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Secondary Structure of DNA Is Recognized by Slightly Cross-Linked Cationic Hydrogel

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Abstract: Interaction of salmon sperm DNA (300−500 bp) and ultrahigh molecular mass DNA (166 kbp) from bacteriophage T4dC with linear poly(*N*-diallyl-*N*-dimethylammonium chloride) (PDADMAC) and slightly cross-linked (#) PDADMAC (#PDADMAC) hydrogel in water has been studied by means of UV-spectroscopy, ultracentrifugation, atomic force, and fluorescence microscopy (FM). It is found that the linear polycation induced compaction of either native (double-stranded) or denatured (single-stranded) DNA by forming PDADMAC-DNA interpolyelectrolyte complexes (IPEC)s. At the same time, #PDADMAC hydrogel is able to distinguish between native and denatured DNA. Native DNA is adsorbed and captured in the hydrogel surface layer, while denatured DNA diffuses to the hydrogel interior until the whole hydrogel sample is transformed into the cross-linked IPEC. Both native and denatured DNA can be completely released from the hydrogel in appropriate conditions with no degradation by adding a low molecular salt. The data observed using conventional physicochemical methods with respect to DNA of a moderate molecular mass remarkably correlate with the pictures directly observed for ultrahigh molecular mass DNA in dynamics by using FM.

Introduction

Slightly cross-linked polyelectrolyte hydrogels are able to sorb oppositely charged linear polyions from aqueous media.[1−4] The sorption is provided by the cooperative electrostatic attraction between the polymeric components giving rise to the formation of interpolyelectrolyte complex constructs, (#IPEC)s, which actually represent polyion networks filled with oppositely charged linear polyions. The transport of linear polyions into oppositely charged polyelectrolyte networks, driven by the free energy of interpolyelectrolyte complexation reaction, was found to be strongly dependent on the chemical structure of the polyelectrolytes and environmental conditions, such as pH, concentration of external salts, etc.

A number of studies were devoted to the interpolyelectrolyte complexes (IPEC)s resulting from the interaction of DNA with synthetic linear and branched polycations.[5−12] On the contrary, interaction between DNA and oppositely charged networks is still in the opening stage of research.[13]

Native double helical DNA is a quite rigid anionic polyelectrolyte. Contrastingly, a single-stranded DNA chain represents a rather flexible polyanion. In alkaline media, at pH > 11, native double-stranded DNA is known to denaturate. On denaturating, a double helix dissociates into the individual polynucleotide copolymers apparently due to disturbing a cooperative sequence of hydrogen bonds between the complementary base pairs caused by ionization (deprotonation) of guanine and thymine residues. Denaturation is reversible, so that the double helix is restored on passing DNA from alkaline into neutral water solution.

Our goal was to clarify the influence of the DNA secondary structure on sorption of DNA polyanions from aqueous media by a cationic hydrogel. In particular, we have studied the interaction between native or denatured DNA molecules with

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slightly cross-linked poly(*N*-diallyl-*N*-dimethylammonium chloride) (#PDADMAC) using a series of experimental techniques including direct observations by means of fluorescence and atomic force microscopy.

PDADMAC is a strong polyelectrolyte, so that the degree of ionization and the swelling capacity of the hydrogel do not depend on pH. Therefore, by switching from neutral to alkaline pH, the peculiarities of the reaction between the hydrogel and native or denatured DNA can be revealed. In fact, the difference if observed would be only due to the change in the DNA secondary structure.

Experimental Section

Materials. #PDADMAC hydrogel was synthesized by a free radical copolymerization of *N*-diallyl-*N*-dimethylammonium chloride with *N*,*N*′-methylenebis(acrylamide) (0.5 mol %) in a 50 wt % aqueous solution of the monomers. The reaction was initiated by a mixture of ammonium persulfate with sodium methabisulfite (0.25% of a monomer mass) and performed at 40 °C for 24 h under argon atmosphere. After the polymerization was completed, the hydrogel was immersed in a large amount of distilled water to wash out the residual chemicals. The wash water was repeatedly changed every 2−3 days during 2 months. The degree of swelling of the hydrogel, $H = (m_s - m_d)/m_d$, determined by gravimetric method in 0.01 M solution of NaCl at pH 7 and pH 12, was about 300 (m_s and m_d are the weights of the equilibrium swollen and the dry sample, respectively). Thus, the concentration of the repeating monomer units in the equilibrium water-swollen gel was about 0.02 base mol/L.

Salmon sperm DNA (300−500 base pairs (bp)) purchased from GosNIIOKhT, Russia, and used for UV and sedimentation measurements required a sufficiently large amount of the DNA samples. Ultrahigh molecular mass T4DNA (166 kilo base pairs (kbp)) from bacteriophage T4dC was purchased from Wako Pure Chemical Industries Ltd., Japan, and was used to observe DNA macromolecules by fluorescence microscopy.

The fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), 2-mercaptoethanol (ME) used as an antioxidant, and linear PDADMAC ($M_w = 3\,000\,000$) were obtained from Wako Pure Chemical Industries Ltd., Japan.

The hydrogel samples used for the investigations were 0.3−0.5 g weighed cubes cut out from #PDADMAC block equilibrium swollen in 0.01 M NaCl at pH 7 or pH 12. In each experiment, such a cube was placed in a weighing bottle containing 19 mL of the DNA solution (1.17 mg/mL) at the corresponding pH. Thus, the overall molar ratio of the quaternary ammonium groups in the gel to the phosphates groups in DNA varied from 0.6 to 1.0.

Methods. UV Spectrophotometry. The amount of DNA sorbed by the #PDADMAC hydrogel was determined as the decrease in DNA concentration in the surrounding solution. The DNA concentration was measured from UV absorption spectra recorded with a Specord M-40 spectrophotometer (Germany). Cells with optical path of 1 cm were used for UV measurements. A molar extinction coefficient at $\lambda = 260$ nm corresponding to the absorbance maximum was assumed to be 6600 L/(mol cm) for the native DNA and to be 8800 L/(mol cm) for the denatured DNA.[14]

The DNA denaturation induced by the change in the solution pH from 7 to 12 and renaturation on returning to the neutral pH region were monitored by spectrophotometry on the basis of the difference in the extinction coefficients of the double- and single-stranded forms. Both transitions occurred over a period of several minutes at room temperature.

Fluorescence Microscopy. The solutions for FM observations were prepared as follows. T4 DNA stock solution was diluted to a preassigned extent with Tris·HCl buffer solution (10 mM, pH 7.2) containing 4 vol % of ME, 0.01 M NaCl, and DAPI as the fluorescent dye (the final molar ratio [DAPI]/[DNA] = 1). ME, a free radical scavenger, was used to reduce fading of the dye and light-induced damage of DNA.

FM observations of the interaction between T4 DNA and the hydrogel have been carried out using a special cell consisting of a plastic plate 2 mm thick with two connecting holes of 5 mm in diameter. The plate was placed between two thin cover glasses so that the holes formed two small communicating reservoirs. The cylindrical specimen cut from an equilibrium swollen gel (60 mg) was put in one of them, while another one was filled with the T4 DNA (0.6 μm in bp) solution. The cell was illuminated with 365 nm UV-light. The samples for microscopic observation of T4DNA interacting with water-soluble reagents were prepared as earlier described.[5] Fluorescence images of DNA were obtained by a Carl Zeiss Axiovert 135TV microscope equipped with a 100× oil-immersed lens. The images were recorded on videotape through a high-sensitivity Hamamatsu SIT TV camera and analyzed with an Argus 10 image processor (Hamamatsu Photonics). Special care was taken to carefully clean the microscope glasses and the cell before each observation as in the previous studies.[15]

Sedimentation. Sedimentation measurements were carried out with a Beckman-E analytical ultracentrifuge at a rotation velocity of $\omega = 48\,000$ rpm and a temperature of 20 °C. The sedimentation profiles were recorded by radial scanning the absorbance of the solution at 260 nm. The sedimentation coefficient for initial DNA from salmon sperm was 6.27 Svedberg units.

Atomic Force Microscopy. Partly air-dried hydrogels samples were investigated by atomic force microscopy (AFM) Nanoscope-III (Digital Instruments). Cantilevers of different stiffness within the range of 0.06−0.6 N/m with silicon carbide needle were used for the contact mode. There were no special precautions for the minimization of the force on the sample. Freshly cleaved mica was used as a substrate. Three-dimensional images were built and processed by the specialized package Femtoscan-001 making it possible to analyze the images obtained by a scanning probe microscope.[16]

Results and Discussion

To clarify the peculiarities of interaction between DNA and #PDADMAC hydrogel, the two sets of preliminary experiments had to be done using individual DNA and its mixtures with linear PDADMAC.

FM Observation of the Behavior of DNA in Alkaline Water Solution. At the beginning of our studies, we have attempted to visualize the denaturation of an individual DNA molecule upon pH shift from a neutral to alkaline pH region by using FM. In doing so, we based our study on the following consideration. It is known that both single- and double-stranded DNA form complexes with DAPI. However, the fluorescence intensity of DAPI-single-stranded DNA is much lower than DAPI-double-stranded DNA complexes.[17] Therefore, we could expect that dissociation of the double helix to form individual polynucleotides may be directly followed by FM on switching pH in the experimental solutions from the neutral to the alkaline region. To trace continuously the dissociation process in dynamics, the FM observation was performed under a continuous increase of pH. The drop of T4DNA water solution at neutral pH was placed in the middle of a glass slide and covered

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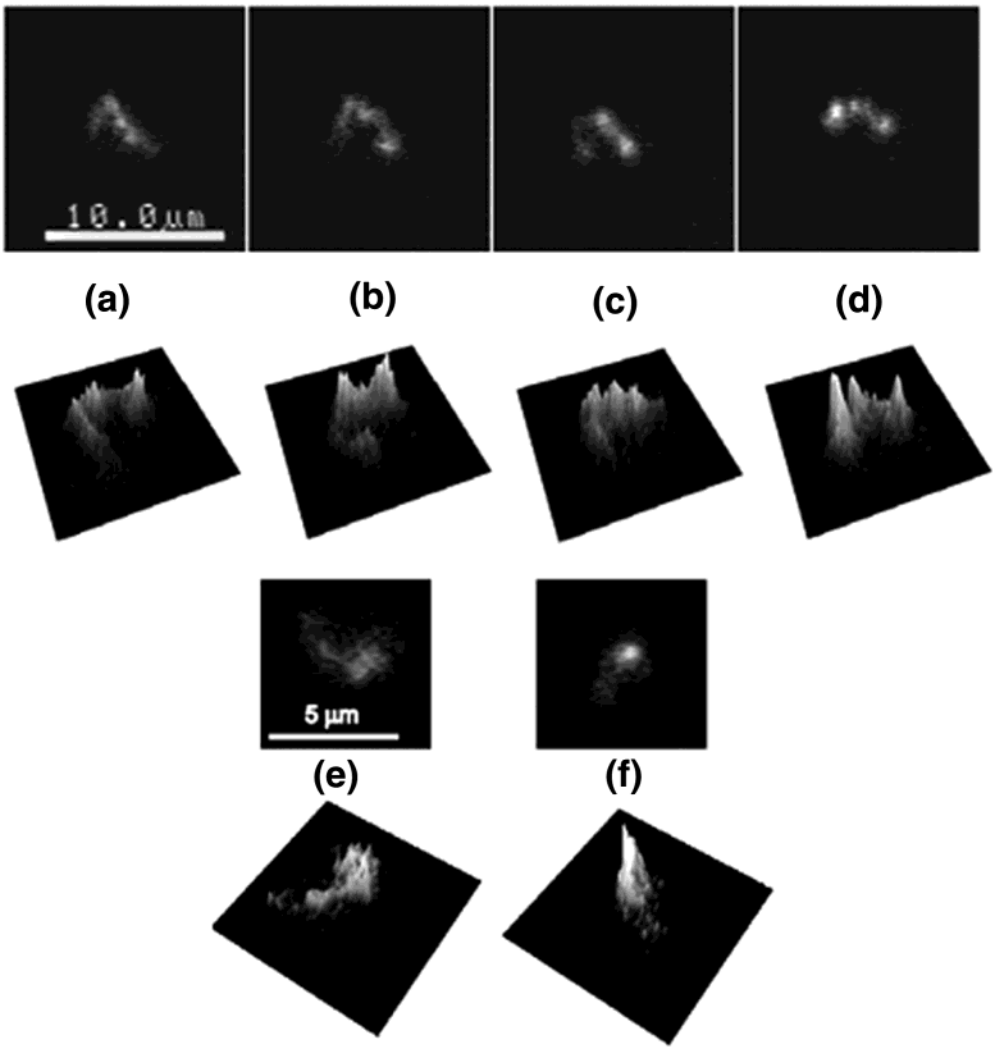

Figure 1. FM images of the individual T4DNA molecule denaturing upon increasing from (a) to (f) concentration of NaOH (upper panels) and the corresponding light-intensity distributions (bottom panels). The concentration of NaOH placed on the edge of the cover slip was 1 M. The time interval between shots was 6 s.

with a cover slip. The drop of concentrated NaOH solution was then placed on the edge of the cover slip to make it contact with the T4DNA solution spread in the gap between the glass slide and the cover slip. The evolution of the shape of T4DNA molecule and the fluorescence intensity distribution within its image upon increasing NaOH concentration are shown in Figure 1. The pictures were taken with a time interval of 6 s. In the first stage, the original image of the individual T4DNA extended coil was drastically changed; expanded bright spots were generated, which fast migrated back and forth in the DNA coil (Figure 1a−d). The image of the T4DNA molecule then became more and more blurred and diffused while the local concentration of NaOH increased further (Figure 1e,f). Finally, it vanished completely for ca. 10 min, apparently indicating a complete dissociation of the double helix.

Interaction of Native and Denatured DNA with Linear PDADMAC. On addition of the aqueous solution of linear PDADMAC to the aqueous solutions of native (pH 7) and denatured (pH 12) salmon sperm DNA, the mixtures remained transparent up to the [PDADMAC]/[DNA] ratio of 0.6 but progressively turned turbid at [PDADMAC]/[DNA] > 0.6, apparently due to formation of the insoluble IPEC.

An elementary step of the cooperative coupling reaction between the two oppositely charged polyelectrolytes is presented in Scheme 1, where B is a purine or pyrimidine base. Sodium chloride released remains in solution in the amount equivalent to a number of the interpolyelectrolyte ion pairs (salt bonds) formed. According to the classical approach by Manning,[12] the driving force, that is, the decrease in the free energy of the system, is provided by the increase in entropy of released counterions originally localized in the vicinity of polyelectrolyte coils. However, broadly speaking, such factors as hydrophobic interaction, hydrogen bonding, charge transfer, specific (non-Coulomb) interaction between counterions, and polyelectrolyte ionic groups should be taken into account. All of these factors, as well as recently considered[18] electrostatic correlation between adsorbed polycations, may contribute in the form of either positive or negative free energy terms.

The insoluble PDADMAC-DNA complexes were separated by ultracentrifugation. The concentration of DNA remaining in the supernatant was then measured spectrophotometrically. Figure 2 shows that the drastic decrease in DNA concentration in the supernatant occurs at [PDADMAC]/[DNA] ratios exceed-

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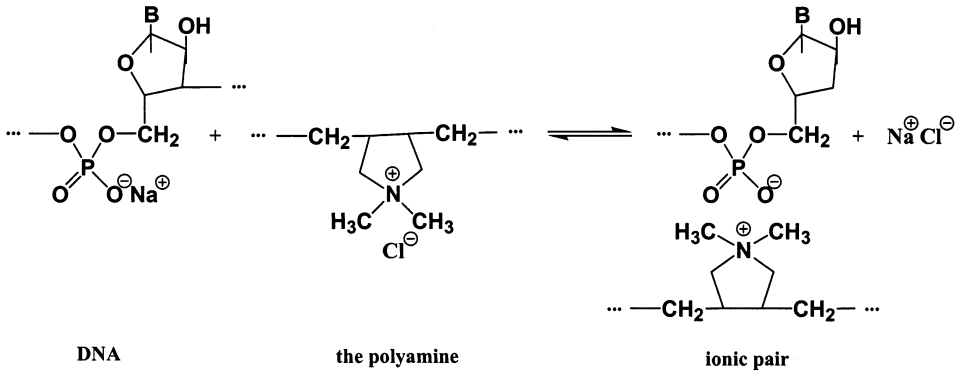
Scheme 1



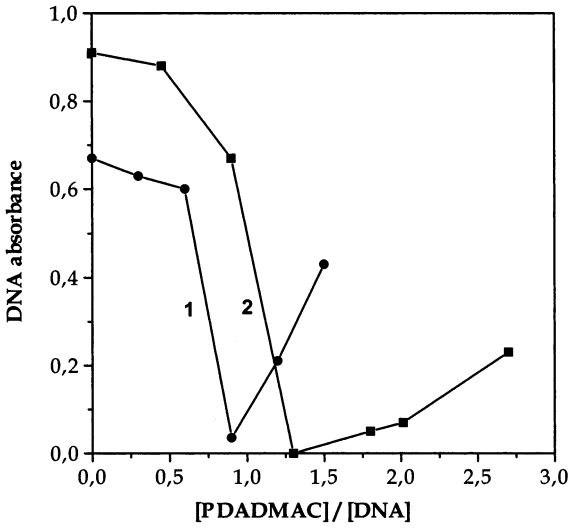


Figure 2. Residual absorbance of salmon sperm DNA in the supernatant after centrifugation vs [PDADMAC]/[DNA] ratios in 0.01 M NaCl solutions at pH 7 (1); and pH 12 (2); $\omega = 11\,000$ rpm, 20 °C.

ing 0.6. At the [PDADMAC]/[DNA] ratio close to 1, the aqueous phase contains no measurable amount of the native DNA (Figure 2, curve 1). A somewhat higher ratio (about 1.4) is required to reach a complete precipitation of denatured DNA (Figure 2, curve 2). The extra amount of PDADMAC is probably consumed for neutralization of deprotonated negatively charged guanine and thymine base residues. It is also seen in Figure 2 that at [PDADMAC]/[DNA] ratios above the neutralization points, the residual DNA concentration in the supernatant increases, apparently indicating formation of the water-soluble IPECs overcharged by the excess polyammonium salt. Positively charged water-soluble DNA containing IPECs was observed earlier for DNA interacting with another linear polycation[6] and poly(propylene imine) dendrimers of high generations taken in excess charge ratios.[5]

To date, fluorescence microscopy has been applied successfully to monitor conformational changes in giant T4DNA chains induced by various agents in dilute aqueous solutions.[5,10,15,19−21] We used FM to visualize the conformational behavior of the individual T4DNA molecules interacting with linear water-soluble PDADMAC. Figure 3 exemplifies the typical images of native giant T4DNA molecules in the presence of different amounts of the polycation. As expected, the T4DNA double helix exhibits an extended coil conformation in neutral Tris·HCl aqueous solution (Figure 3a). Addition of linear PDADMAC causes compaction of the DNA chains. Partially compacted structures with two collapsed parts edging a single chain (dumbbells) are observed as intermediates between an extended coil and a fully collapsed form (Figure 3b). Some compact globular-like IPEC species formed at [PDADMAC]/[DNA] > 1 exhibit thermal motion in the bulk solution. However, the main fraction of the similarly looking globules sticks to the microscope slides and remains motionless (Figure 3c). Thus, a native T4DNA extended coil interacting with PDADMAC to form IPEC collapses just as it does upon complexation with other positively charged condensing agents.[22−25] The exhaustive review on DNA compaction caused by polycations was recently published in ref 25.

As already noted, a single-stranded T4DNA chain exhibits no fluorescence image on FM observation. However, on addition of linear PDADMAC solution to the alkaline solution of denatured T4DNA, the images of compact single globules appear, apparently indicating that the formation of polycation-single-stranded DNA IPEC species captured some additional amount of the fluorescent dye. Such globules are small in size as compared to the globules observed for polycation-native T4DNA complex. In other words, PDADMAC provides compaction of DNA molecules either in neutral or in alkaline media, that is, either in a double helical or in a single-stranded form.

Recognition of the Double- and Single-Stranded DNA by #PDADMAC Hydrogel. On the basis of the scope of the above observations, the complexation of DNA molecules can be expected when they interact with the slightly cross-linked #PDADMAC hydrogel. Indeed, immediately after immersing the #PDADMAC hydrogel sample in 0.01 M NaCl solution of either native or denatured DNA, a thin iridescent film appears on the gel−solution interface. Formation of such a film indicates that DNA is actually adsorbed on the hydrogel surface to form the corresponding #IPEC. From iridescence observed, it follows that the thickness of the #IPEC skin is of the DNA molecular size.

However, the subsequent behavior of the native and denatured DNA interacting with #PDADMAC hydrogel has been abso-

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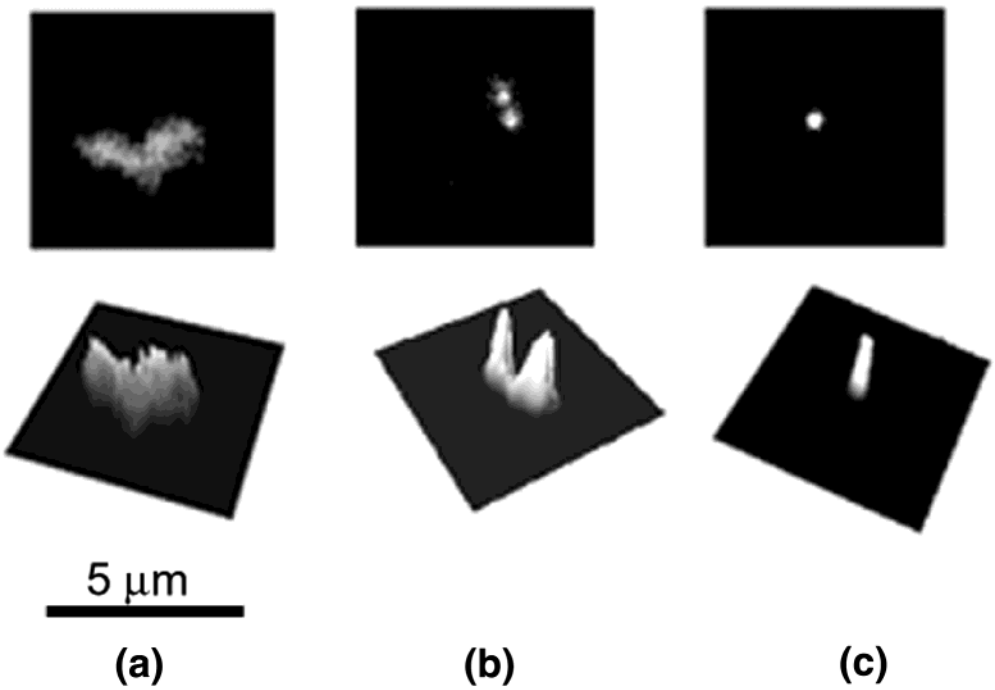

Figure 3. FM images of the individual T4DNA molecule at different [PDADMAC]/[DNA] ratios (upper panels) and the corresponding light-intensity distributions (bottom panels). (a) [PDADMAC]/[DNA] = 0; (b) [PDADMAC]/[DNA] = 0.5; (c) [PDADMAC]/[DNA] = 1.10.

lutely different. In the case of native DNA, the sorption process ends at the above stage. The rigid double helical DNA polyanions do not penetrate deeper into the gel. Further DNA uptake is inhibited by the thin #IPEC surface layer formed by the native DNA and #PDADMAC. Contrastingly, in the case of denatured single-stranded DNA, the #IPEC layer propagates frontally inward from the hydrogel surface. The #IPEC propagation is accompanied with a drastic volume contraction, that is, a collapse of a polyelectrolyte network. Finally, the original highly swollen #PDADMAC sample transforms into a compact #IPEC. On the cuts of the samples at intermediate stages, a turbid #IPEC shell surrounding the transparent unreacted hydrogel core is observed. The equilibrium degree of #IPEC swelling is at least 50 times lower than that of the initial hydrogel sample. The kinetic curves demonstrating the different character of the native and denatured single-stranded DNA uptake by the #PDADMAC hydrogel are presented in Figure 4. From this figure it is evident that the secondary structure of the DNA polyanions has a profound effect either on sorption kinetics or on the ultimate extent of the DNA uptake.

One other type of interaction between different polyions and oppositely charged hydrogels has been already observed and explained in refs 1, 3, and 4. The mechanism that provides unlimited transformation of the hydrogel into the corresponding #IPEC actually includes two interrelated interpolyelectrolyte reactions. The adsorption of polyions on the surface of an oppositely charged hydrogel (i.e., the formation of the #IPEC in the surface layer) occurs very rapidly as polyions diffuse from the environmental solution. The attachment of a polyion to the surface fragment of the oppositely charged polyelectrolyte network is accompanied by the release of counterions as shown in Scheme 1 for our case. The #IPEC formation apparently is driven and controlled by the same factors as in the case of linear polyions.

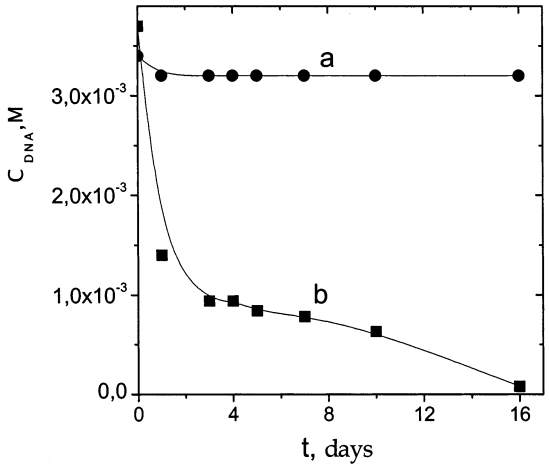

Figure 4. Time dependence of the DNA concentration above the #PDADMAC hydrogel: (a) pH 7 and (b) pH 12; 0.01 M NaCl, 20 °C.

It is clear that at full coating of the hydrogel surface by the #IPEC, all cationic vacancies on the surface are consumed, preventing the addition of the next portions of linear polyions. To regenerate such a vacancy, the polyion fragment that has been already involved in formation of an #IPEC should migrate inward from the hydrogel surface by, at least, one "step". Such migration can occur only upon the cooperative ion exchange reaction involving few charged repeating units of either the adsorbed polyion or the polyelectrolyte network, as well as an equal number of the network counterions (in our case, Cl^- ions). As a result, the vacant cationic groups of the polyelectrolyte network neutralized by the counterions appear again on the sample surface. These groups are capable of adding the next polyion fragment coming from the solution. Thus, the rate of transformation of the initial hydrogel into #IPEC is determined by the rate of the interpolyelectrolyte exchange at the #IPEC−hydrogel interface inside the sample. It is evident that the rate

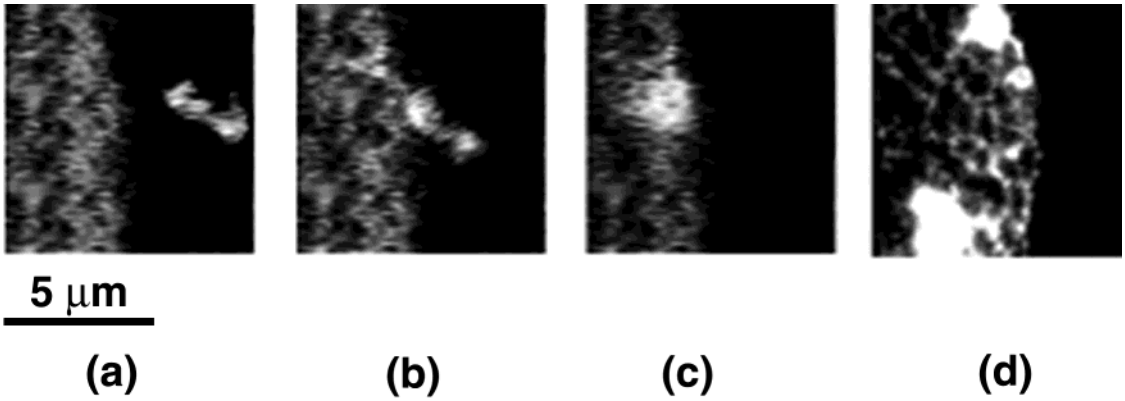

Figure 5. FM images of #IPEC skin forming on the surface of the #PDADMAC gel. The time interval between shots was 2 s.

of such an exchange reaction is equal to the rate of migration of the cationic vacancies of the network through the #IPEC layer to the outside of the sample. If the interpolyelectrolyte exchange reaction is forbidden for some reason, the #IPEC layer does not propagate beyond the hydrogel−solution interface. The #IPEC surface skin itself serves as the barrier. As applied to the systems under consideration, it means that such a prohibition, effective for the double-stranded DNA polyanions, is eliminated when DNA has been split into relatively flexible single strands.

To visualize the peculiarities of the interaction of individual DNA molecules with the hydrogel, the FM observations were carried out. Figure 5 shows the typical fluorescence image of a native T4DNA molecule in the vicinity of and at the gel−solution interface. Immediately, after placing T4DNA solution into the above-described special cell containing a hydrogel sample, the T4DNA molecule diffusing toward the gel surface (Figure 5a) attaches to the surface by one part of the coil, whereas another extended part exposed to the solution still exhibits thermal motion (Figure 5b). The DNA molecule remains in such a partially fixed state for a few seconds but then adopts a compact conformation (Figure 5c). Finally, the continuous #IPEC layer of a very high fluorescence intensity covers the whole hydrogel surface, so that the bright border is clearly detected at the gel−solution interface (Figure 5d). Figure 6 represents an AFM image of the edge of the specimen treated with the solution of salmon sperm DNA, then thoroughly washed and partly air-dried. It is seen that the surface of the #IPEC skin formed is rather smooth.

Single-stranded T4DNA chains not detectable by FM in the solution are collapsing and become visible when they approach the #PDADMAC hydrogel surface, just as they do interacting with linear water-soluble PDADMAC. Figure 7 demonstrates typical FM images of the hydrogel surface contacting with T4DNA solution at pH 12. One can see the bright spot apparently corresponding to compacted T4DNA single chains (Figure 7a), the number of which continuously increases in time (Figure 7b). Note that early in the development of DNA sorption, the bright spots dispose at a certain distance from each other and do not segregate, forming some kind of a cellular-like structure. It appears as a sorbed DNA chain collapsing upon its complexation with #PDADMAC fragments and produces a local "dead" zone around, which is not favorable to adopt another DNA chain. This is probably because of local strains in the hydrogel network caused by the local collapse. However, eventually a continuous bright layer is formed on the hydrogel

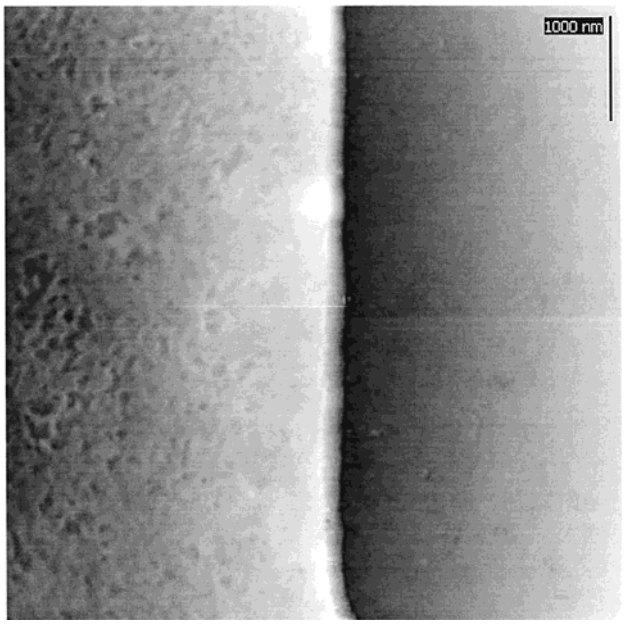

Figure 6. AFM image of #IPEC layer formed at the exterior of the #PDADMAC gel treated with a solution of salmon sperm DNA and then thoroughly washed and partly air-dried; [DNA] = 10^{-4} M (in bp).

surface while #IPEC proliferates inward from the surface. At this stage, a noticeable volume contraction of the whole sample occurs.

The quite unexpected phenomenon was revealed on lengthy (ca. 1 h) FM observations of native T4DNA giant molecules in a T4DNA-#PDADMAC hydrogel mixture. At times, some double helical coils collapsed in a bulk solution being pretty far removed from the gel/solution interface (up to 10^3 μm). Figure 8 represents the evolution with time of the distribution of an effective long-axis length (L) for T4DNA molecules being not in contact with the hydrogel. Each pattern (d, f, and g) was obtained by measuring the effective size of no less than 100 DNA molecules for an assigned period of time using the corresponding videotape recording. It is seen that the distribution gradually becomes narrower and shifts to the image sizes characteristic for fully collapsed T4DNA species.

Nothing of this kind happens in the bulk T4DNA solution surrounding the #PDADMAC hydrogel if it has been pretreated with DNA solution, rinsed with water, and then placed into the FM cell. Figure 9a shows a typical image of the hydrogel sample coated with #IPEC layer and the native T4DNA molecule in

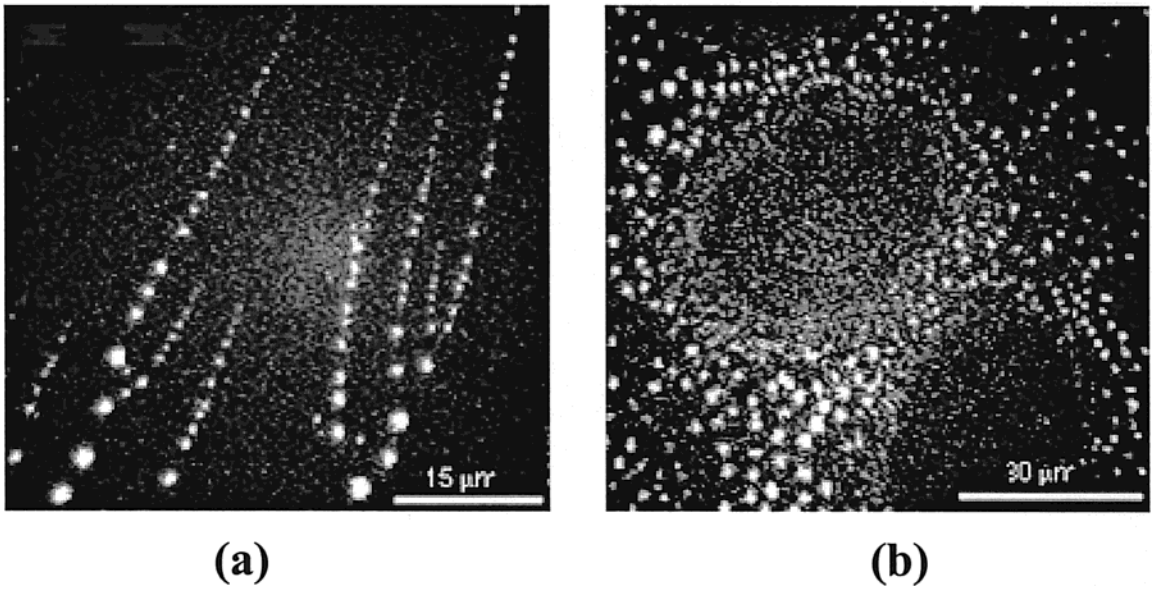

Figure 7. FM images of #IPEC layer forming at the surface of the #PDADMAC gel immersed in the solution of the denatured T4DNA at pH 12. The time interval between the shots was 30 min.

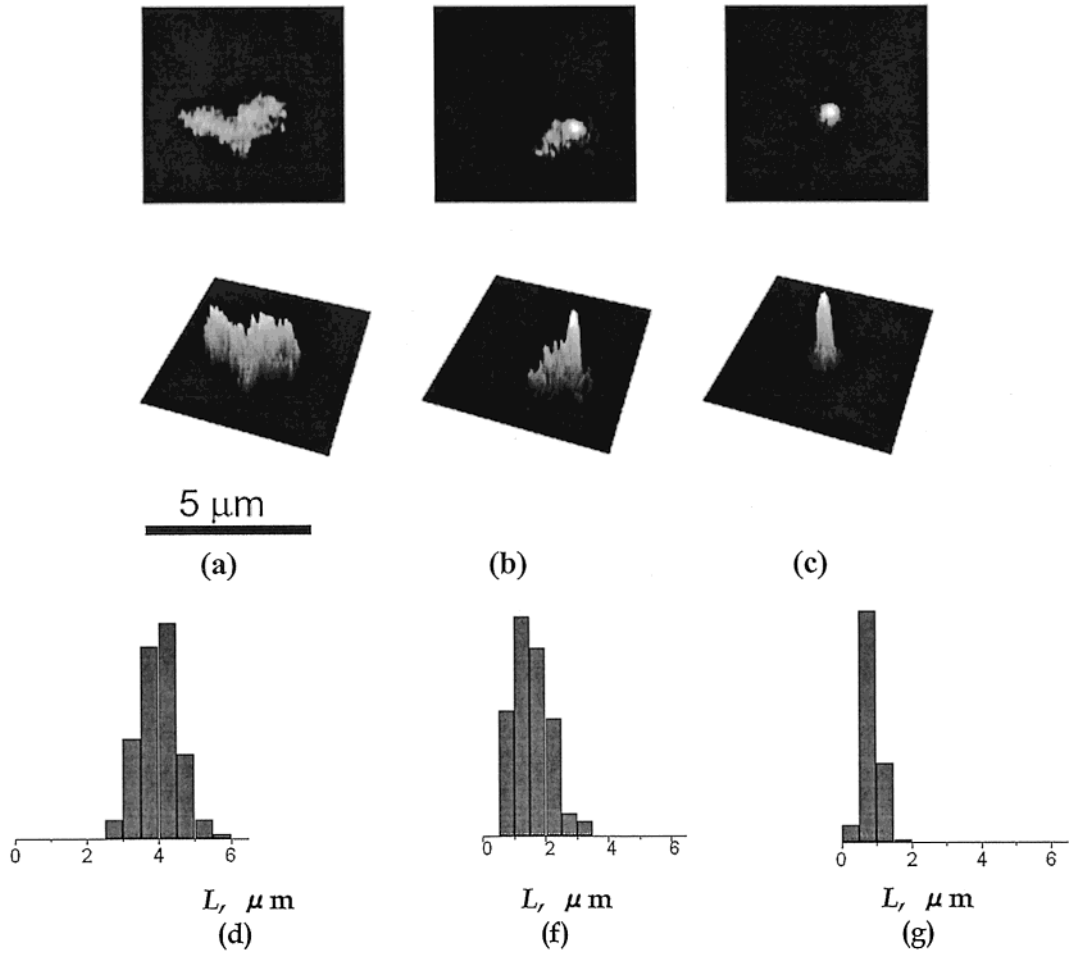

Figure 8. FM images of free coiled (a), partially compacted (b), and fully compacted (c) T4DNA individual molecule observed at a distance of 10^3 μm from a border of gel−solution interface (upper panels) and the corresponding light-intensity distributions (bottom panels). Temporal evolution of the distribution of a long-axis length, *L*, for T4DNA molecules in the bulk solution contacting with the #PDADMAC gel sample (to get each distribution pattern, at least 100 T4DNA molecules were counted). The elapsed time after contact was 0−15 min (d); 15−30 min (f); and 30−45 min (g).

an extended coil conformation. Lengthy observation has shown neither collapse of such coils diffusing in the vicinity of the gel surface nor change in the distribution of the long-axis length (Figure 9b).

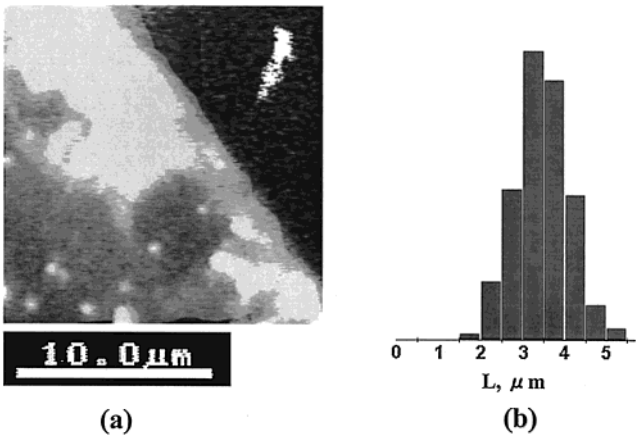

Figure 9. FM image of an individual T4DNA coil exhibiting thermal motion in the vicinity of the #PDADMAC gel sample preliminary coated with #IPEC layer (a); and the corresponding distribution of the T4DNA long-axis length (b).

The only explanation of the above phenomena is in the assumption that the bulk T4DNA solution contacting with the initial #PDADMAC hydrogel sample, in fact, contains some trace amount of PDADMAC microgel species, probably formed on cutting the specimens for FM observation. Such species complexing with native DNA molecules induce their "strange" collapse shown in Figure 8. However, the microgels are bound and removed upon pretreatment of the hydrogel specimen with DNA solution with water washing. No collapse in solution is observed (Figure 9). The above explanation looks very likely in the light of well-known data on DNA compaction upon its complexation with highly branched polycations.[5] Anyway, compaction of DNA molecules using a nanosize microgel may become a promising area for future research.

It is well known that IPECs may dissociate into initial polyelectrolyte components at certain concentrations of the external salts in the solution. The concentration of the salt at which dissociation takes place characterizes the stability of an IPEC.[4] In the case of #IPECs, dissociation is accompanied by release of a liner polyelectrolyte component into the environmental solution and regeneration of the initial hydrogel.

To estimate the concentration of NaCl required for dissociation of the IPECs in question, the water-insoluble charge-stoichiometric complexes formed from the native or denatured salmon sperm DNA and linear PDADMAC were prepared in 0.01 NaCl at pH 7 and pH 12, respectively, separated by centrifugation, and washed. The series of both IPEC samples were then placed in equal volumes of water with different NaCl concentrations at pH 7. After 24 h (a time sufficient for equilibration), the amount of DNA released into the aqueous phase was determined by measuring the absorbance at $\lambda = 260$ nm (PDADMAC does not absorb light in this region of spectrum). Figure 10 shows the corresponding dependences of a relative absorbance. As can be seen in Figure 10, both types of DNA remain trapped in the insoluble IPEC, until a certain NaCl concentration is attained; this is ranging from ca. 0.25 to 0.9 M for the double-stranded DNA (curve 1) and ca. 0.5 to 1.25 M for single-stranded DNA (curve 2). Importantly, no DNA molecules have been detected in solution at lower concentrations. At the same time, at higher concentrations, both IPECs completely dissociate, so that the DNA entirely transfers to the solutions. The Manning's analysis[12] shows that the entropic

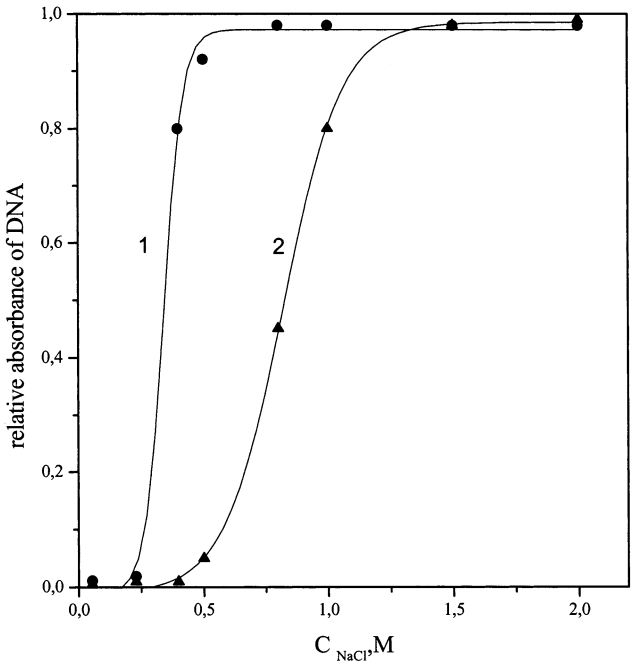

Figure 10. Relative absorbance of DNA released from precipitates of DNA-linear PDADMAC IPEC prepared at pH 7 (1) or pH 12 (2), vs NaCl concentration; 20 °C, pH 7.

driving force arising from release of counterions is highly dependent on salt concentration. Counterions are released from a region of high local concentration near the polyion surface. If the environmental salt concentration is about the same as this high local concentration, there is no entropic gain in the release of counterions. The high local concentration of sodium counterions condensed on native DNA is about 1.2 M.[12] We have observed dissociation in roughly this range of the environmental salt. However, more studies are required to check whether the above consideration is sufficient to describe dissociation of the DNA containing IPECs in solutions of other simple salts. In particular, for the soluble nonstoichiometric poly(methacrylate)-poly(*N*-ethyl-4vinylpyridinium) complex (ca. 3:1), the concentration range, at which dissociation occurs, strongly depends on the nature of an external salt. In the series LiCl, NaCl, KCl, $(CH_3)_4NCl$, it ranges from ca. 0.3 to 1.5 M, while in the series $(CH_3)_4NBr$, $(CH_3)_4NCl$, $(CH_3)_4NF$ it ranges from ca. 0.6 to more than 5.0 M. The latter figures indicate a role of certain specific interactions.[26,27]

The difference in stability of the IPECs containing native or denatured DNA with respect to the action of the small counterions might be due to a better adjustment of the ion pairs forming the salt bonds between PDADMAC and the single-stranded DNA, which is more flexible as compared with the double helix. The data represented in Figure 10 also reveal that the IPECs containing either native or denatured DNA dissociate completely into initial components in 1 M NaCl. Thus, one can assume that this salt concentration is also sufficient for the complete dissociation of the #IPEC under study.

Indeed, when the initially compact #IPEC sample containing denatured DNA is immersed into 1 M aqueous solution of NaCl

(26) Gulyaeva, Zh. G.; Aldoshina, I. V.; Zansokhova, M. F.; Rogacheva, V. B.; Zezin, A. B.; Kabanov, V. A. *Vysokomol. Soedin.* **1990**, *32A*, 776−783.
(27) Pergushev, D. V.; Izumrudov, V. A.; Zezin, A. B.; Kabanov, V. A. *Polym. Sci.* **1993**, *35*, 940−944.

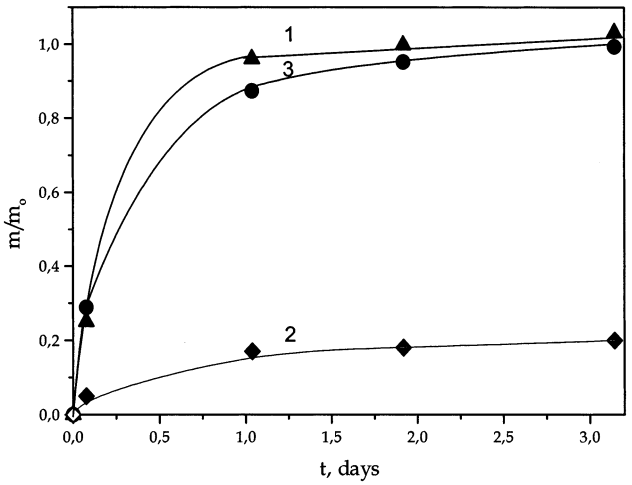

Figure 11. Relative amount of DNA released from the #PDADMAC hydrogel to the surrounding 1 M solution of NaCl vs time; 20 °C; *m* is current amount of DNA in solution, m_0 is the total amount of DNA in the original #PDADMAC hydrogel sample. (1) Original #IPEC prepared at the pH 7 and immersed in a solution at pH 7; (2) original #IPEC prepared at pH 12 and immersed in a solution at pH 7; (3) original #IPEC prepared at pH 12 and immersed in a solution at pH 12.

at pH 12, a transparent highly swollen outer layer is formed, which is separated from the inner dense opaque core by a clearly defined boundary. The boundary gradually moves toward the center of the sample while the total volume of the sample increases. Finally, the #IPEC turns into a uniformly transparent hydrogel, returning to its initial size and shape. This transformation is accompanied by the quantitative release of DNA to the surrounding solution (Figure 11, curve 1). When the solution pH has been brought to 7, the DNA molecules undergo renaturation with almost complete recovery of the double helix conformation. This is indicated by the magnitude of the hyperchromic effect measured at 260 nm after repeated alkalization (33%). The sedimentation coefficient measured for the renatured DNA (6.12 Svedberg units) was close to that measured for the initial DNA before the experiment (6.27 units).

The behavior of the same #IPEC immersed in 1 M aqueous solution of NaCl at pH 7 is somewhat different. Some portion of DNA is released from the #IPEC to the aqueous phase and renatured as in the previous case (Figure 11, curve 2). However, the sedimentation coefficient of this DNA fraction is somewhat lower than the initial one (5.5 Svedberg units). Moreover, about 80% of DNA remains in the hydrogel and is not extracted even with 4 M NaCl. This difference has a natural explanation. The dissociation of the salt bonds between the DNA chains and the #PDADMAC network is accompanied by two competing processes, the diffusion of single-stranded chains from the hydrogel to the solution and the formation of a double-stranded secondary structure upon the reconstruction of the hydrogen bonds between the complementary base pairs. During the second process, loop defects can arise in the intervening space between the coupled DNA sections. Such loops may enclose fragments of the #PDADMAC network and thus lock DNA molecules inside the hydrogel as shown in the scheme at Figure 12. First of all, this kind of locking should involve the DNA fraction of the highest molecular mass. It is likely that exactly this fact rather than rupture of the chains is responsible for the decrease in the sedimentation coefficient of the fraction released from the hydrogel to the solution.

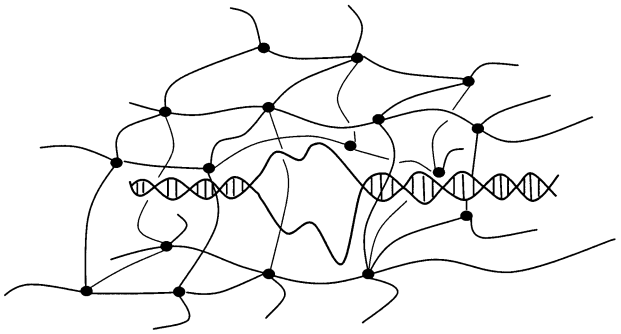

Figure 12. Possible mechanism of fixing DNA in the polyelectrolyte hydrogel.

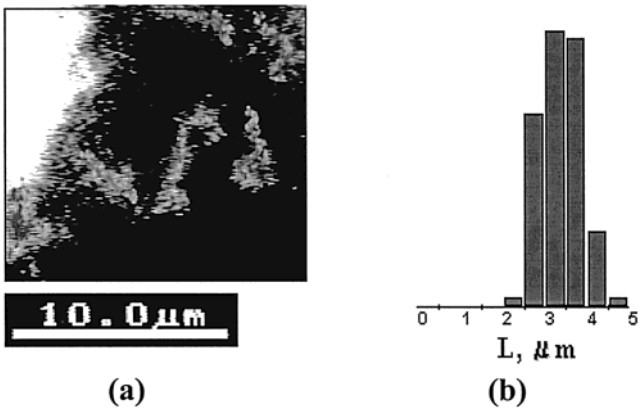

Figure 13. (a) FM image of individual T4DNA molecules releasing from #IPEC(DNA-#PDADMAC) hydrogel sample to the experimental solution: [NaCl] = 1 M, [TrisHCl] = 10 mM, ME - 4 vol %, DAPI - 0.3 μM. (b) Distribution of the long-axis length of T4DNA molecules, *L*, released from the hydrogel.

Native DNA which forms a thin barrier #IPEC layer on the hydrogel surface is completely desorbed in 1 M NaCl aqueous solution at pH 7 (Figure 11, curve 3). The individual T4DNA extended coils releasing from the bright hydrogel surface layer are seen on FM observation (Figure 13a). The brightness of the gel surface layer vanishes gradually and finally disappears completely. Importantly, the distribution of the long-axis length, *L*, for the DNA chains desorbed from the gel surface is virtually the same as that in the initial T4DNA solution (Figure 13b). It means that the reversible sorption of native T4DNA by the #PDADMAC gel does not lead to the distraction of the giant double helixes.

Conclusion

The scope of the above data shows that the linear polycation interacting with DNA induces compaction of either native (double-stranded) or denatured (single-stranded) DNA polyanions in water solution. Such behavior observed for PDADMAC, apparently, can be extended to other cationic polyelectrolytes. At the same time, the slightly cross-linked cationic hydrogel (#PDADMAC) can easily distinguish between native and denatured DNA. Native DNA is adsorbed and captured on the hydrogel surface, while the denatured DNA penetrates to the hydrogel interior until the whole hydrogel sample is transformed into #IPEC. Importantly, both native and denatured DNA can be completely released from the hydrogel in appropriate conditions by adding a low molecular salt.

The data obtained using conventional physicochemical methods with respect to DNA of a moderate molecular mass

remarkably correlate with the pictures directly observed for ultrahigh molecular mass DNA in dynamics by using FM.

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