Responsive Polymer Gels: Double-Stranded versus Single-Stranded DNA

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Cross-linking of polyelectrolytes such as DNA gives gels that are osmotically highly swollen but contract upon addition of electrolytes and, in particular, upon association of oppositely charged cosolutes with the polyelectrolyte chain. The deswelling behavior of cross-linked DNA gels thus reflects the DNA−cosolute interactions and provides a basis for the development of responsive DNA formulations. Gels of both single- and double-stranded DNA have interesting applications, and a comparison between them provides the basis for understanding mechanisms. Denaturation of cross-linked ds-DNA gels was induced by heating them above the melting temperature and then cooling. This process, studied by fluorescence using ethidium bromide, appeared to be reversible when a heating/cooling cycle was performed. The swelling behavior upon addition of different cosolutes, such as metal ions, polyamines, charged proteins, and surfactants, was investigated for different DNA gel samples, including long and short ds-DNA and long and short ss-DNA. The DNA molecular weight was found to have only a slight effect on the deswelling curves, whereas conformation exhibited a pronounced effect. In general, single-stranded DNA gels exhibited a larger collapse in the presence of cations than did double-stranded DNA. This difference was more pronounced with surfactants than with the other cosolutes investigated. The difference between double- and single-stranded DNA was attributed to differences in linear charge density, chain flexibility, and hydrophobicity. For surfactants with different chain lengths, the swelling behavior displayed by ss-DNA can be interpreted in terms of an interplay between hydrophobic and electrostatic interactions, the latter being influenced by polymer flexibility. Increasing hydrophobicity of the network leads to a decreased critical aggregation concentration (cac) for the surfactant/gel complex, as a result of the strengthened hydrophobic attractive force between the surfactant and the gel chain. The swelling of DNA gels appears to be reversible and to be independent of DNA conformation. Surfactant-induced deswelling of DNA gels under some conditions appears to be quite homogeneous, whereas under other conditions, there is a separation into a collapsed region in the outer parts of the gel sample and an inside swollen part. Such "skin" formation is quite different for ss- and ds-DNA, with ss-DNA giving more pronounced skin formation over a wider range of binding ratio, β. For example, no macroscopic separation into collapsed and swollen regions was observed at intermediate degrees of binding for ds-DNA gels, whereas a dense surfactant-rich surface phase (skin) was found to coexist with a swollen core network for ss-DNA gels with $\beta > 0.5$. One explanation for this difference is the large deformation energy required for the compression of the very stiff ds-DNA chains.

Introduction

Polymer gels that respond to changes in the surrounding environment with a volume transition, often referred to as responsive gels, have attracted much interest in the past few years.[1−8] In this group of materials are polyelectrolyte gels, which consist of charged polymer networks, counterions, and solvent and are usually synthesized by chemically cross-linking charged or titrating polymers. The environmental conditions to which such polymers respond include changes in different parameters such as pH,[6,9−11] solvent composition,[12] ionic strength,[13] temperature,[14,15] pressure,[16] buffer composition,[17] surfactants,[18−27] and photoelectric stimuli.[28] Because of their significant swelling and syneresis in response to external stimuli, these polymeric networks are used for a variety of applications such as contact lenses,[29] diapers, wound dressings, membrane materials, pharmaceutical products,[30] monolithic drug delivery systems,[31] chromatography packing materials, and agricultural products.[32] Moreover, cross-linked gels have been investigated for many biomedical uses including tissue cultures,[33] enzyme activity controlling systems,[34] and materials with improved biocompatibility,[35] as well as in the design and analysis of artificial muscles and biosensors and in the design of intelligent controlled drug release devices for site-specific drug delivery. The scientific interest in polymer gels is due to the complex interplay of counteracting forces that are of equal order of magnitude and that determine the swelling of the gels.[35] The main contributions are osmotic pressure originating from electrostatically confined small ions inside the gel,[36] effective electrostatic interactions between the charged groups,[37−40] and hydrophobic interactions.

The swelling of polymer gels is restricted by the cross-linking density in the network, and the deswelling is restricted by the volume occupied by the polymer network. The extent of collapse is affected by molecular parameters of the network such as its

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cross-linking density, its hydrophobic/hydrophilic balance, its content of charged groups, its flexibility, and its ability to interact with added solutes.

DNA compaction in living cells is a process of extraordinary biological importance. The compaction of DNA in vivo is achieved by histone proteins.[41] The condensation of DNA in vitro can also be induced by a number of small polyvalent ions[42,43] and by lowering of the dielectric constant.[44] Electrostatic interactions have significant effects on the physical properties of macromolecules such as DNA, and electrostatic ion−ion correlations that go beyond traditional mean-field theory can explain the phenomenon known as DNA condensation.[45] It has been shown that the simplest way to achieve an effectively attractive interaction between equally charged aggregates, through a strong coupling, is by using multivalent counterions. The observed attraction is thought to contribute to the condensation and aggregation of DNA and other highly charged polymers.[46]

Complexes between DNA and cationic surfactants have attracted increased interest lately because of the possibility of using these systems for gene transfection.[47] The nature of both the DNA[48] and the surfactant[49−51] has been found to influence the phase separation limits, as well as the structure of the D

factant complexes. When polyelectrolyte gels interact with oppositely charged surfactants, interesting features can arise.[52−55] Swollen polyelectrolyte networks undergo collapse after absorbing equimolar amounts of surfactant, whereas when insufficient surfactant is available to form complexes involving all polyion chains in the network, a region in the network remains in the swollen state coexisting with the collapsed part. The collapsed region, containing the surfactant aggregates, makes up a surface phase (skin) surrounding the water-swollen network (core)[56] This phenomenon has also been observed in gels absorbing proteins or polyions of opposite charge to the network.[57,58]

In a previous study,[59] we reported on the swelling behavior of covalently cross-linked DNA upon the addition of different cosolutes, including inorganic salts with different cation valencies; polyamines such as spermine and spermidine; cationic macromolecules such as chitosan, lysozyme, poly(L-lysine), and poly(L-arginine); and surfactants. We found that DNA gels are very interesting as "responsive" systems, because dramatic volume changes can be induced by (often very small) changes in the composition of the swelling medium. The swelling of the gels appears to be reversible, as exemplified by the deswelling/swelling process induced by subsequent addition of cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) or chitosan and NaCl. The contraction of DNA gels in certain cases displays a two-step behavior not observed for other polyelectrolyte gels; this is attributed to an initial compaction of DNA.

In this work, the DNA denaturation process induced by changes in temperature is characterized. The effects of DNA molecular weight and conformation on the swelling behavior were investigated using the same cosolutes as in the previous study. For this purpose, the following well-defined DNA samples were used to prepare cross-linked DNA gels: long double-stranded DNA (ds-DNA; 2000 base pairs), long single-stranded DNA (ss-DNA; heat-denatured 2000 bases) short ds-DNA (146 base pairs), and short ss-DNA (580 and 831 base-pair fragments). The reversibility of the swelling process was checked by the addition of SDS to a DNA gel collapsed by CTAB. Furthermore, the formation of a surfactant surface phase (skin) surrounding the water-swollen network as a function of the degree of binding is discussed. The DNA network/surfactant complex might offer a possibility for many uses in medicine as drug delivery systems and drug control during administration. Also, future development in the use of covalent DNA gels in separation seems to be a great challenge.

Materials and Methods

Materials. Deoxyribonucleic acid sodium (DNA) from salmon testes (Sigma) was used as supplied. Its molecular weight was around 2000 base pairs (bp). Salmon DNA was thermally denatured to produce ss-DNA by heating the sample at 90 °C for 10 min and then cooling it rapidly by injecting it into a beaker that was cooled by immersion into a mixture of cold ice and ethanol. The conformation of DNA in aqueous solution, that is, whether the chains were single- or double-stranded, was verified by differential scanning calorimetry (DSC) measurements. Short double-stranded DNA (146 base pairs) and short single-stranded DNA from salmon testes was purchased from Sigma. This ss-DNA was precipitated in ethanol and sonicated to produce single-stranded fragments that comigrated with the 580 and 831 base-pair-marked fragments. *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED), sodium hydroxide (NaOH), and sodium bromide (NaBr), all from Sigma, and ethylene glycol diglycidyl ether (EGDE), from Aldrich, were used as supplied. Sodium, lithium, potassium, rubidium, cesium, calcium, magnesium, and strontium chlorides (NaCl, LiCl, KCl, RbCl, CsCl, $CaCl_2$, $MgCl_2$, and $SrCl_2$, respectively) were purchased from Sigma. Cationic alkyltrimethylammonium bromide surfactants (C_nTAB) and sodium dodecyl sulfate (SDS) were obtained from Serva and used without further purification. Spermine tetrahydrochloride, spermidine trihydrochloride, poly(L-lysine) (PL, M_w = 5 kDa), and poly(L-arginine) (PA, M_w = 5 kDa), all from Sigma, were utilized without further purification. Stock solutions of spermine and spermidine in deionized water were prepared from chloride salts of the polyamines and were adjusted to pH 6.5 ± 0.2 with HCl or NaOH. Stock solutions of poly(L-lysine) (PL) and poly(L-arginine) (PA) were prepared using 10 mM phosphate buffer, pH 7.4. Lysozyme (95%) from chicken egg white, recrystallized three times, dialyzed, and lyophilized, and chitosan from crab shells obtained by alkaline deacetylation from chitin were obtained from Sigma (85% deacetylation, M_w = 22 kDa). As lysozyme is very stable and largely self-buffering, no buffer solution was used. Without any buffer, the pH of the solution was 6.5, and because the isoelectric point of lysozyme is pH 11, the protein had a positive net charge of 8+ at this pH. A chitosan stock solution was prepared by dissolving it in sterile 25 mM acetate buffer, pH 6.0, and then diluted to the final desired concentration. In the cases of polyamines and polycations, gel swelling measurements were carried out at pH 6.5, and concentrations are presented per charge. Millipore filtered water was used in all experiments.

Preparation of Gels. Double- and single-stranded DNA was dissolved in water containing 3.7 mM NaBr to a DNA concentration of 9 wt %. The DNA was chemically cross-linked using ethylene glycol diglycidyl ether (EGDE) at pH 9. After the addition of 1 M NaOH and TEMED, the sample was mixed and then transferred to test tubes and incubated for 2 h in a water bath at 50 °C. Freshly synthesized gels were neutralized and rinsed with large amounts of 1 mM NaOH solution. The DNA gels swelled considerably in the NaOH solution, and as a result, the DNA concentration in the gels was lowered. The concentration of DNA in the gels equilibrated with 1 mM NaOH (reference state) was obtained by weighing the gels before and after freeze-drying. A decrease in the DNA concentration from 9 wt % at preparation time to 1 wt % after immersion of the

gels in the NaOH solution was observed. Thus, the reference state of the experiments was selected as the equilibrium swelling state in 1 mM NaOH solution. CTAB/gel samples were prepared by placing cylindrical gel pieces of known mass (1−3 g) into 0.1 mM or 0.5 mM solutions of CTAB containing 1 mM NaOH. Different charge ratios (CRs) (0.2, 0.4, 0.8, 1, and 1.2) of surfactant to DNA in the system were obtained by using the appropriate volume of solution. All samples were equilibrated in sealed containers for 4 weeks at 25 °C and shaken slowly several times during that period of time. After 1 month, the concentration in equilibrium with the gels was determined using a surfactant-sensitive electrode, as described in detail elsewhere.[60] The binding ratio β in the gels was obtained by subtracting the number of moles of surfactant in the solution from the initial amount added to the solution and then dividing by the number of moles of fixed charges in the gel.

Static Fluorescence. For luminescence spectral measurements, a Spex Fluorolog 111 instrument was used in the 90° configuration. Emission spectra for the system of ethidium bromide and DNA were obtained with the monochromator set at appropriate wavelengths.

Swelling Experiments. In the swelling experiments, four to six gel pieces were immersed in vials containing 8 mL of aqueous solutions of additive. Because of the much larger volume of the swelling medium (a factor of more than 100) compared to the volume of the gels, the fraction of surfactant and other additives bound to the gels was negligible. The equilibrium concentration of additive in the swelling medium was, therefore, equal to the initial concentration. All samples were equilibrated in sealed containers for 1 week at 25 °C and shaken slowly during that time to reach the equilibrium degree of swelling. The swelling ratios are given as $V/V_0 = (D/D_0)^3$, where V is the volume and D is the diameter of the gel and where the subscript 0 denotes the corresponding value at preparation time ($D_0 \approx 1.4$ mm). The diameters of the gels were measured with a video camera calibrated with a 0.1-mm scale using an image computer program.[61] Each data point represents the average of ca. five measurements of D, with a variation of ca. 5%.

Results and Discussion

Reversibility ds-DNA/ss-DNA in the Gels. Before starting the investigation of the swelling behavior of DNA gels upon addition of different additives using different DNA gel samples, we were interested in studying the reversibility of the transition from double- to single-stranded DNA conformation in the covalent gels. For this purpose, we chose one of the four DNA gel samples used in this work: long ds-DNA (2000 base pairs, see Experimental Section).

Under physiological conditions, the B-DNA structure is most stable. Changes in solution parameters, such as temperature, pH, and electrolyte concentration, can lead to the loss of its secondary structure, and the DNA molecules can undergo melting or denaturation. This mechanism, where a double-stranded DNA (ds-DNA) falls apart and forms two single strands (ss-DNA), has been followed by techniques such as UV/vis spectroscopy and differential scanning calorimetry (DSC).[62,63]

Information about the conformation of the DNA molecules in the gels was obtained in this work by fluorescence measurements using ethidium bromide as a fluorescent probe. Ethidium bromide is a dye that binds to double-stranded DNA by intercalation between the base pairs and is characterized by a high quantum yield of fluorescence.[64] It is known from previous work[55] that, initially, the DNA molecules in the network are in their double-stranded conformation. In this study, we changed the conformation of the DNA molecules in the ds-DNA gels (2000 bp) by increasing the temperature from 10 to 95 °C and thus determined the melting temperature. Figure 1 shows the fluorescence spectra of ethidium bromide in the presence of a DNA gel after the sample had been heated to 80 and 95 °C for a DNA concentration of 1 wt %. As a reference and for the same concentration, the spectrum is represented in the absence of the DNA gel. In part C of this figure are shown the spectra of ethidium bromide in aqueous solution and in aqueous solutions of double-stranded DNA and single-stranded DNA at the same concentration as in the gel (1 wt %). When EtBr is

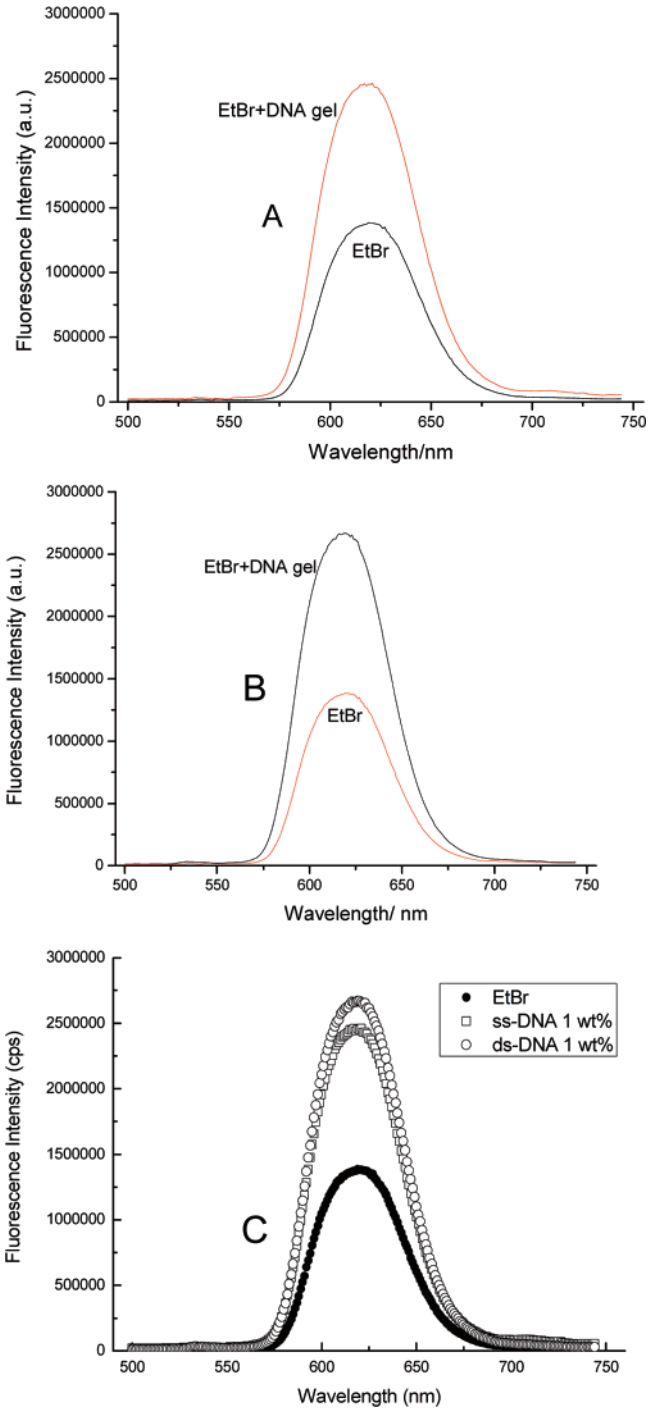

Figure 1. Fluorescence spectra of ethidium bromide in the absence and presence of a long ds-DNA gel (1% cross-linker density) after the sample had been heated to (A) 95 and (B) 80 °C and (C) in the presence of reference solutions of 1% ds-DNA and 1% ss-DNA at 25 °C.

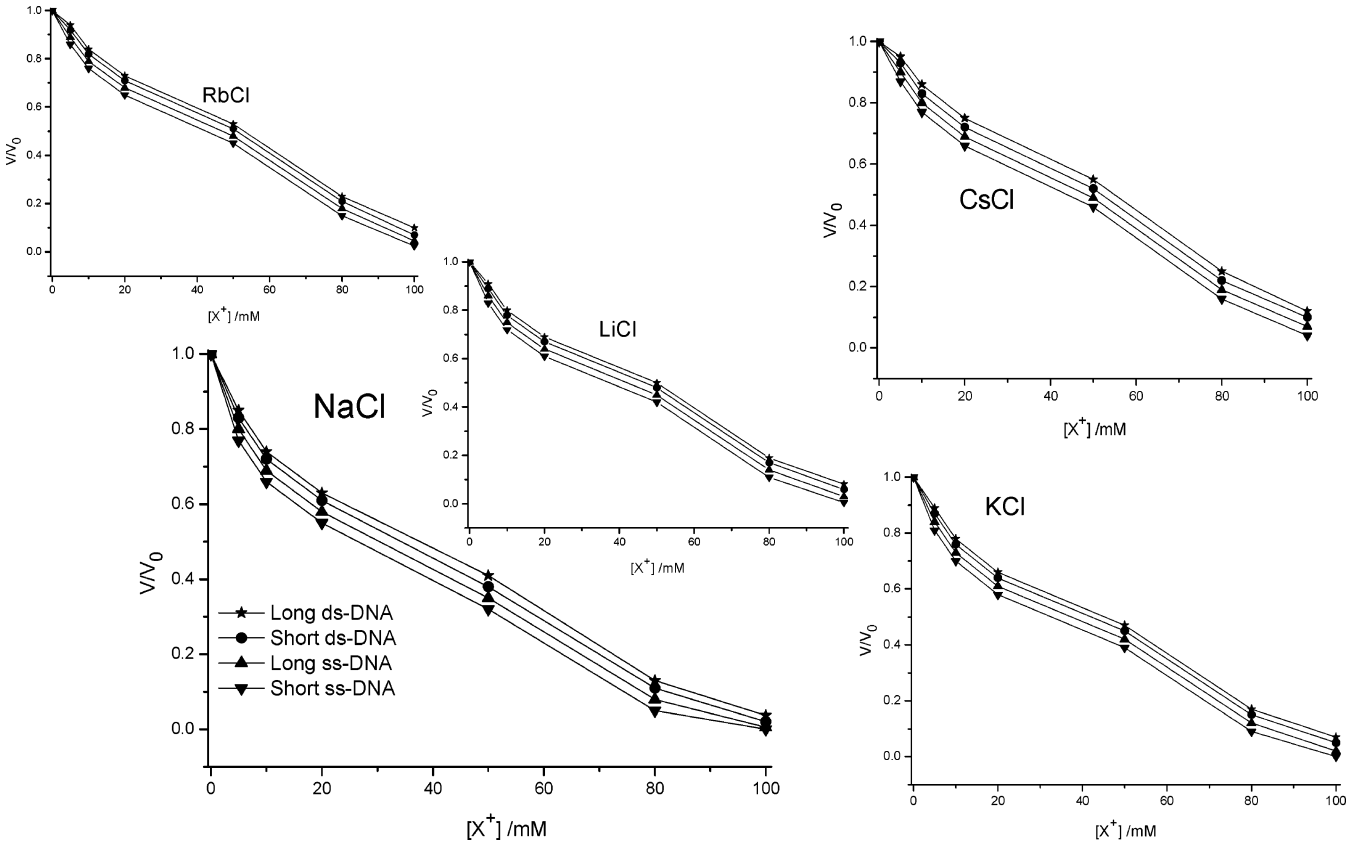

Figure 2. Swelling isotherms (V/V_0) for long ds-DNA, short ds-DNA, long ss-DNA, and short ss-DNA gels (1% cross-linker density) immersed in solutions of the monovalent ions NaCl, KCl, LiCl, RbCl, and CsCl. Temperature 25 °C, pH 9.

bound to nucleic acids, a marked enhancement in its fluorescence is observed; however, this increase depends on the configuration of the DNA. As ethidium bromide in the presence of the heated DNA gels presents the same fluorescence intensity as in the solution of single-stranded DNA, we can assume that ethidium bromide binds to the heated gel in the same way as it binds to ss-DNA. We conclude that, as the temperature is increased, the DNA molecules change their conformation from a double- to a single-stranded conformation. For the determination of the melting temperature (T_m), several temperatures from 60 to 95 °C were tested using the fluorescence technique mentioned above. It was observed that the transition occurred at 85 °C. The presence of salt during gel preparation and a higher concentration of DNA in the network (see Experimental Section) are two factors that could increase the temperature at which melting occurs and lead to more cooperative binding between strands. In a study on solution behavior by DSC,[65] it was shown that, for higher DNA concentrations (6 mM), the presence of DNA counterions is sufficient to stabilize the double-stranded conformation at room temperature, indicating a self-screening effect. The amount of free counterions in solution, and thus the ionic strength, increases when the DNA concentration is increased. Also, by increasing the salt concentration, one can stabilize the ds-DNA molecules at higher temperatures and increase the cooperativity of the transition.

We report here also on the effect of an increase and then a decrease of the temperature of DNA gels, illustrating the reversibility of the denaturation process. This process of reversibility was studied by heating the gels to 95 °C and then decreasing the temperature to 10 °C. In this case, the ethidium bromide spectra show the same fluorescence intensity as in the solution of double-stranded DNA (data not shown). Thus, the denaturation of DNA gels appears to be reversible. It is possible, however, that not all of the DNA molecules return to their double-helix form.

Effects of Mono- and Divalent Metal Ions, Polyamines, and Polycations on Gel Deswelling. In the absence of salt, polyelectrolyte gels in water are generally highly swollen. Because of the condition of macroscopic electroneutrality, the counterions of the charged network have to be confined inside the gel. The large swelling capacity of gels is attributed to the increase of the counterion entropy. In the presence of salt, the osmotic pressure in the solution is higher than that inside the gel. As shown in Figure 2, this leads to a decrease of the gel volume. Figure 2 shows the volumes of the long and short ds-DNA and the long and short ss-DNA gels in the presence of the monovalent salts NaCl, KCl, LiCl, RbCl, and CsCl (V) relative to the volume in 1 mM NaOH (V_0), as a function of metal ion concentration. For all DNA gel samples, the nature of the monovalent counterion had only a moderate effect on the deswelling of the DNA gels. The degree of collapse followed the order $Na^+ > K^+ > Li^+ > Rb^+ > Cs^+$, with sodium ion showing the highest ability to collapse the DNA gels. Except for lithium, this dependence follows the Hofmeister series (lyotropic series), i.e., the observed deswelling effect increases with decreasing ionic radius Li < Na < K < Rb < Cs. In fact, the order observed suggests that monovalent cations with smaller atomic numbers are more efficient in the collapse of DNA gels. This order seems not to change as a function of DNA molecular weight and conformation.

The interaction between cross-linked long and short ds-DNA/ss-DNA gels and divalent metal ions is shown in Figure 3. The deswelling upon addition of the divalent salts $CaCl_2$, $MgCl_2$, and $SrCl_2$ occurs at considerably lower salt concentrations and appears to be more pronounced than that occurring with the monovalent metal ions mentioned above. This can be explained

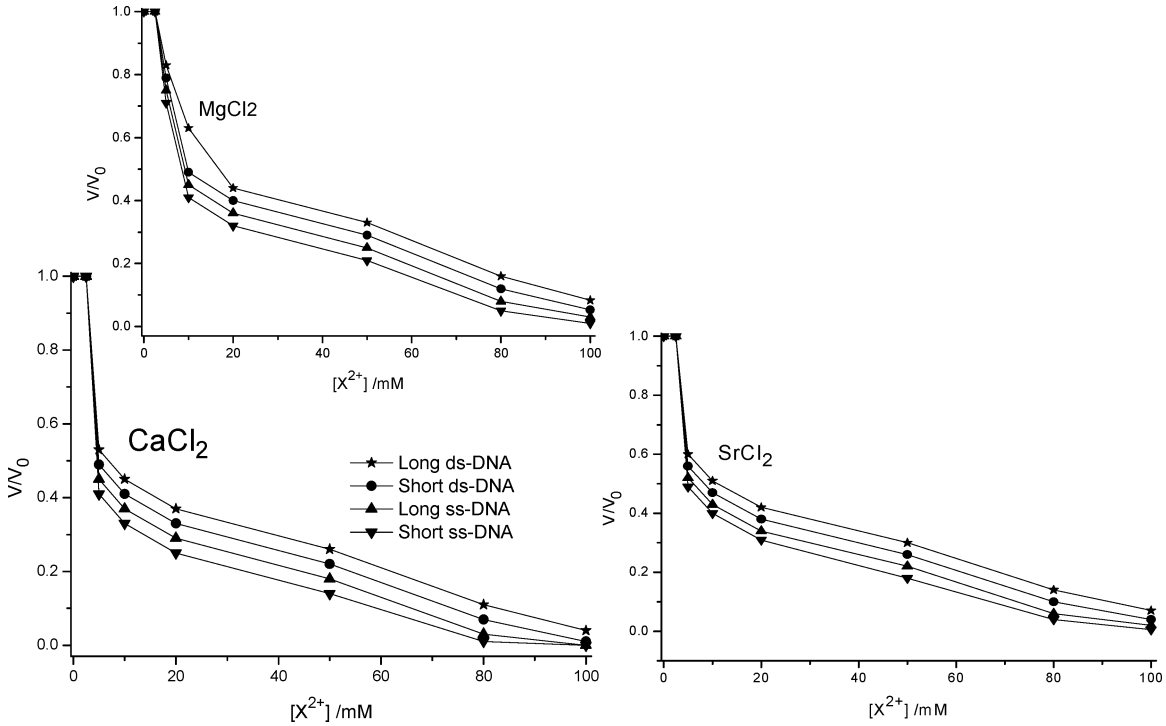

Figure 3. Swelling isotherms (V/V_0) for long ds-DNA, short ds-DNA, long ss-DNA, and short ss-DNA gels (1% cross-linker density) immersed in solutions of the divalent ions $CaCl_2$, $MgCl_2$, and $SrCl_2$. Temperature 25 °C, pH 9.

by the smaller osmotic contribution from the counterions owing to the smaller number of them and the stronger electrostatic attraction to the network in the system containing divalent counterions. As was observed in a previous study,[59] the shrinking process of DNA gels in the presence of divalent metal ions seems to follow a different behavior; there is a marked deswelling at low metal ion concentrations, followed by a region where deswelling occurs more progressively with concentration. This two-step process is suggested to involve, in the first step, a compaction of individual DNA chains induced by the presence of divalent ions and, in the second step, a general osmotic deswelling. This two-step process seems to be specific for DNA because there is no evidence in the literature for this kind of behavior involving other polyelectrolytes or polymers. This effect is independent of the DNA molecular weight and conformation.

Figure 4 shows the swelling isotherms for the four DNA gel samples in the presence of chitosan, spermine (Spm), spermidine (Spd), lysozyme, poly(L-lysine) (PL), and poly(L-arginine) (PA). In all cases, chitosan has the highest potential to induce the collapse of the DNA gels under the range of concentrations studied. Using other cations, higher concentrations of the cosolute are needed. This is the case for the polyamines, which are needed in higher amounts to induce the collapse of the gels. Moreover, the cationic protein lysozyme has the capability to collapse DNA gels but to a smaller extent than the above-mentioned agents. Polypeptides, such as poly(L-arginine) and poly(L-lysine), lead to a gel deswelling, but at higher concentrations than the other cationic polyamines studied.

For a deeper understanding of the mechanism of binding of polycations to DNA, further experiments based on modeling will be reported soon.

When studying the effect of mono- and divalent metal ions, polyamines, and polycations on the swelling behavior of double- and single-stranded DNA gels, we found that the extent of swelling depends on both the molecular weight and conformation of the DNA. The DNA molecular weight had only a slight effect on the deswelling curves; short DNA gels showed a slightly larger deswelling. The DNA conformation showed a more pronounced effect on the degree of swelling. For all systems mentioned above, single-stranded DNA gels collapsed more efficiently than double-stranded did.

Thus, the general trend seems to be that a low molecular weight and a single-stranded conformation lead to a larger gel deswelling. To explain this observation, the differences between double- and single-stranded DNA molecules were considered. These are that ss-DNA has greater flexibility (smaller persistence length), whereas ds-DNA is quite rigid and characterized by a large persistence length (~500 Å[66]), and ss-DNA has a lower linear charge density and a larger hydrophobicity than ds-DNA. It can be argued that the increased chain flexibility and decreased linear charge density are the main factors that cause the ss-DNA gels to collapse more than gels with the double helix. In general, higher flexibility implies a larger loss of configurational entropy upon deswelling because of the loss of translational degrees of freedom. The loss in entropy during the deswelling is smaller for networks of stiff chains than for flexible chains. Regarding the elasticity of the network, it has been demonstrated that, for stiff chains, an energetic penalty restricts bending and the preferred conformation of stiff polyelectolyte chains is extended even in the unswollen state of the gel.[67] Also, the role of the flexibility of the polyelectrolyte in an association process has been investigated in some detail, and it was found that, in general, a flexible chain interacts more strongly with an oppositely charged macroion than a rigid chain.[68]

The swelling ability is also affected by the network charges and the counterions. Together with the charge density, the density of counterions in the gel increases upon deswelling. The counterions are not homogeneously distributed around the polyelectrolyte chains. The concentration close to the polymer chain is higher than that far from the chain. According to Manning theory, the ion distribution is better described by a two-phase model. In this type of model, one fraction of the counterions is assumed to be condensed on the chain[69] or

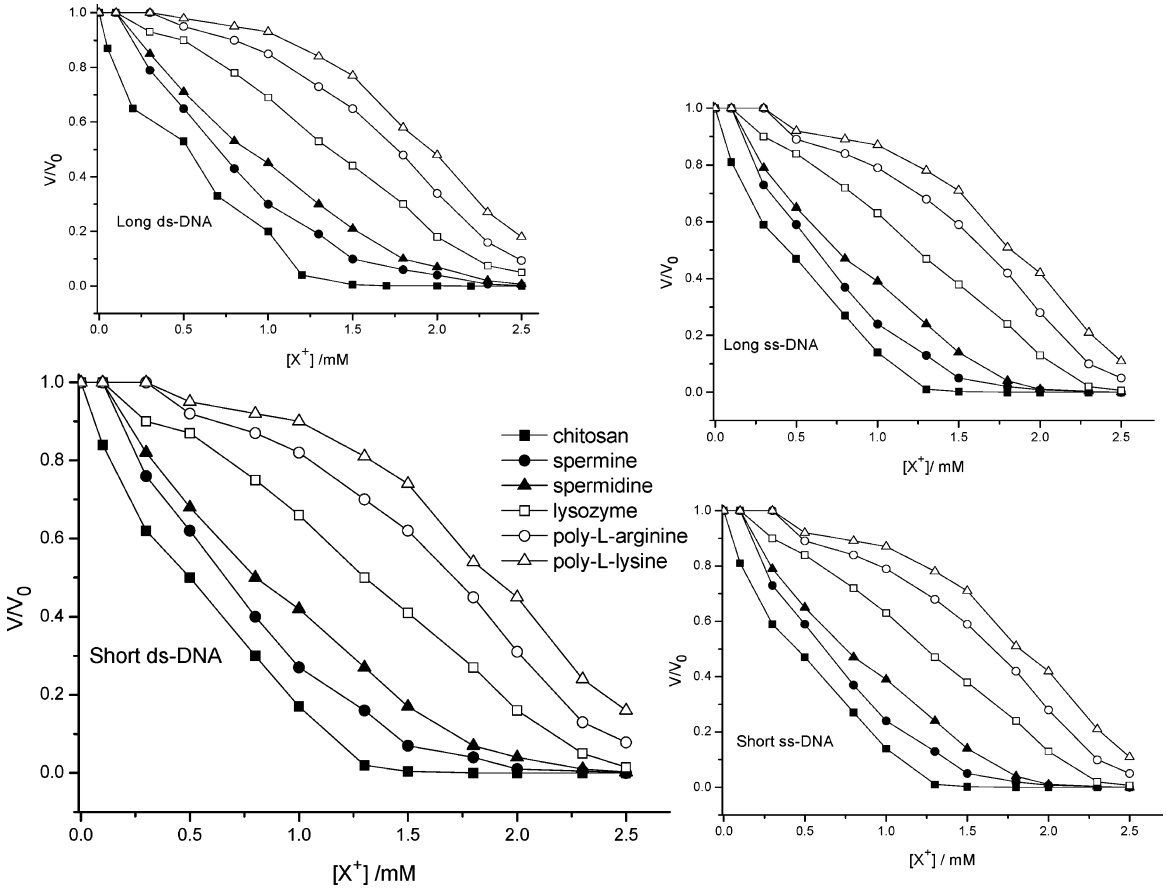

Figure 4. Swelling isotherms (V/V_0) for long ds-DNA, short ds-DNA, long ss-DNA, and short ss-DNA gels (1% cross-linker density) immersed in solutions of chitosan, spermine, spermidine, lysozyme, poly(L-lysine), and poly(L-arginine). Temperature 25 °C, pH 6.5.

confined to a small volume around the chains,[70] which leads to a reduction of the effective charge density on the chain. Thus, at high charge densities, some of the counterions are condensed on the chains and contribute less to the osmotic pressure.

In terms of hydrophobicity, there are also differences between the two conformational states of DNA. DNA is, because of its bases, an amphiphilic polyelectrolyte. The hydrophobic interaction between the bases drives the association of the two DNA chains into the double helix. Clearly, the amphiphilic character is very different for the DNA states: In ds-DNA, the hydrophobic groups are hidden, whereas in ss-DNA, they are exposed to the solution. Thus, the single-stranded arrangement shows a larger hydrophobicity than the double-stranded configuration does, and in this case, hydrophobic interactions would be much more significant. The results show that, as the hydrophobicity of the network increases, the swelling degree decreases. The uptake of water from single-stranded DNA gels is low when compared to that of the corresponding double-stranded gels.

The hydrophobic effect plays a determining role in the deswelling process of DNA gels, especially in the presence of surfactants. Below, we discuss in some detail the influence of hydrophobic interactions on the existence and extent of the transition from a swollen to a collapsed gel state induced by surfactants.

The swelling of DNA gels also increases with the size of the DNA molecule for both single- and double-stranded DNA. This effect of molecular weight, however, is less relevant because the differences between gels based on long and short DNA chains are very small.

There is good qualitative agreement between our experimental results and the theoretical studies performed by Schneider and Linse.[71] These authors investigated model systems of cross-linked polyelectrolyte gels by means of Monte Carlo simulations. They characterized the swelling properties of polyelectrolyte gels at different charge densities, cross-linking densities, chain flexibilities, and counterion valencies. An increased linear charge density, a decreased cross-linking density, an increased chain stiffness, and a decreased counterion valence led to an increase in the osmotic pressure and, therefore, to an increased swelling of the gels. Moreover, studies from other groups have shown that an increased swelling with increased charge density occurs for charged gels prepared by copolymerization of nonionic and ionic monomers.[72] Analogous observations have been made for *i*-poly(acrylamide) (PAA_m) gels with increasing content of sodium acrylate (NaA) groups[72] or sodium 2-acrylamido-2-methyl propanesulfonate (NaAMPS) groups[73] and for phosphorylated poly(vinyl alcohol) (PVA) gels, which also swell with increasing charge density.[74] The effect of counterion valency has been extensively studied,[75,76] the main observation being that divalent counterions reduce the gel volume much more efficiently than monovalent ions. This behavior has, for example, been shown for *N*,*N*-dimethylacrylamide/2-acrylamide-2-methylpropyl sulfonate copolymer gels immersed in salt solutions containing Na^+ and Ca^{2+} as the counterions.[75]

Prior to our study, the influence of chain stiffness on the swelling behavior does not seem to have been investigated experimentally.

Effects of Cationic Surfactants on Gel Deswelling. Figures 5 and 6 show swelling isotherms for DNA gels upon addition of the cationic surfactants C_{16}TAB, C_{14}TAB, C_{12}TAB, and C_8TAB. As can be seen, the surfactants have no effect at lower concentrations, but there is a marked deswelling at higher

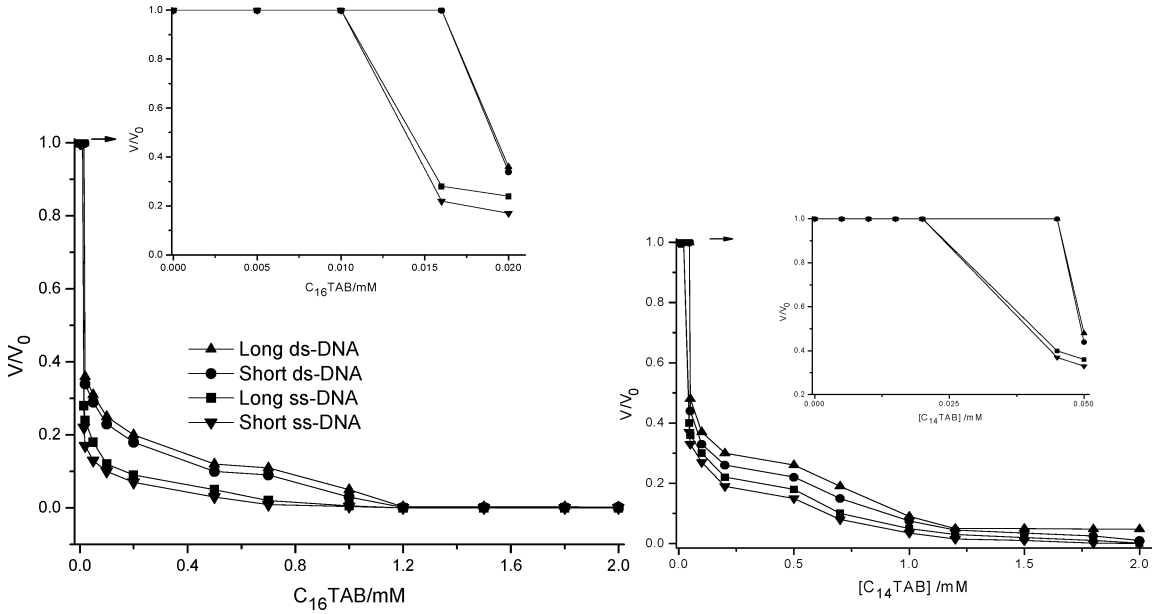

Figure 5. Swelling isotherms (V/V_0) for long ds-DNA, short ds-DNA, long ss-DNA, and short ss-DNA gels (1% cross-linker density) immersed in solutions of C_{16}TAB and C_{14}TAB. The C_{16}TAB concentration range from 0 to 0.2 mM and the C_{14}TAB concentration range from 0 to 0.05 mM are also represented in detail (see insets). Temperature 25 °C, pH 9.

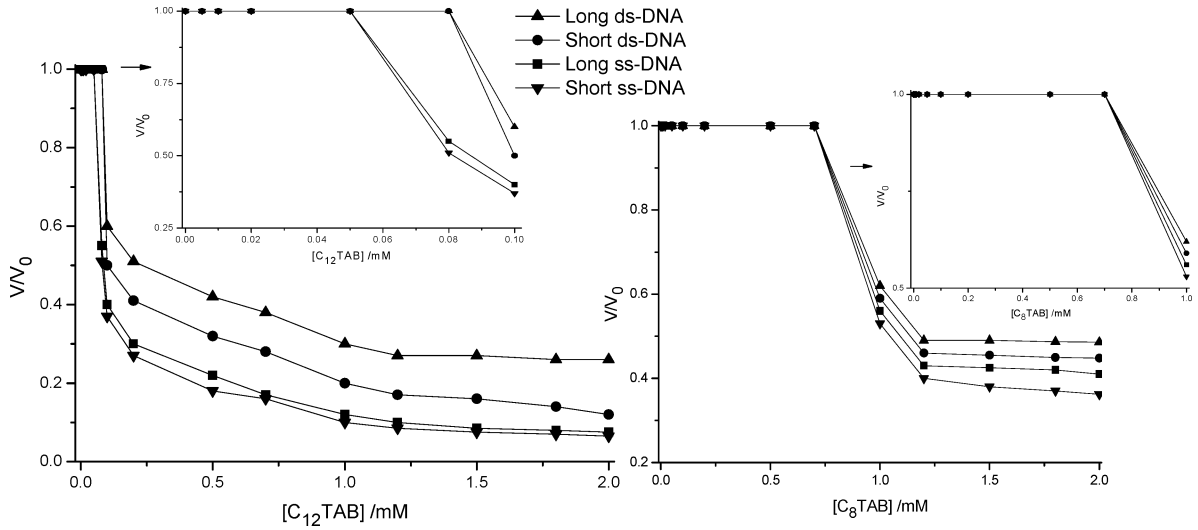

Figure 6. Swelling isotherms (V/V_0) for long ds-DNA, short ds-DNA, long ss-DNA, and short ss-DNA gels (1% cross-linker density) immersed in solutions of C_{12}TAB and C_8TAB. The C_{12}TAB concentration range from 0 to 0.1 mM and the C_8TAB concentration range from 0 to 1 mM are also represented in detail (see insets). Temperature 25 °C, pH 9.

concentrations that becomes more evident the longer the surfactant alkyl chain. We note that the concentration of onset of deswelling varies by orders of magnitude between different surfactants. The pronounced chain length dependences directly suggest a dominant role of surfactant self-assembly.

In the bulk phase and at very low concentrations, the surfactant molecules are present as unimers, whereas at higher surfactant concentrations, self-assembly into aggregates occurs. For the single-chain surfactants, the aggregates formed in this self-assembly are commonly spherical micelles, with micelle formation starting at a well-defined concentration, the critical micelle concentration (cmc). The cmc depends on the chemical structure of the surfactant, surfactants with longer hydrophobic tails having lower cmc values, with the decrease being by a factor of 2 for each additional methylene group in the alkyl chain. In the presence of an oppositely charged polyelectrolyte, the micelle formation of an ionic surfactant is strongly facilitated, leading to a marked lowering of the cmc; the cmc in the presence of a polymer is often referred to as the critical association concentration, cac. The stabilization of micelles by an oppositely charged polyelectrolyte is mainly an entropic effect, because of a release of counterions. Therefore, the critical aggregation concentration for the surfactant in the presence of DNA, cac, is lower than the critical micellization concentration, cmc. The fact that the cationic surfactant binding occurs preferentially to anionic polyelectrolytes of high charge density further illustrates this behavior.[77,78]

The DNA gels are highly swollen because of the osmotic pressure arising from the counterions that are confined to the gel. After the immersion of the swollen DNA gels in the solutions of the oppositely charged surfactants, the surfactant ions migrate into the network and replace the network counterions, which are released. In comparison to the regular micellization involving counterion binding and confinement, this process is, as for bulk solutions of polyelectrolytes and surfactants, extremely favorable from the point of view of translational entropy of the counterions, because of the release of condensed counterions of both polymer network and surfac-

tant micelles. Adsorption of a considerable amount of C_nTA^+ ions leads to a transition of the swollen network to the collapsed state. The main reason for this transition is thus the aggregation of surfactant ions within the DNA gel resulting from the hydrophobic interactions between their hydrocarbon chains. As a consequence, the mobile counterion concentration in the network decreases, leading to a significant decrease in the internal osmotic pressure in the gel. Furthermore, the surfactant aggregates act as multivalent counterions and, by ion correlation effects, contribute to the contraction of the gel.[79,80]

In particular, these results for the different alkyl chain lengths confirm that the deswelling occurs well below the normal critical micelle concentration of the surfactant. We found that the surfactants induce a volume transition starting at a certain rather well-defined concentration (critical aggregation concentration): For both the long and short ds-DNA gels, cac $\approx$ 0.02 mM for C_{16}TAB,[55] cac $\approx$ 0.05 mM for C_{14}TAB, cac $\approx$ 0.1 mM for C_{12}TAB, and cac $\approx$ 1 mM for C_8TAB, and for both the long and short ss-DNA gels, cac $\approx$ 0.015 mM for C_{16}TAB, cac $\approx$ 0.045 mM for C_{14}TAB, cac $\approx$ 0.08 mM for C_{12}TAB, and cac $\approx$ 1 mM for C_8TAB. Similarly to the cosolutes mentioned above, the swelling behavior of DNA gels varies with the length and conformation of DNA. Whereas DNA size does not significantly influence the collapse of the gels, with only a small difference between long and short DNA samples regarding the extent of collapse, DNA conformation does. Single-stranded DNA gels show considerably more pronounced deswelling and a larger collapse than double-stranded gels. We note that the difference between ss- and ds-DNA is more significant with surfactants than with the other cosolutes investigated. This suggests the importance of hydrophobic interactions; see below. Except for C_8TAB, the decreased cac values displayed by C_nTAB/ss-DNA gels systems also demonstrate that, with this DNA conformation, the association starts at lower surfactant concentrations.

As was previously discussed,[79] a longer alkyl chain (a lower cmc) gives earlier deswelling and a more pronounced collapse, as in the case of C_{16}TAB, than a shorter chain such as the C_8TAB/gel system, which has the highest cac and the lowest percentage of gel collapse. The initial plateau, indicating no volume transition of the gels, in the concentration range under study is very narrow for C_{16}TAB but increases for C_{14}TAB and C_{12}TAB, whereas C_8TAB shows a very extended plateau. The interaction disappears when the hydrophobic tail is too short. The differences in the swelling ratio, V/V_0, and also aggregate size should at least partly be due to differences in the concentration of the free surfactant, which acts as a screening electrolyte. However, the degree of binding is also higher for a surfactant with a longer hydrophobic tail. This contributes to a larger collapse of the gels.

The interaction between polyelectrolytes and oppositely charged surfactants in solution has been extensively studied, because of its importance both in fundamental polymer physics/biophysics and in industrial applications. These interactions are quite strong and can induce the formation of complexes with highly ordered structures.[24,81] Both electrostatic interactions between charged species and hydrophobic interactions between the polymer backbone and the alkyl tail of the surfactant are important issues. Similarly to the solution situation, the polyelectrolyte gel/surfactant interactions are primarily governed by three effects: the translational entropy of counterions and the electrostatic and hydrophobic polyelectrolyte/surfactant interactions. Electrostatic interactions have a great influence on the existence and extent of the transition from a swollen to a collapsed gel state. In addition, hydrophobic interactions also affect the extent of the collapse.

It is known from previous work on polyelecrolyte/surfactant systems, both experimental and theoretical, that the linear charge density of the polyelectrolyte, its flexibility, and any amphiphilic character play a significant role.[82,83] The linear charge density of ds-DNA is considerably higher than that of ss-DNA, and from a simple electrostatic mechanism, ds-DNA should interact more strongly with oppositely charged polyelectrolytes or surfactant micelles. Single-stranded DNA shows a higher flexibility than ds-DNA, which is quite stiff and has a large persistence length. As mentioned above, it was found that a flexible chain interacts more strongly with an oppositely charged macroion than a rigid chain.[68] When a polyelectrolyte contains some hydrophobic groups, the association of oppositely charged surfactants is much strengthened because of the combination of electrostatic and hydrophobic attractions. As mentioned above, the hydrophobic character varies with DNA conformation: In ds-DNA, the hydrophobic groups are largely hidden, whereas in ss-DNA, they are exposed to the solution. Thus, the hydrophobic interactions should be more significant for the latter DNA conformation.

As demonstrated in this work, all of these factors influence the extent of gel collapse. The low chain stiffness and charge density and the higher hydrophobicity of ss-DNA lead to a decreased swelling of the gels in the presence of added cosolutes such as metal ions, polyamines, and polycations. A cationic surfactant should associate with DNA mainly by electrostatic interactions, and both linear charge density and DNA flexibility have significant influences. Whereas ds-DNA has a higher charge density, ss-DNA is more flexible and hydrophobic. The cac decreases with increasing hydrophobicity of the network as seen here, except for the surfactant with the shortest alkyl chain. The cac for C_8TAB has the same value for both double- and single-stranded DNA gels. Because of the short hydrophobic tail of C_8, the effect of increasing the hydrophobic attractive force with ss-DNA becomes less relevant.

Other authors have shown that, when the hydrophobicity of a polymer gel increases, the cac decreases.[84] Recently, it was found that there is a minimum hydrophobicity threshold that must be overcome in order for an association to occur.[85] As an example, the anionic surfactant SDS does not bind to poly(acrylamide) gels. However, when poly(ethylene oxide) (PEO) groups are grafted onto the network, the gels become slightly hydrophobic and therefore start to interact with SDS.[86] Similarly, ethyl hydroxyethylcellulose (EHEC) gels interact with cationic surfactants, whereas more hydrophilic hydroxyethylcellulose (HEC) gels do not.[7]

In a previous study,[59] we reported on the effect of the addition of an anionic surfactant to collapsed long ds-DNA gels, demonstrating the reversibility of the swelling process. This reversibility of the swelling of ds-DNA gels was studied by the addition of different concentrations of a cationic surfactant, C_{16}TAB, followed by the addition of different concentrations of an anionic surfactant, SDS. We found that the swelling of DNA gels is essentially fully reversible. Here, we were interested in studying the influence of DNA molecular weight and conformation on the reversibility of swelling. The same kinds of experiments were performed for both long and short ss-DNA gels. Because the molecular weight has no large effect on the swelling ability, we present the data only for short ss-DNA gels. Figure 7 shows the behavior when collapsed ss-DNA gels were immersed in SDS solutions. The most interesting fact is that the relative V/V_0 value returned to between 90% and 100% of

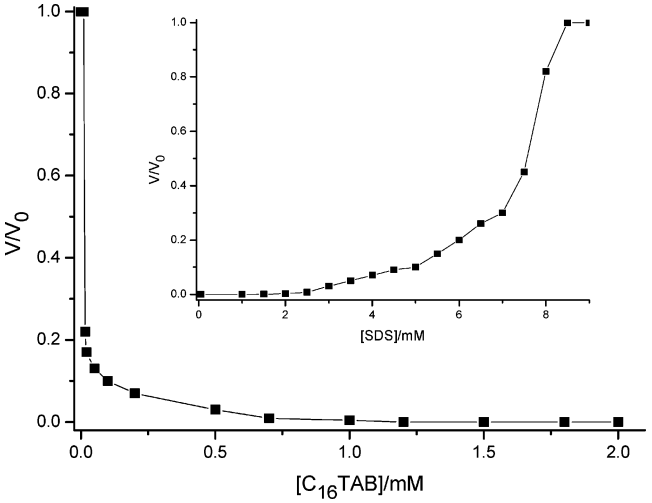

Figure 7. Swelling isotherm (V/V_0) for short ss-DNA gels (1% cross-linker density) precollapsed first in solutions of the cationic surfactant C_{16}TAB and then immersed in solutions of the anionic surfactant SDS. Temperature 25 °C, pH 9.

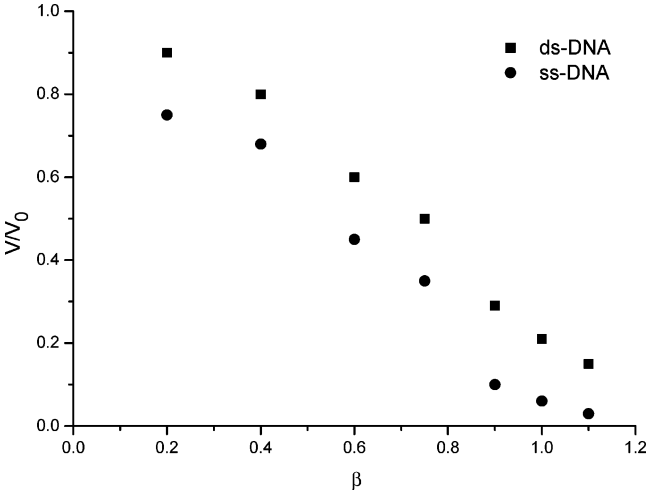

Figure 8. Dependence of relative volume (V/V_0) of ds-DNA and ss-DNA gels (1% cross-linker density) on degree of surfactant binding (β). All gels were equilibrated in 0.50 mM CTAB solutions of different volumes.

the initial state. Thus, the swelling of the DNA gels appears to be reversible and independent of the DNA conformation. We argue that, as in our previous studies of the behavior of DNA in the presence of a mixed cationic/anionic surfactant system,[87] the interaction between the two surfactants is stronger than that between a cationic surfactant and DNA. This dynamic deswelling−swelling process could be useful in the control of the release rate of solutes from gels via "on−off" switching.[88]

Skin Formation on Surfactant Binding. All cross-linked double- and single-stranded DNA gels in their swollen state, in equilibrium with 1 mM NaOH, are clear and transparent and have the same refractive index as water. When both long and short ds-DNA and ss-DNA gels came into contact with a cationic surfactant solution, a cloudy layer formed on the surface of the gel, and water was expelled to the bulk solution. The boundary between the collapsed surface layer and the highly swollen interior of the gel was sharp. However, the final state depended both on the concentration and on the total amount of surfactant available in the solution and the conformation of DNA. The same observations were made earlier by Khandurina et al.[56] working on the reaction of cross-linked polyacrylate gels with alkyltrimethylammonium bromides and by Hansson et al.[54] who also studied the interaction of slightly cross-linked sodium polyacrylate (cl-NaPA) with cetyltrimethylammonium bromide (CTAB). Additionally, we observed here that ds-DNA and ss-DNA gels shrink in an irregular fashion with CTAB when the surfactant concentration is higher than 0.02 and 0.015 mM, respectively. Observations by the naked eye allowed us to verify the differences concerning the formation of a surfactant skin layer as a function of the DNA conformation. A strongly collapsed skin formed for ss-DNA gels at low degrees of surfactant binding ($\beta > 0.5$), whereas for ds-DNA gels, no macroscopic separation of collapsed and swollen regions was observed at low or intermediate degrees of binding, suggesting that CTAB aggregates are evenly distributed in the gels. There is thus a large difference in the conditions of skin formation for different DNA conformations, with ss-DNA giving rise to skin over a much broader range of surfactant binding. Skins from both double- and single-stranded DNA gels are stable and remain unchanged for several months.

In Figure 8, the V/V_0 parameter is represented as a function of the degree of binding, β. For ds-DNA gels, no macroscopic separation of collapsed and swollen regions was observed at intermediate degrees of binding. Only the ds-DNA gel with the largest β value appeared to have a core/shell structure (1 mM CTAB, $\beta = 0.84$, CR = 1.0). As the DNA molecular size does not substantially affect the swelling degree of the gels, it is assumed that short ds-DNA gels present the same kind of behavior. A rather different situation was thus verified for ss-DNA gels upon binding of CTAB. In the case of ss-DNA gels, a surfactant-rich surface phase (skin) was found to surround a swollen core network for gels with $\beta > 0.5$. Also, it was observed that the ss-DNA gel volume was smaller than the volume of the ds-DNA gels. The amount of surfactant was not sufficient to collapse the entire network, and it was intriguing, therefore, that CTAB distributed evenly in the gels. As pointed out elsewhere,[54] the network in a collapsed surface phase is nonuniformly deformed. Thus, in the direction parallel to the gel surface, it is stretched out to the same extent as the swollen core network, whereas in the direction perpendicular to the surface, it is compressed to an extent depending on how collapsed the phase is. One explanation for the absence of a surface phase at low and intermediate β values in ds-DNA gels can thus be that the formation of a strongly collapsed skin is associated with a deformation energy that is too large for the compression of the very stiff DNA chains in the direction perpendicular to the gel surface. The formation of a very concentrated phase might not be critical for the interaction between DNA and CTAB. However, an even distribution of surfactant in the gel is a better alternative for the system as a whole than no surfactant binding. As mentioned above, the intrinsic characteristics of ss-DNA, such as the greater flexibility and hydrophobicty and low charge density, lead to a more pronounced shrinking of the gels in the presence of cationic surfactants. In the formation of the skin layer, the larger flexibility of ss-DNA chains plays a determining role in decreasing the deformation energy for the compression of the DNA chains in the direction perpendicular to the gel surface.

As noted above, the interactions of polyelectrolytes with oppositely charged surfactants are strong and can lead to complex formation with ordered structures. In this process, both electrostatic and hydrophobic interactions are important in driving the self-assembly of surfactant molecules to form ordered structures inside the complexes. Surfactants in water exhibit very rich and complex self-organized structures that are also the basis of the structure-forming abilities of polyelectrolyte/surfactant complexes.

To confirm the existence of ordered structures, the skins of both double- and single-stranded DNA gels were checked for optical birefringence by positioning them between crossed polarizers. The samples appeared birefringent (anisotropic), indicating the existence of ordered structures. In a previous study,[55] fluorescence decay curves from ds-DNA/CTAB gels containing small amounts of a fluorescent probe (pyrene) and a quencher (cetylpyridinium chloride, CPC) were recorded with the single-photon-counting technique. The surfactant self-assemblies were found to be either small globular micelles or long rod-like structures, depending on the surfactant concentration.

The fact that the gel is statistically disordered does not seem to inhibit the regular arrangement of surfactant aggregates. The average mesh size of the gel is much smaller than the spatial scale over which the surfactant aggregates are arranged in a perfect crystalline order.[89] We studied the structure of both DNA/CTAB complexes by means of small-angle X-ray diffraction (SAXS). No characteristic ordered structure, such as rod-like micelles in a hexagonal packing or a lamellar phase, was revealed. It has been shown[89] that gels with higher charge densities exhibit more defined crystalline structures. Also, the ss-DNA/CTAB complex structure in aqueous medium was found to be more disorganized than the corresponding ds-DNA/CTAB complex.[90] From these results, we can predict that skins from ds-DNA gels display a more defined and organized structure than the ss-DNA skins.

Conclusions

The denaturation of cross-linked ds-DNA gels was induced by changes in temperature and was found to be reversible when a heating/cooling cycle was performed. The DNA gels prepared offer a novel opportunity for monitoring DNA−cosolute interactions by simply following the change in gel volume. Both ds- and ss-DNA gels were investigated with respect to their volumetric response to the addition of cationic cosolutes. The deswelling behavior upon addition of metal ions, polyamines, charged proteins, and surfactants is considerably influenced by the DNA conformation, whereas this behavior is only slightly affected by DNA molecular weight. The general trend seems to be that the deswelling degree increases for single-stranded and short-chain DNA gels; the difference between ss-DNA and ds-DNA is larger for surfactants than for the other cosolutes. This finding was attributed to differences in linear charge density, chain flexibility, and hydrophobicity. We demonstrated that the binding of the surfactant to the polyelectrolyte network is accompanied by shrinking of the gel. Monitoring a volume change of a gel, therefore, can give first indications of the binding behavior of a certain surfactant to a polymer. In the presence of surfactants with different chain lengths, the swelling behavior displayed by ss-DNA can be interpreted in terms of an interplay between hydrophobic and electrostatic interactions, the latter being related to polymer flexibility. It was found that the cac decreases with increasing hydrophobicity of DNA, except for the C_8TAB/gel system, where the alkyl tail of the surfactant is too short and the effect of increasing the hydrophobic attractive force becomes irrelevant. The deswelling of DNA gels appears to be reversible and to be independent of the DNA conformation. No macroscopic separation of collapsed and swollen regions was observed at intermediate degrees of binding for ds-DNA gels, whereas a dense surfactant-rich surface phase (skin) was found to coexist with a swollen core network for ss-DNA gels with $\beta > 0.5$. The difference in chain flexibility between the two states of DNA conformations can explain this phenomenon. A large deformation energy is required for the compression of the very stiff ds-DNA chains in the formation of the skin-surfactant layer. Evidence for the existence of ordered structures comes from optical birefringence. A more defined and organized structure is predicted for ds-DNA skins.

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