, which may indicate that the globules have charged surfaces and repel each other. Therefore, the binding of excessive chitosan molecules seems to not destroy the inner structure of the globules, but to just modify their surface.

The data presented in Figure 2 concern the ensemble of DNA macromolecules. To provide a quantitative description of the coil−globule transition at the level of individual DNA duplexes, the distribution of L values at a given chitosan/DNA ratio q was considered (Figure 3). At low content of chitosan ($q < 0.3$) the distribution of L for single DNA duplexes is rather broad and centered at 4−4.5 μm, which is characteristic of DNA in the coil state (Figure 3a,b). At high content of chitosan ($q > 1$) DNA is mainly in the globular state with much less fluctuation in the apparent size (Figure 3f).

The most interesting situation is observed at intermediate q values ($0.3 < q < 1$). At $q = 0.4$ almost no coils or globules are detected (Figure 3d). At these conditions DNA adopts an intrachain segregated structure, in which elongated and collapsed parts coexist within a single DNA chain. A typical fluorescent image of such a structure is presented in Figure 1c. At somewhat smaller q values (e.g., $q = 0.35$) two definite populations of DNA chains are observed (Figure 3c),

indicating the coexistence of coils and segregated molecules. At somewhat higher q values ($0.4 < q < 1$) the segregated molecules coexist with collapsed ones (Figure 3e). Therefore, in the case of chitosan as condensing agent the conformation of DNA at the collapse transition changes from elongated coil to intramolecularly segregated state and then to the globule.

It is known that at the level of individual macromolecules the collapse transition can proceed by one of two ways: (a) by direct transition, coil → globule; or (b) via the intermediate state, coil → intrachain segregated state → globule. The first way was observed for DNA collapse induced by cationic surfactants;[31] low-molecular-weight amines with two (1,3-diaminopropane), three (spermidine), or four (spermine) positively charged amino groups;[32] cationic polypeptides (PLL[29] and poly(L-arginine)[9]); uncharged polymer poly(ethylene glycol) (PEG);[33] block copolymers with one cationic (PLL) block and one uncharged (PEG) block;[29] etc. The second way was observed for DNA collapse induced by 2-propanol[34] and some polycations including PEG with pendant amino groups[35] and histone H1 protein,[36] etc.

The possibility of the formation of a necklace structure by polyelectrolyte in salt-free solutions in a poor solvent was predicted theoretically.[38−41] It occurs for polymers with short-range attraction and long-range repulsion. In the case of chitosan/DNA complexes, the parts of DNA chain with bound chitosan molecules are uncharged and hydrophobic (note that uncharged chitosan is insoluble in water[42]) and therefore attract each other in water. At the same time, electrostatic repulsion in DNA parts free from chitosan opposes the collapse, because it leads to a too-close approach of similarly charged units. As a result a compromise necklace structure is formed, in which the attraction is realized inside the beads, while the similarly charged units get the possibility to separate by larger distances.

Thus, at low salt content the coil−globule transition of DNA duplex induced by interaction with oppositely charged polyelectrolyte−chitosan proceeds via the formation of intermediate (intramolecularly segregated) structures.

High Salt Content. To observe the salt effect, 0.1 M NaCl was added to the solutions under study. The results of this experiment are presented in Figure 2 (curve 2). It is seen (cf. curves 1 and 2 in Figure 2) that in 0.1 M NaCl the collapse transition for the ensemble of DNA chains has the following peculiarities: (a) it is shifted to lower chitosan/DNA ratio; (b) it has a smaller amplitude (i.e., smaller difference in size between the coil and globule states); (c) it is smoother. The observed effect of salt on the collapse transition of DNA is similar to that found for many other polyelectrolytes.[43−45] It can be explained by the screening of electrostatic repulsion between similarly charged DNA units by salt: it reduces the size of DNA molecules in the coil state and allows the chain contraction at a lower amount of condensing agent.

A significant influence of salt is also observed at the level of single chains (cf. Figures 3 and 4). From Figure 4 it is seen that in 0.1 M NaCl the globules start to appear at as low a chitosan/DNA ratio q as 0.05. Therefore, already the addition of a minor amount of condensing agent induces the collapse of some individual chains. This may be explained by the suppression of the electrostatic repulsion of similarly charged DNA units that favors the collapse. Also, salt is known to enhance the hydrophobic interactions that additionally stabilize the collapsed state.

An important observation consists of the fact that, by contrast to the case of low salt content, in 0.1 M NaCl only a few segregated structures were detected. What is the reason for such different behaviors of the same system depending on the salt content? As was mentioned above, at DNA collapse induced by polycations the formation of the segregated state instead of the globular one can be assigned to electrostatic repulsion of uncompensated charges of DNA. When 0.1 M NaCl is added, the uncompensated charges become screened, and the system follows the direct collapse route: coil → globule. Another effect of salt may consist in the enhancement of hydrophobic interactions between parts of DNA chains that are already coupled with chitosan molecules.

From Figure 4 it is seen that in 0.1 M NaCl the coils coexist with the globules. With the increase of the amount of added chitosan the fraction of globules in the mixture of coils and globules becomes higher. When the chitosan/DNA ratio gets as high as 2.5, mainly globules are observed (Figure 4f), while a minor amount of coils is still present. Therefore, in this system the width of the region of coexistence of coils and globules is rather broad. It is generally accepted that the coexistence of coils and globules results from the cooperative binding of the condensing agent onto DNA helices,[29,46] and the width of the coexistence region characterizes the degree of cooperativity in binding.[29] In other words, in such systems the condensing agent when choosing among free and partially complexed DNA chains prefers to bind to the latter. In this way the system gets an additional gain in free energy due to hydrophobic interactions between different hydrophobic sites formed on the DNA chain by bound chitosan molecules.

Thus, at high salt content the coil−globule transition of DNA duplex induced by chitosan proceeds without formation of an appreciable amount of intermediate structures. A broad region of coexistence of coils and globules indicates a high degree of cooperativity of the transition.

Chitosan/DNA Globules. Now let us consider in more detail the globular state of chitosan/DNA species, because it is of particular interest to deliver genes into living cells. Direct fluorescence microscopy observations do not allow one to determine the exact size of globules because of the blurring effect that is due to the resolution limit originating from the wavelength of the observation light and also partly due to the high sensitivity of the TV camera.[31] To obtain information about the actual sizes of globules, their hydrodynamic radii R_H were determined from the translational diffusion constants D of Brownian motion of single globules. The results obtained are presented in Figure 5.

It is seen that the mean hydrodynamic radii of the globules are around 40−50 nm. This is consistent with the data obtained in ref 45 by transmission electron microscopy (TEM) and atomic force microscopy (AFM). The size of globules is quite important for a favorable cellular uptake. It was reported[47,48] that polycation/DNA gene delivery systems mostly enter the cell by endocytosis or pinocytosis, having a size requirement less than ca. 100 nm. Hence, the chitosan/DNA nanoparticles have the required size, which makes them suitable for the uptake by cells.

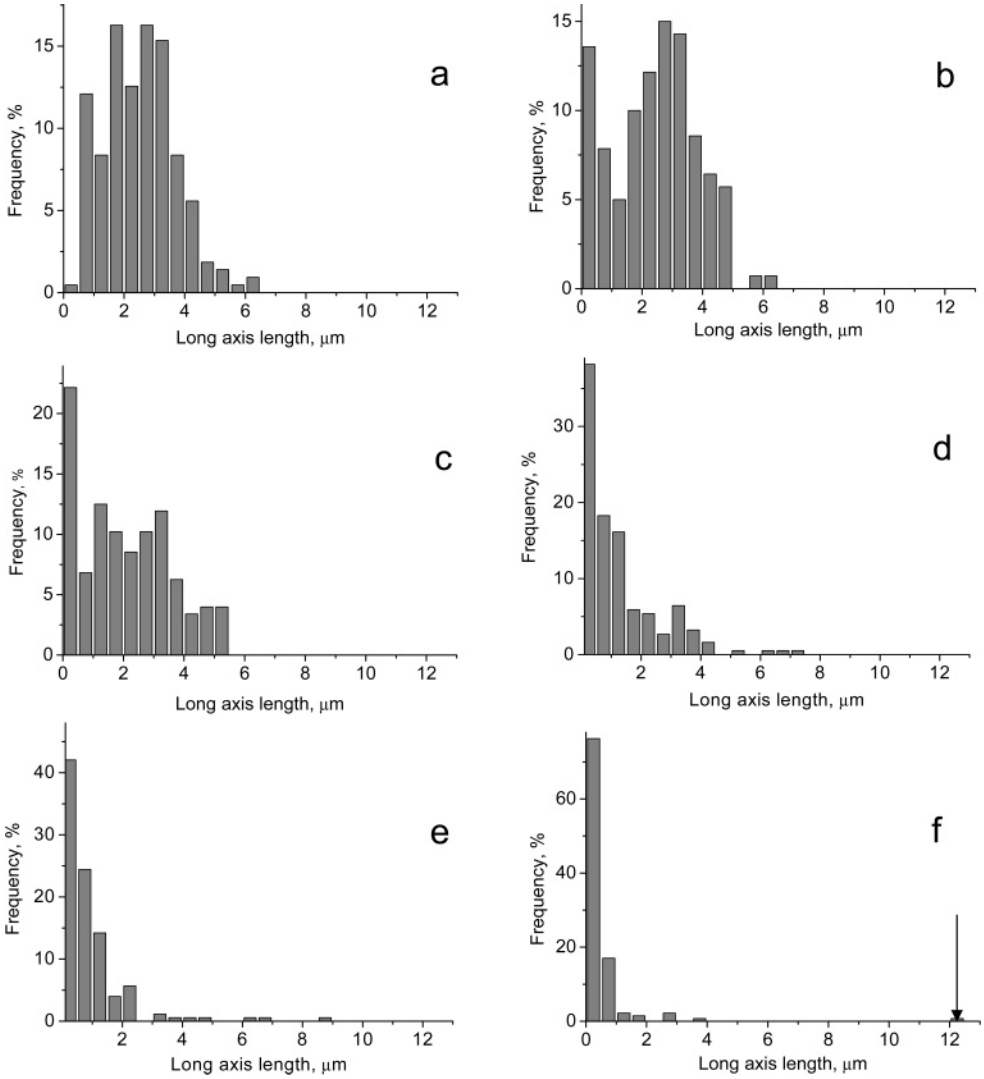

Figure 4. Histograms of the distribution of long-axis lengths of DNA molecules at different chitosan/DNA monomolar ratios in the presence of 0.1 M NaCl: 0 (a), 0.05 (b); 0.10 (c); 0.30 (d); 0.50 (e); 2.0 (f). The number of analyzed DNA molecules is ca. 200. The area is normalized to be equal for each histogram.

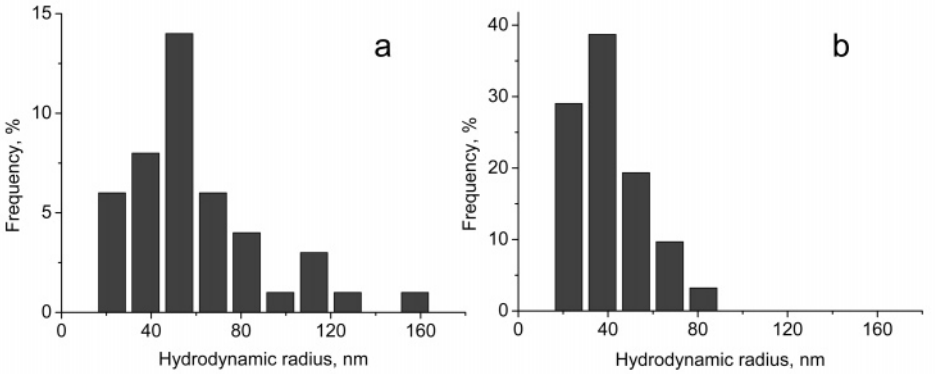

Figure 5. Histograms of the distribution of hydrodynamic radii of globules in aqueous solutions of DNA at chitosan/DNA monomolar ratio of 5.0 in the absence (a) and presence (b) of 0.1 M NaCl. The number of analyzed DNA molecules is ca. 50. The area is normalized to be equal for each histogram.

A significant effect of salt is also observed in the collapsed state of DNA. Figure 5 shows that upon addition of 0.1 M NaCl the mean R_H of globules decreases from 50 to 40 nm, which may be due to the contraction of the swollen shells of the globules containing chitosan chains bound in excess of the equimolar ratio. TEM and AFM data indicate that chitosan/DNA globules have mainly toroidal and rodlike structures.[18] For T4dC DNA samples under study the typical radius of a toroid is about 40 nm,[49,50] which is consistent with the obtained R_H data (Figure 5). Fluorescence microscopy does not allow one to determine the form of globules, but we could suggest that, probably, some population of globules with R_H values of 110 and 160 nm observed at low salt content (Figure 5a) may be due to the contribution of elongated particles that are formed

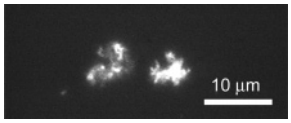

Figure 6. Fluorescence image of aggregates of globules formed in aqueous solution at chitosan/DNA monomolar ratio of 1.5 in 0.1 M NaCl.

because of strong electrostatic repulsion between similarly charged groups, which are located on the surface of globules. This suggestion coincides with the AFM observation[18] that the increase of the net charge of chitosan/DNA complexes leads to a higher fraction of rods in the mixture of toroidal and rodlike globules.

As mentioned above, at low salt content (0.01 M) single globules are quite stable and do not show any tendency to aggregation. At these conditions even a small net charge of globules is enough to induce electrostatic repulsion between them. It should be noted that the chitosan/DNA ratio corresponding to uncharged globules ($q = 1.05$, taking into account the degree of acetylation of chitosan) was not explored in this paper. By contrast, at high salt content (0.1 M), when the electrostatic repulsion is sufficiently screened, a significant number of globules aggregate with each other (Figure 6). The aggregation is most pronounced in the q range of 1−1.5. Similar results were obtained[17] by TEM in 0.15 M NaCl. They were attributed to a low net charge of globules under these conditions according to zeta-potential measurements.[17]

Thus, in the presence of salt the size of single globules decreases, but simultaneously very big intermolecular aggregates appear, indicating the tendency for precipitation. These data show that salt screens the electrostatic repulsion both inside one globule and among different globules.

It is known that IPEC can be destroyed upon addition of excess salt[37] as a result of the screening of electrostatic interactions between oppositely charged polyelectrolyte chains. Such a dissociation was observed recently[20] for IPEC formed between DNA and short oligomeric chitosan chains, when 0.003−0.1 M salt was added. The results obtained in this paper show that for rather long chitosan chains 0.1 M concentration of NaCl is not enough to produce such an effect because of stronger cooperativity in interpolymer interactions.

Conclusions

Salt does not disrupt the interpolyelectrolyte complex of DNA with chitosan, but it affects its morphology.

The most significant finding of this paper is the evidence that salt suppresses the formation of intramolecularly segregated structures and forces the chains to follow the direct transition from coils to globules without any intermediate states.

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References and Notes

(1) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. *Molecular Biology of the Cell*; Garland Publishing: New York, London, 1983.
(2) Cui, D.; Gao, H. *Biotechnol. Prog.* **2003**, *19*, 683−692.
(3) Wu, G. Y.; Wu, C. H. *J. Biol. Chem.* **1987**, *262*, 4429−4432.
(4) Wagner, E.; Zenke, M.; Cotton, M.; Beug, H.; Birnstiel, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3410−3414.
(5) Zhou, X. H.; Klibanov, A. L.; Huang, L. *Biochim. Biophys. Acta* **1991**, *1065*, 8−14.
(6) Kabanov, V. A. In *Macromolecular Complexes in Chemistry and Biology*; Dubin, P., Bock, J., Davies, R. M., Schulz, D. N., Thies, C., Eds.; Springer-Verlag: Berlin, Heidelberg, 1994; pp 151−174.
(7) Kabanov, A. V.; Kabanov, V. A. *Bioconjugate Chem.* **1995**, *6*, 7−20.
(8) Garnett, M. C. *Crit. Rev. Ther. Drug Carrier Syst.* **1999**, *16*, 147−207.
(9) Minagawa, K.; Matzusawa, Y.; Yoshikawa, K.; Matsumoto, M.; Doi, M. *FEBS Lett.* **1991**, *295*, 67−69.
(10) De Smedt, S. C.; Demeester, J.; Hennink, W. E. *Pharm. Res.* **2000**, *17*, 113−125.
(11) Boussif, O.; Lezoualch, F.; Zanta, M. A.; Mergny, M. D.; Scherman, D.; Demeneix, B.; Behr, J.-P. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7297−7301.
(12) Tang, M. X.; Szoka, F. C. *Gene Ther.* **1997**, *4*, 823−832.
(13) Dash, P. R.; Toncheva, V.; Schacht, E.; Seymour, L. W. *J. Controlled Release* **1997**, *48*, 269−276.
(14) Rao, S. B.; Sharma, C. P. J. *Biomed. Mater. Res.* **1997**, *34*, 21−28.
(15) Richardson, S. C.; Kolbe, H. V.; Duncan, R. *Int. J. Pharm.* **1999**, *178*, 231−243.
(16) MacLaughlin, F. C.; Mumper, R. J.; Wang, J.; Tagliaferri, J. M.; Gill, I.; Hinchcliffe, M.; Rolland, A. P. *J. Controlled Release* **1998**, *56*, 259−272.
(17) Erbacher, P.; Zou, S.; Bettinger, T.; Steffan, A.-M.; Remy, J.-S. *Pharm. Res.* **1998**, *15*, 1332−1339.
(18) Danielsen, S.; Varum, K. M.; Stokke, B. T. *Biomacromolecules* **2004**, *5*, 928−936.
(19) Roy, K.; Mao, H.-Q.; Huang, S.-K.; Leong, K. W. *Nat. Med.* **1999**, *5*, 387−391.
(20) Köping-Höggård, M.; Mel'nikova, Y. S.; Varum, K. M.; Lindman, B.; Artursson, P. *J. Gene Med.* **2003**, *5*, 130−141.
(21) Yamaoka, T. Gene Delivery. In *Supramolecular Design for Biological Applications*; Yui, N., Ed.; CRC Press: Boca Raton, 2002; pp 269−286.
(22) Yoshikawa, K.; Matsuzawa, Y.; Minagawa, K.; Doi, M.; Matsumoto, M. *Biochem. Biophys. Res. Commun.* **1992**, *188*, 1274−1279.
(23) Rinaudo, M.; Le Dung, P.; Gey, C.; Milas, M. *Int. J. Biol. Macromol.* **1992**, *14*, 122−128.
(24) Rinaudo, M.; Milas, M.; Le Dung, P. *Int. J. Biol. Macromol.* **1993**, *15*, 281−285.
(25) Buhler, E.; Guetta, O.; Rinaudo, M. *Int. J. Polym. Anal. Charact.* **2000**, *6*, 155−175.
(26) Rinaudo, M.; Desbrieres, J.; Pavlov, G. *Polymer* **1999**, *40*, 7029−7032.
(27) Schatz, C.; Pichot, C.; Delair, T.; Viton, C.; Domard, A. *Langmuir* **2003**, *19*, 9896−9903.
(28) Matsuzawa, Y.; Yoshikawa, K. *Nucleosides Nucleotides* **1994**, *13*, 1415−1423.
(29) Yamasaki, Y.; Katayose, S.; Kataoka, K.; Yoshikawa, K. *Macromolecules* **2003**, *36*, 6276−6279.
(30) Matsumoto, M.; Sakaguchi, T.; Kimura, H.; Doi, M.; Minagawa, K.; Matsuzawa, Y.; Yoshikawa, K. *J. Polym. Sci., B: Polym. Phys.* **1992**, *30*, 779−783.
(31) Mel'nikov, S. M.; Sergeev, V. G.; Yoshikawa, K. *J. Am. Chem. Soc.* **1995**, *117*, 2401−2408.
(32) Takahashi, M.; Yoshikawa, K.; Vasilevskaya, V. V.; Khokhlov, A. R. *J. Phys. Chem. B* **1997**, *101*, 9396−9401.
(33) Yoshikawa, K.; Matsuzawa, Y. *Physica D* **1995**, *84*, 220−227.
(34) Ueda, M.; Yoshikawa, K. *Phys. Rev. Lett.* **1996**, *77*, 2133−2136.
(35) Yoshikawa, K.; Yoshikawa, Y.; Koyama, Y.; Kanbe, T. *J. Am. Chem. Soc.* **1997**, *119*, 6473−6477.
(36) Yoshikawa, Y.; Velichko, Y. S.; Ichiba, Y.; Yoshikawa, K. *Eur. J. Biochem.* **2001**, *268*, 2593−2599.
(37) Kabanov, V. A.; Zezin, A. B. *Macromol. Chem., Suppl.* **1984**, *6*, 259−276.
(38) Kantor, Y.; Kardar, M. *Phys. Rev. E* **1995**, *51*, 1299−1312.
(39) Dobrynin, A. V.; Rubinstein, M.; Obukhov, S. P. *Macromolecules* **1996**, *29*, 2974−2979.
(40) Solis, F. J.; Olivera de la Cruz, M. *Macromolecules* **1998**, *31*, 5502−5506.
(41) Dobrynin, A. V.; Rubinstein, M. *Macromolecules* **2000**, *33*, 8097−8105.

(42) Desbrieres, J.; Rinaudo, M.; Chtcheglova, L. *Macromol. Symp.* **1997**, *113*, 135−142.
(43) Hirokawa, Y.; Tanaka, T.; Sato, E. *Macromolecules* **1985**, *18* (12), 2782−2784.
(44) Vasilevskaya, V. V.; Khokhlov, A. R.; Matsuzawa, Y.; Yoshikawa, K. *J. Chem. Phys.* **1995**, *102*, 6595−6602.
(45) Filippova, O. E. *Polym. Sci., C* **2000**, *42*, 208−228.
(46) Hill, T. L. *Thermodynamics of Small Systems*; Benjamin: New York, 1963.
(47) Corsi, K.; Chellat, F.; Yahia, L.; Fernandes, J. C. *Biomaterials* **2003**, *24*, 1255−1264.
(48) Wolfert, M. A.; Seymour, L. W. *Gene Ther.* **1996**, *3*, 269−273.
(49) Matsuzawa, Y.; Kanbe, T.; Yoshikawa, K. *Langmuir* **2004**, *20*, 6439−6442.
(50) Yamasaki, Y.; Yoshikawa, K. *J. Am. Chem. Soc.* **1997**, *119*, 10573−10578.

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