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Formation of Hydrogels by Simultaneous Denaturation and Cross-Linking of DNA

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DNA hydrogels with a wide range of tunable properties are desirable for applications to make use the characteristics of DNA. This study describes formation conditions of DNA hydrogels using ethylene glycol diglycidyl ether (EGDE) cross-linker and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) catalyst under various reaction conditions. Rheological measurements indicate that the cross-linking of DNA in semidilute solutions proceeds by alternate gel−sol and sol−gel transitions due to two antagonistic effects of EGDE-TEMED pair; the one destroying the physical bonds (denaturation), the other creating chemical bonds (cross-linking). The viscoelastic properties of the hydrogels and the conformation of DNA network chains could be tuned by adjusting the synthesis parameters. Increasing concentration of DNA at the gel preparation stabilizes its structure so that double stranded (ds-) DNA hydrogels form. The average distance between the effective cross-links in single stranded DNA gel is much larger than that in ds-DNA gel making the former gel stable in aqueous solutions. Creep-recovery tests show that heating a semidilute solution of DNA above the DNA melting temperature (87.5 °C) and subsequently cooling down to 25 °C increases the elastic response of the solution and produces an elastic DNA mesh. DNA hydrogel undergoes a volume phase transition in aqueous solutions of polyethylene glycol's at which the gel changes about 5 times its volume.

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Introduction

Deoxyribonucleic acid (DNA) is composed of building blocks called nucleotides consisting of deoxyribose sugar, a phosphate group, and four amine bases. In its native form, DNA is a semiflexible polymer with a double-helical conformation stabilized by hydrogen bonds between the amine bases.[1] When a DNA solution is subjected to high temperature, the hydrogen bonds holding the two strands together break and the double helix dissociates into two single flexible strands having a random coil conformation. This transition from double stranded (ds) to single stranded (ss) DNA is known as denaturation or melting and can be reversed by slow cooling of the DNA solution.[2] Aqueous solutions of DNA have been investigated with regard to its response to various external stimuli. Because DNA is a polyanion, it interacts with positively charged molecules by electrostatic interactions[3,4] and results in DNA compaction as occurred in cells by histones, positively charged proteins.[5,6] DNA in dilute solutions undergoes a transition between an elongated coil and a compact globule by various condensation agents such as polyethylene glycol (PEG), cationic surfactants, alcohol, multivalent inorganic cations, and polyamine.[7−18]

DNA hydrogel is a network of chemically cross-linked DNA strands swollen in aqueous solutions.[19] Such soft materials are a good candidate to make use of the characteristics of DNA such as coil−globule transition, biocompatibility, selective binding, and molecular recognition.[20,21] They have a wide range of potential applications, including drug and gene delivery, selective sorbents, and biosensors. DNA hydrogels have been prepared starting from branched DNA molecules via ligase-mediated reactions.[22] These hydrogels can also be prepared by the solution cross-linking of DNA using a chemical cross-linker such as ethylene glycol diglycidyl ether (EGDE).[23] EGDE contains epoxide groups on both ends that can react with nucleophiles, including the amino groups on the nucleotide bases to form a three-dimensional DNA network.[24] Similar to single DNA molecules,[9,25] the gels derived from DNA undergo volume phase transitions in response to changes in the external stimuli.[23,26−30] Tanaka observed that DNA gels exhibit a first-order volume phase transition in acetone/water mixtures at about 63 v/v % acetone, at which the gel changes 15 times its volume.[23] Further, Mayama et al. observed first-order volume phase transitions in gels and solutions of DNA induced by spermidine, a trivalent cation.[25]

Although DNA hydrogels attracted interest in recent years,[26−30] formation conditions of a cross-linked DNA network starting from single DNA strands as well as the hydrogel properties such as viscoelasticity, swelling and thermal behavior have not been investigated in detail. Recently, we investigated the rheological behavior of DNA gels formed by solution cross-linking of ds-DNA at a concentration of 9.3 w/v %.[31] Although strong DNA gels were obtained in the presence of ≥10% EGDE cross-linker, swelling measurements showed that the rigid ds-DNA strands in gels cannot withstand the swelling pressure of the surrounding water so that they gradually dissolve in aqueous solutions. In the present work, we have focused on the gelation behavior of DNA in semidilute solutions. The cross-linking reactions were carried out under various experimental conditions to find the formation conditions of DNA hydrogels that are stable and do not dissolve in aqueous solutions. As will be seen below, the cross-linking of DNA using EGDE cross-linker and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) catalyst proceeds by alternate gel−sol and sol−gel transitions. As a consequence, formation of stable DNA gels requires critical concentrations of the reactants. The results also show that DNA hydrogels undergo a volume phase transition in aqueous PEG solutions whose extent is much smaller than that observed in single DNA molecules.

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Experimental Section

Materials. DNA hydrogels were made from DNA sodium salt from salmon testes (Sigma). According to the manufacturer, the % G-C content of the ds-DNA used is 41.2%, and the melting temperature is reported to be 87.5 °C in 0.15 M sodium chloride plus 0.015 M sodium citrate. The molecular weight determined by ultracentrifugation is 1.3×10^6 g/mol, which corresponds to approximately 2000 base pairs. The cross-linker EGDE (Fluka), the catalyst TEMED (Merck), PEG's of molecular weights 8000 and 20000 g/mol (PEG-8K, PEG-20K, respectively, Fluka), ethidium bromide (Sigma), and sodium bromide (NaBr, Merck) were used as received.

Cross-Linking Reactions. DNA was first dissolved in 4.0 mM NaBr at 35 °C overnight. DNA solution was then mixed with various amounts of EGDE and stirred at 500 rpm and 35 °C for 1 h. After addition of TEMED catalyst and rigorous stirring for a further 5 min, the solution was transferred between the parallel plates of rheometer. The cross-linker (EGDE) content of the reaction solution was expressed as

$$\text{EGDE\%} = \frac{\text{mass of pure EGDE}}{\text{mass of DNA}} \times 10^2 \qquad (1)$$

Because the molecular weights of EGDE and the nucleotide repeat unit of DNA are 174.2 and 324.5 g/mol, respectively, using the known % G-C content, a multiplication factor of 0.18 converts EGDE % into the moles of epoxide groups added per mole of guanine base in the ds-DNA. DNA and TEMED concentrations were expressed as the mass of DNA or the volume of TEMED in 100 mL of reaction solution, respectively.

Rheological Experiments. Gelation reactions were carried out between the parallel plates of the rheometer (Gemini 150 Rheometer system, Bohlin Instruments) equipped with a Peltier device for temperature control. The upper plate (diameter 40 mm) was set at a distance of 500 μm before the onset of the reactions. During all rheological measurements, a solvent trap was used to minimize the evaporation. Further, the outside of the upper plate was covered with a thin layer of low-viscosity silicone oil to prevent evaporation of solvent. A frequency of $\omega = 1$ Hz and a deformation amplitude $\gamma^o = 0.01$ were selected to ensure that the oscillatory deformation is within the linear regime. The reactions were monitored in the rheometer at a fixed temperature between 30 and 60 °C up to a reaction time of 3 h 15 min, following 1 h at 25 °C. Thereafter, frequency- and strain-sweep tests (both in up and down directions) at $\gamma^o = 0.01$ and $\omega = 1$ Hz, respectively, were carried out.

Thermal behavior of DNA gels was investigated by heating the samples formed between the parallel plates of the rheometer from 25 to 90 °C with a heating rate of 3.25 °C/min, keeping at 90 °C for 10 min, subsequently cooling down to 25 °C with a rate of 1.08 °C/min, and finally keeping at 25 °C for 40 min. Each gelation reaction and the heating−cooling cycle of the gels were carried out twice to check the reproducibility of the results. The experimental setup was also checked by subjecting polyacrylamide gels prepared within the rheometer to the same heating−cooling cycles.[32] No changes in the dynamic moduli of gels were observed after this procedure, indicating that the evaporation during the measurements is negligible.

Creep and creep-recovery experiments were performed using gels and solutions of DNA both before and after the heating−cooling cycle. Creep compliances $J_c(t)$, that is, $\gamma(t)/\tau_0$ were evaluated by applying a constant shear stress τ_0 and measuring the time dependent increase of the strain $\gamma(t)$ for 4000 s. After 4000 s, τ_0 was set to zero and the recovery compliance $J_r(t)$ was measured also during 4000 s.

Hyperchromicity Measurements. For the hyperchromicity measurements, DNA gels were prepared in an oven under the same experimental condition as in the rheological experiments. At predetermined time intervals, samples were taken and diluted to a concentration of 26 mg/L with 4.0 mM NaBr. The degree of denaturation was estimated from the optical absorbance at 260 nm measured with a T80 UV−visible spectrophotometer. The results were presented as the normalized absorbance A_{rel} with respect to that measured at 25 °C before the onset of the reactions. Because melting of DNA strands leads to a rise of the normalized absorbance up to 1.4,[33] the fraction of ss-DNA fragments in DNA (ss-DNA %) was estimated as $\text{ss} - \text{DNA}\% = 250\,(A_{rel} - 1)$.

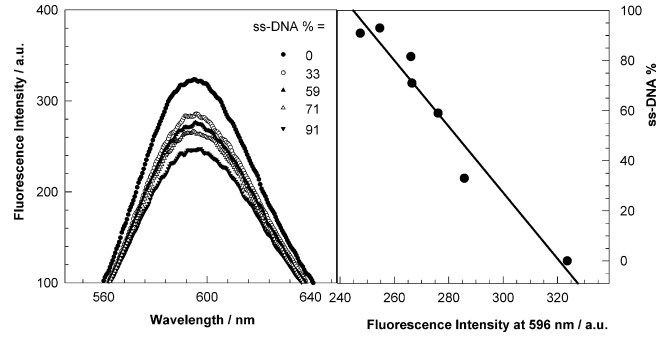

Figure 1. (A) Fluorescence spectra of EtBr in the presence of solutions of DNA with various ss-DNA%. DNA = 0.695 w/v %. EtBr = 4.0×10^{-3} mM. (B) ss-DNA% shown as a function of the fluorescence intensity of EtBr−DNA solutions at 596 nm. Solid line is the linear best fit to the data.

Fluorescence Measurements. The fluorescence measurements were performed both on solutions and gels of DNA containing ethidium bromide (EtBr) as a fluorescent probe. At the state of the measurements, DNA and EtBr concentrations in gels and in solutions were fixed at 0.695 w/v % and 4.0×10^{-3} mM, respectively. Gel samples were prepared in the presence of EtBr under the same experimental condition as described above; they were then diluted with 4.0 mM NaBr to adjust the final concentrations. Solutions of DNA with various ss-DNA % were obtained by thermal denaturation of 0.695 w/v % ds-DNA solutions at 95 °C for various time intervals between 0 and 30 min and then immediately dipping into ice-ethanol for fast cooling to prevent renaturation. EtBr was then dissolved in DNA solutions before the measurements. The measurements were performed using fluorescence spectrometer (Perkin-Elmer LS 55) at an excitation wavelength of 520 nm. Then, the emission spectra were recorded and the peak at 596 ± 1 nm was used for calculations. Figure 1A shows the fluorescence spectra of EtBr in DNA solutions with various ss-DNA%, calculated using the hyperchromicity measurements. In Figure 1B, ss-DNA % is plotted against the maximum intensity at 596 nm ($I_{max,596}$). The solid line is the best linear fit to the data ($R^2 = 0.95$), which gives the equation, $\text{ss-DNA}\% = 423 - 1.3\, I_{max,596}$. Because EtBr intensity in DNA solution is the same as that in DNA gel of the same concentration,[29] this equation was used as the calibration curve to estimate ss-DNA% in gels.

Swelling Measurements. Part of the reaction solution prepared for the rheological tests was transferred into plastic syringes of about 4.5 mm internal diameters and the cross-linking reactions were carried out in an oven at 50 °C for 3 h 15 min. Then, the gels taken from the syringes were cut into samples of about 5 mm in length and they were placed in an excess of 4.0 mM aqueous NaBr solution at 21 ± 0.5 °C. To reach swelling equilibrium, the samples were immersed in solution for at least four weeks replacing the solution many times. The swelling equilibrium was tested by measuring the diameter of the gel cylinders by using an image analyzing system consisting of a microscope (XSZ single Zoom microscope), a CDD digital camera (TK 1381 EG), and a PC with the data analyzing system Image-Pro Plus. The relative volume swelling ratio V_{rel} (gel volume at swelling equilibrium/gel volume just after preparation) was calculated as

$$V_{rel} = \left(\frac{D}{D_o}\right)^3 \qquad (2)$$

where D and D_o are the diameters of the gel sample at swelling equilibrium and after-preparation state, respectively. Swelling tests of

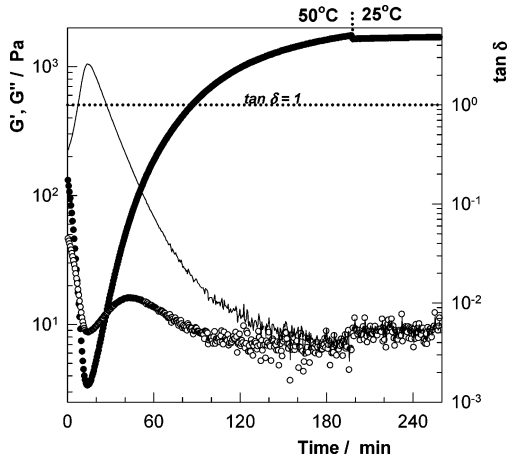

Figure 2. Elastic modulus G' (●), the viscous modulus G'' (○) and the loss factor tan δ (solid curve) during the solution cross-linking of ds-DNA at 50 °C in the presence of 10% EGDE. TEMED = 0.44%. DNA = 6.3%. The dotted horizontal line represents the condition tan $\delta = 1$.

the gels in 4.0 mM aqueous NaBr solutions containing PEG-8K or PEG-20K of various amounts were also carried out as described above by increasing PEG concentration in the external solution.

Results and Discussion

Cross-Linking versus Melting During Gelation of DNA Solutions. The cross-linking reactions were investigated in aqueous solutions of DNA using EGDE as a cross-linker and TEMED as a catalyst. The concentration of ds-DNA in the reaction solution was varied between 3 and 9.3%, which is well above its critical overlap concentration (0.043%).[31] The reactions were carried out isothermally between the parallel plates of the rheometer at a given temperature between 30 and 60 °C for 3 h 15 min, following at 25 °C for 1 h. Dynamic rheological measurements were performed during the cross-linking process to follow the gradual formation of the three-dimensional DNA network. In parallel to the rheological measurements, swelling tests were conducted on the final hydrogels to find the formation conditions of DNA gels that are stable in aqueous solutions.

Swelling tests showed that DNA gels that do not dissolve in water form by conducting the cross-linking reactions at 50 °C in the presence of 0.44% TEMED, if enough DNA and the cross-linker EGDE are present in the reaction solution. Figure 2 shows the gelation profile of the reaction system at a DNA concentration of 6.3% in the presence of 10% EGDE. Here, the elastic modulus G', the viscous modulus G'', and the loss factor tan δ ($= G''/G'$) are shown as a function of the reaction time. Semilogarithmic plot was chosen for clearer representation of the changes in the dynamic moduli of the reaction system at short reaction times. Initially, DNA solution exhibits an elastic modulus G' of about 130 Pa, which is larger than its viscous modulus G'' (50 Pa), typical for a semidilute polymer solution above its crossover frequency.[34] With the onset of the reactions, both G' and G'' rapidly decrease, while the loss factor tan δ increases and becomes unity after 7 min, indicating that the reaction system undergoes a gel - sol transition at this time. G' and G'' start to increase again after crossing a minimum, and a sol−gel transition occurs after 27 min. G' keeps increasing, while G'' decreases at longer times. During the second isothermal reaction period at 25 °C, both moduli remain almost unchanged indicating that the cross-linking reactions stop by reducing the temperature from 50 to 25 °C.

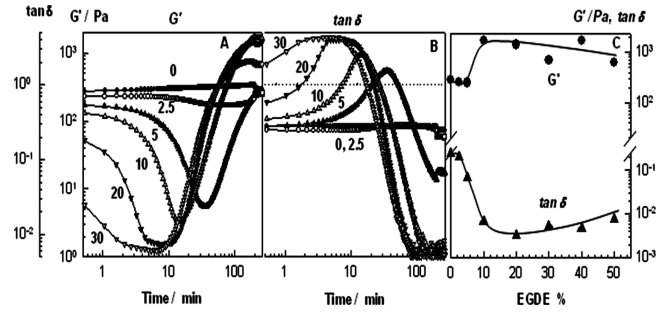

Figure 3. (A,B) Elastic modulus G' (A) and the loss factor tan δ (B) during the cross-linking of ds-DNA at 50 °C in the presence of various amount of EGDE indicated. The dotted horizontal line in B represents the condition tan $\delta = 1$. (C) Final values of G' (●) and tan δ (▲) shown as a function of the EGDE content. TEMED = 0.44%. DNA = 6.3%.

Similar gelation profiles were also observed at various EGDE contents. Double-logarithmic plots in Figure 3A and B show the variations of G' and tan δ during the cross-linking reactions of DNA (6.3%) at EGDE contents between 0 and 30%. In the absence of the cross-linker EGDE, both moduli remain unchanged, indicating that no reaction occurs during the whole course of the heating period at 50 °C. In the presence of EGDE, a minimum appears in G' versus time plot and both the minimum in G' and the gel−sol transition shift to shorter reaction times as the EGDE content is increased. In Figure 3C, the values of G' and tan δ after the reaction period at 50 °C are shown against the cross-linker content. The quantity tan δ represents the ratio of dissipated energy to stored energy during one deformation cycle. With increasing the cross-linker concentration from 5 to 10%, that is, with increasing molar ratio of epoxide groups in EGDE to the guanine base in DNA from 0.9 to 1.8, G' increases from 250 to 1700 Pa; further increase in EGDE content from 10 to 50% slightly decreases the final modulus of the hydrogels. Thus, at or above 10% EGDE, DNA hydrogels with an elastic modulus of the order of 1 kPa and with a loss factor of below 0.01 were obtained indicating formation of strong gels with negligible viscous properties.

Gelation of a semidilute polymer solution is known to result in a continuous increase of the elastic modulus and a decrease of the loss factor due to the formation of elastically effective cross-link points between the polymer chains. However, Figures 2 and 3 clearly show that the gelation profile of DNA solution at a concentration of 6.3% DNA is distinctly different in that the reaction system undergoes alternate gel−sol and sol−gel transitions during the cross-linking reactions. The results are also different from those previously reported for a DNA concentration of 9.3%, where an initial lag phase of about 30 min was observed in the gelation profiles.[31] Because conformational changes of ds-DNA may be responsible for this unusual gelation profile, hyperchromicity measurements were performed to get information about the conformation of DNA molecules during the reactions. The results are shown in Figure 4A, where the normalized absorbance A_{rel} and the fraction of ss-DNA fragments (ss-DNA%) are plotted against the reaction time at 50 °C for three different levels of EGDE.

At or below 2.5% EGDE, A_{rel} was found to be below 1.08, indicating that less than 20% of DNA segments melt during the cross-linking reactions. However, the fraction of ss-DNA fragments rapidly increases with the cross-linker content; at 10% EGDE, it increases to about 70% after a reaction time of about 20 min. It must be noted that the UV measurements could not be conducted at longer reaction times or at larger EGDE contents

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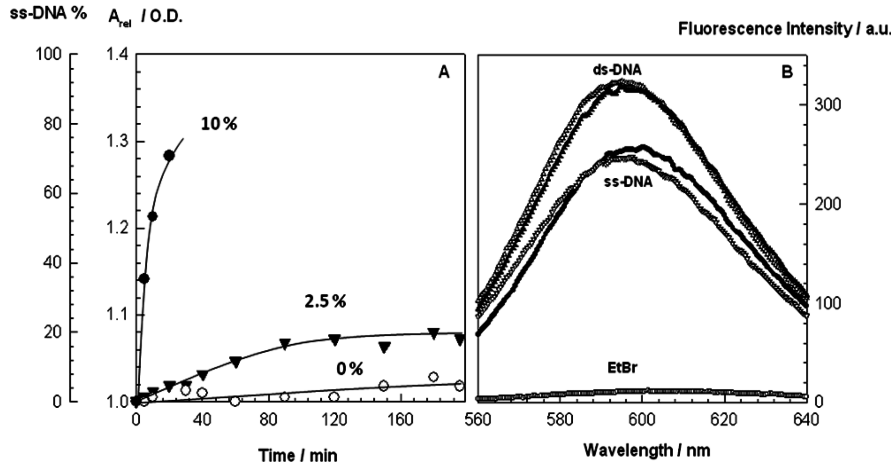

Figure 4. (A) The normalized absorbance (A_{rel}) and the fraction of ss-DNA fragments (ss-DNA%) shown as a function of the reaction time at 50 °C. DNA = 6.3%. TEMED = 0.44%. EGDE % indicated. (B) Fluorescence spectra of EtBr in the absence (○) and in the presence of solutions of ds-DNA (△) and of denatured DNA with 91% ss-DNA (▽). Filled circles represent the spectra in the presence of DNA gels prepared at 6.3 (●) and 9.3% DNA (▲). EGDE = 10%. TEMED = 0.44%.

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due to the formation of an infinite gel, which prevented dilution of the reaction solution for the measurements. Therefore, fluorescence measurements were performed on DNA gels prepared in the presence of ethidium bromide (EtBr) as a fluorescent probe. For comparison, solutions of single- and double-stranded DNA molecules containing EtBr were also subjected to the measurements. It is known that, when EtBr is bound to DNA, its fluorescence increases and this increase depends on the DNA conformation.[28−30,35,36] In Figure 4B, open symbols represent fluorescence spectra of EtBr in the absence and in the presence of DNA solutions; the upper spectrum is for ds-DNA, while the spectrum in the middle is for a denatured DNA with 91% ss-DNA. Due to the intercalation mechanism, ds-DNA leads to a higher increase in EtBr intensity than ss-DNA so that these two spectra represent the limiting values for the fluorescence intensity of DNA gels. Filled circles in Figure 4B represent the spectrum of EtBr in a DNA gel with 10% EGDE. It is seen that its maximum intensity is close to that of EtBr in the presence of ss-DNA. When the technique described in the Experimental Section is used, ss-DNA % for this gel was estimated as 90%. At EGDE contents above 10%, ss-DNA% was always larger than 90% indicating that the gels formed under the reaction conditions consist of mainly ss-DNA network chains.

The results in Figure 4 demonstrate that the gelation reactions in the presence of EGDE are accompanied by the denaturation of DNA fragments; the rate of denaturation increases with increasing amount of the cross-linker EGDE in the reaction solution. The dramatic decrease of both moduli during the initial period of gelation shown in Figures 2 and 3 can thus be related to the denaturation of DNA strands. According to Figure 4A, the hydrogen bonds holding the two strands together break on heating at 50 °C in the presence of 10% EGDE so that the double helix partially dissociates into two single strand fragments. Compared to ds-DNA, ss-DNA is a more flexible polymer because its persistence length p is about 1 nm, while that of ds-DNA is around 50 nm.[37−41] Because DNA used in this work has a molecular weight of 2000 bp or 1.3×10^6 g/mol, the radii of gyration R_g were estimated as 106 and 20 nm for its double stranded and single stranded conformations.[31] Calculation of the critical concentration c^* at which DNA molecules in water start to overlap indicates that it increases from 0.043 to 3.2 w/v % during the DNA melting process.[31] Thus, the semidilute solution of ds-DNA becomes less helical and more flexible on melting so that its viscosity significantly decreases. As a consequence, G'' exceeds G', that is, the loss factor tan δ becomes larger than unity at short reaction times, indicating liquid-like response of the reaction system due to the melting of DNA. However, with the progress of the cross-linking reactions, ss-DNA chains connect each other through EGDE bridges to form larger molecules so that tan δ decreases again below unity, indicating reformation of a viscoelastic gel. Thus, two antagonistic effects of EGDE, the one destroying the physical bonds and the other creating chemical bonds, play a prominent role in the gelation of DNA in aqueous solutions.

Because at or below 2.5% EGDE, no substantial melting of DNA occurs (Figure 4A), G' as well as tan δ remain almost unchanged. As the EGDE content is increased, the minimum in G' shifts to shorter reaction times and the gel-to-sol transition occurs earlier due to the increasing rate of the melting process. At longer times, however, the cross-linking dominates over denaturation so that the elastic modulus rapidly increases. In our previous work, no substantial denaturation was observed at a DNA concentration of 9.3%.[31] As a consequence, instead of a minimum, an initial lag phase was observed in G' versus time plots during which both moduli remained almost unchanged (Figures 1 and 2 in ref 31). Because 9.3% DNA is 0.3 M in counterions, this high counterion concentration in the gel or in the solution increases the stability of ds-DNA in the solution.[42] Indeed, the solid triangles in Figure 4B, representing EtBr spectrum in a DNA gel prepared at 9.3% DNA, are close to the EtBr spectrum in ds-DNA solution; calculations indicate that only 8% of the DNA segments melt to form ss-DNA regions in this gel. Thus, increasing DNA concentration at the gel preparation from 6.3 to 9.3% stabilizes DNA structure leading to the formation of ds-DNA gels.[43] Moreover, decreasing DNA concentration promoted the melting process during the reactions and, at a DNA concentration of 3%, more than 90% of ds-DNA were melted under the gelation conditions.

After the gelation reactions, frequency-sweep tests at $\gamma^o = 0.01$ were carried out at 25 °C over the frequency range 0.01−40 Hz. The results of the measurements are shown in Figure 5. DNA gels with a cross-linker content of 10% or above show a solid-like response, that is, G' shows a plateau over the whole frequency range while G'' remains on a low level about 2 orders of magnitude smaller than G'. Such rheological behavior

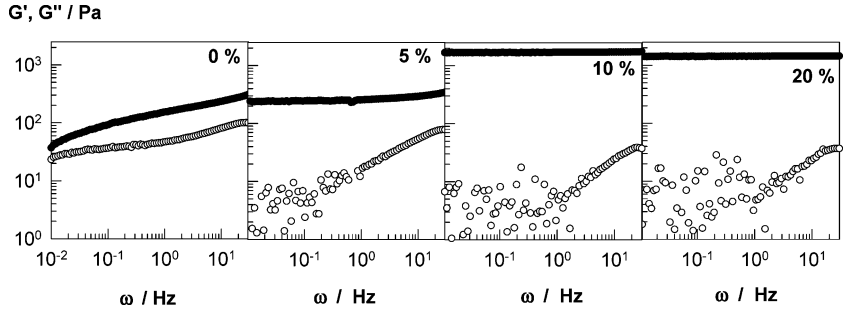

Figure 5. G' (●) and G'' (○) of DNA gels at 25 °C shown as a function of the frequency ω. DNA = 6.3%, TEMED = 0.44%, gelation temperature = 50 °C, EGDE % indicated.

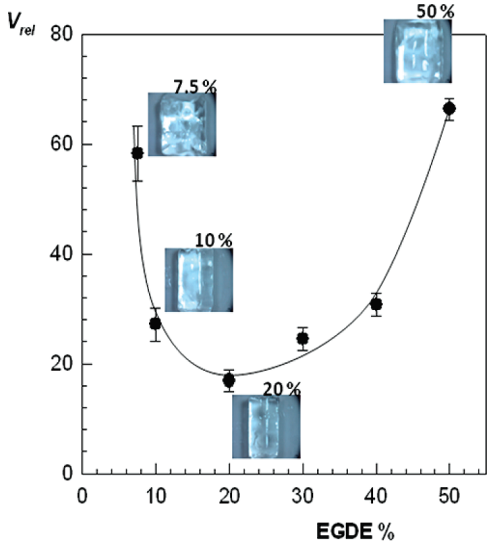

Figure 6. Relative volume swelling ratio V_{rel} of DNA gels in 4.0 mM NaBr solution plotted against the EGDE content. DNA = 6.3%, TEMED = 0.44%, gelation temperature = 50 °C. Pictures show equilibrium swollen DNA gel samples with EGDE contents indicated.

matches of the characteristics of a strong gel such as the chemically cross-linked hydrogels.[44] Note that, compared to ds-DNA gels reported before,[31] present gels with ≥10% EGDE consist of mainly ss-DNA network chains (Figure 4B). At lower cross-linker contents, so-called weak gels were obtained exhibiting frequency dependent moduli. Thus, the viscoelastic properties of DNA gels can be tuned by the amount of EGDE.

Gelation reactions were also conducted in plastic syringes to obtain gel samples with rod-shape for swelling measurements in aqueous 4 mM NaBr solution. The hydrogels formed below 7.5% EGDE were too weak to withstand the swelling pressure while those formed at larger cross-linker contents were stable in solutions. Figure 6 shows the relative volume swelling ratio V_{rel} of DNA gels plotted against the EGDE concentration. Pictures in the figure taken from the equilibrium swollen DNA gels of various EGDE contents indicate that the gels are homogeneous over the whole range of the cross-linker content. V_{rel} decreases with increasing EGDE content up to about 20% due to the increasing cross-link density. However, in accord with the rheological measurements (Figure 3C), V_{rel} increases again at larger EGDE contents. In highly swollen DNA gels, the swelling ratios V_{rel} are between 60 and 70, indicating that the DNA concentration in gels is about 0.1%, that is, the strands forming the network chains are in a 4-fold extended conformation compared to their conformation after the gel preparation.

Because the frequency independent elastic modulus of strong gels corresponds to the equilibrium shear modulus G, one may calculate the molecular weight $\bar{M}_c$ of the network chains in DNA gels. Assuming affine network behavior, G at the state of gel preparation is given by[45,46]

$$G = (\rho/\bar{M}_c)RT\nu_2^0$$

where ρ is the DNA density, ν_2^0 is the volume fraction of cross-linked DNA in the gel, and R and T are their usual meanings. Assuming complete conversion of DNA to the cross-linked material ($\rho\ \nu_2^0 = 63$ kg/m^3), calculations show that $\bar{M}_c$ increases from 93 to 250 kg/mol with increasing EGDE from 10 to 50%. Further, because the distance between the bases in ss-DNA is 0.58 nm,[41,47] the result implies that the average distance between the effective cross-link points in the gel network, that is, the counter length of the network chains is 166 and 447 nm for 10 and 50% EGDE, respectively. These distances are much larger than the persistence length of ss-DNA (about 1 nm), suggesting that the strands between the cross-link points are in a coiled conformation. Thus, DNA gels prepared in the present study considerable differ from those prepared before from ds-DNA strands,[31] in which the average distance between the effective cross-link points is much smaller than the persistence length of ds-DNA (50 nm), that is, rigid segment of ds-DNA is connected each other using EGDE bridges. The stability of the present ss-DNA gels in aqueous solutions, as compared to the ds-DNA gels reported before can thus be related to the flexibility of the network chains.

Other synthesis parameters affecting the properties of DNA gels are the concentrations of DNA and TEMED as well as the preparation temperature. In Figure 7, the effects of TEMED concentration (upper panel), the preparation temperature (middle panel), and DNA concentration (bottom panel) on the gelation profile of DNA are given in terms of the variations of G' and tan δ during the cross-linking reactions of DNA. In the absence of TEMED, no denaturation or gelation was observed, indicating that both of these processes require TEMED−EGDE pair to proceed. The presence of EGDE and TEMED promotes the dissociation of strands by breaking the hydrogen bonds, while EGDE irreversibly connects the separated strands by chemical bonds. The initial decrease in G', that is, the extent of denaturation increases as the amount of TEMED or the gelation temperature are increased or as the DNA concentration is decreased. Mechanical spectra of DNA gels given in Supporting Information, Figure S1 show that, depending on the synthesis parameters, weak to strong DNA gels could be obtained. Final values of G' and tan δ of gels are shown in Figure 8A as functions of TEMED concentration and the gelation temperature. A maximum in the elastic modulus and a minimum in the loss factor require 0.44% TEMED, that is, 0.8 mol of TEMED per

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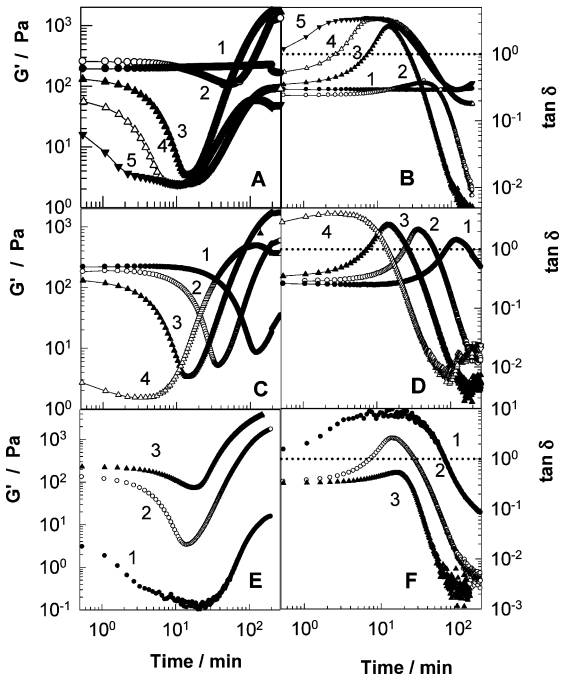

Figure 7. Variations of G' (left panel) and tan δ (right panel) during cross-linking of DNA in aqueous solutions: (A, B) DNA = 6.3%, EGDE = 10%, temperature = 50 °C, TEMED = 0 (1), 0.22 (2), 0.44 (3), 0.88 (4), and 1.26% (5); (C, D) DNA = 6.3%, EGDE = 10%, TEMED = 0.44%, temperature = 30 (1), 40 (2), 50 (3), and 60 °C (4); (E, F) EGDE = 10%, TEMED = 0.44%, temperature = 50 °C, DNA = 3 (1), 6.3 (2), and 9.3% (3).

mole of EGDE at a temperature of 50 °C, which are the experimental parameters selected for the preparation of the gel samples given in Figure 6. Thus, competition between denaturation and cross-linking processes requires a critical concentration of TEMED and a critical temperature to obtain a gel with a high degree of elasticity.

We have to note that, in all previous works on the preparation of DNA gels, pH was used as the experimental parameter, which was adjusted by the addition of TEMED or TEMED + NaOH.[23,26−30] Figure 8B shows pH of the reaction solution as a function of TEMED %. In the absence of EGDE and DNA (open circles), pH rapidly increases with the addition of the weak base TEMED to values greater than 10 but then slightly increases upon further increase of TEMED content beyond 0.1%. In the presence of 10% EGDE cross-linker (open triangles), pH of the solution with >0.4% TEMED is about one pH unit greater than that found without EGDE. This indicates formation of a quaternary base by the nucleophilic attack of the ternary amine TEMED on one of the ring carbon atoms of EGDE.[48−51] From the increase of pH, it was estimated that about 4 mol % of EGDE are participating into this side reaction. Moreover, when DNA is also present in the reaction solution (filled circles), pH slightly decreases due to the acidic character of DNA, but it remains between 11 and 11.5 over the concentration range 0.3−1.3% TEMED. Comparison of Figure 8A and B thus show that, although the properties of DNA gels formed between 0.3 and 1.3% TEMED vary significantly, pH remains around 11, indicating that not the pH but the amount of TEMED in the reaction solution is important for the preparation of stable DNA gels.

Thermal Behavior of DNA Gels. Thermal behavior of DNA gels was investigated by heating the gels from 25 to 90 °C with a heating rate of 3.25 °C/min, keeping at 90 °C for 10 min, subsequently cooling down to 25 °C with a rate of 1.08 °C/min, and finally keeping at 25 °C for 40 min. Our previous work has shown that, for dilute DNA solutions, this heating procedure resulted in the formation of approximately 95% ss-DNA fragments, that is, in almost complete melting of ds-DNA.[31] On cooling back to 25 °C, the amount of ss-DNA fragments decreased to 60%.[31] In the following experiments, the changes in the dynamic moduli of gels were monitored during the course of the heating−cooling cycle as a function of temperature and time. Figure 9 shows the results obtained using three DNA gels prepared at 6.3% DNA and in the presence of 10−30% EGDE cross-linker, corresponding to 1.8−5.4 epoxide groups per guanine base in DNA, respectively. It is seen that G' slightly decreases, while G'' increases during the heating or cooling periods. The result suggests that the denaturation of ds-DNA segments remaining in the gel network (≤10%) decreases the number of elastically effective strands while the formation of dangling ss-DNA strands results in an increase of the viscous modulus. The larger the cross-linker content, the larger the decrease in the elastic modulus of gels during the cycle, probably due to the effect of excess EGDE accelerating the melting process. Moreover, all the gel samples subjected to the heating−cooling cycle remain in the gel state indicating the stability of the cross-links between the DNA strands.

However, different behavior was observed at or below 2.5% EGDE. Figure 10 shows the variations of G', G'', and tan δ during heating−cooling cycles of DNA gels with 0 and 2.5% EGDE. Because these gels originally consisted of ds-DNA fragments (≥80% ds-DNA, Figure 3), at temperatures above 50 °C, denaturation of ds-DNA results in an abrupt decrease of both G' and G'', and the gels convert into a viscous liquid at 90 °C, as evidenced from the value of tan δ approaching unity. Thus, heating provides thermal energy that breaks hydrogen bonds and allows the strands to dissociate, which make the DNA less bulky, reducing the viscosity of the system. However, on cooling back, both moduli increase while tan δ decreases indicating reformation of the hydrogen bonds. At 0% EGDE, the heating−cooling cycle results in a gel with $G' = 4$ kPa compared to its initial value of 0.3 kPa. This 13-fold increase in G' is due to the high DNA concentration in the solution;[31] the strands, which were melted during the heating period, cannot reorganize to form the initial double-stranded conformation. Hence, the hydrogen bonds forming during the cooling period connect the portions of the strands belonging to different ds-DNA molecules so that this portions act as physical junction zones in the solution. The presence of 2.5% EGDE significantly suppress the increase in G'' probably due to the steric effects of chemical cross-links preventing hydrogen bond formation in the system. Similar results such as those given in Figure 10, but at a much higher extent, were observed before at a DNA concentration of 9.3%.[31]

The drastic change in the viscoelastic properties of semidilute DNA solutions as a result of the heating−cooling cycle was also investigated by the frequency and strain sweep experiments as well as by the creep-recovery tests. Figure 11A and B show the moduli versus frequency plots of solutions before and after the cycle, respectively. Both moduli increase and G' becomes frequency independent after the heating−cooling cycle of the DNA solution, confirming formation of a highly elastic network. Figure 11C shows up and down strain sweep experiments at a frequency of 1 Hz for the DNA solution after the cycle. The measurements were carried out for strain amplitude $\gamma°$ ranging from 0.01 to 20.3. The upward curves show a distinct linear viscoelastic region at low strains ($\gamma° < 0.1$) beyond which G'

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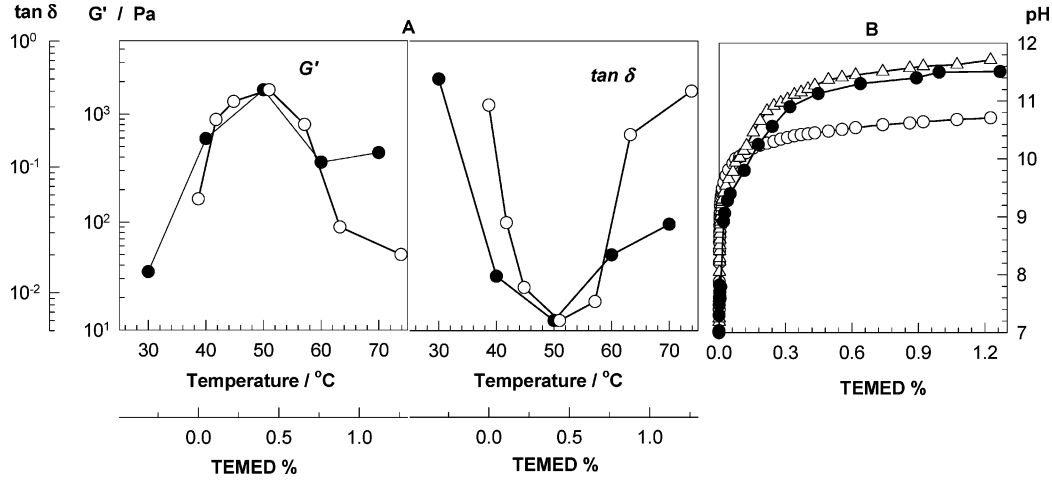

Figure 8. (A) Final elastic modulus G' (left) and the loss factor tan δ (right) of DNA gels shown as functions of TEMED concentration at 50 °C (○), and of the gelation temperature at 0.44% TEMED (●). DNA = 6.3%; EGDE = 10%. (B) pH of the solutions shown as a function of TEMED %. Solutions: 4.0 mM NaBr (○), 4.0 mM NaBr containing 10% EGDE (△), and 4.0 mM NaBr containing 10% EGDE + 6.3% DNA (●).

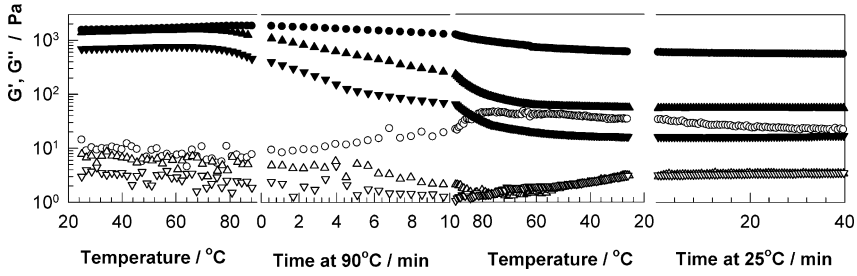

Figure 9. G' (filled symbols) and G'' (open symbols) of DNA gels during the heating−cooling cycle: $\omega = 1$ Hz; $\gamma^\circ = 0.01$; DNA = 6.3%; TEMED = 0.44%; gelation temperature = 50 °C; EGDE = 10 (●,○), 20 (▲,△), and 30% (▼,▽).

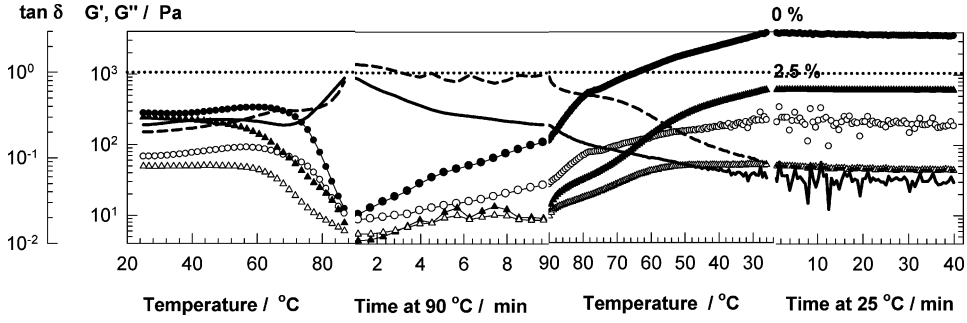

Figure 10. G' (filled symbols), G'' (open symbols), and tan δ (curves) during the heating−cooling cycle of DNA gels. DNA = 6.3%; TEMED = 0.44%; gelation temperature = 50 °C; EGDE = 0 (circles and solid curves) and 2.5% (triangles and dashed curves). The dotted horizontal line represents the condition tan $\delta = 1$.

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decreases rapidly, while G'' increases slightly to a maximum value and then decreases again less rapidly than G'. The initial increase of G'' with strain in the low strain range, as also reported before for colloidal suspensions,[52] is attributed to breakdown of ds-DNA domains, that were formed during the heating−cooling cycle, to a larger number of smaller size ds-DNA regions, resulting in a more dissipative system. At higher strains, further breakdown of the structure leads to shear-thinning viscous flow behavior. Comparison of the up and down curves indicates that the gel system exhibits an almost reversible strain sweep spectrum; the breakdown of the microstructure caused by the strain is recovered at very low strain amplitudes.

Figure 11D and E show creep-recovery curves of DNA solutions before and after the heating−cooling cycle, respectively. Here, the values of creep compliance $J_c(t)$ are shown as a function of creep time t between 0 and 4000 s. For the interval $4000 < t < 8000$ s, recovery compliance $J_r(t)$ is represented. The creep compliance was analyzed by means of the Burger model composed of a Maxwell element in series with one Kelvin-Voigt element, that is,

$$J_c(t) = J_o + J_1[1 - \exp(-t/\tau)] + \frac{t}{\eta} \qquad (4)$$

where J_o and J_1 are the compliances of the Maxwell and Kelvin−Voigt springs corresponding to the instantaneous and retarded compliances, respectively, τ is the retardation time, and η is the Newtonian viscosity.[53] Each creep curve in the figures is characterized by an initial elastic response corresponding to the instantaneous creep compliance J_o, followed by a time-dependent creep region related to a viscoelastic response. Further, sudden removal of the applied stress τ_0 at time $t = 4000$ s allows a reversible deformation that partially recovers the initial shape of the gel. The recoverable deformation, γ %, was characterized by the recoverable compliance,

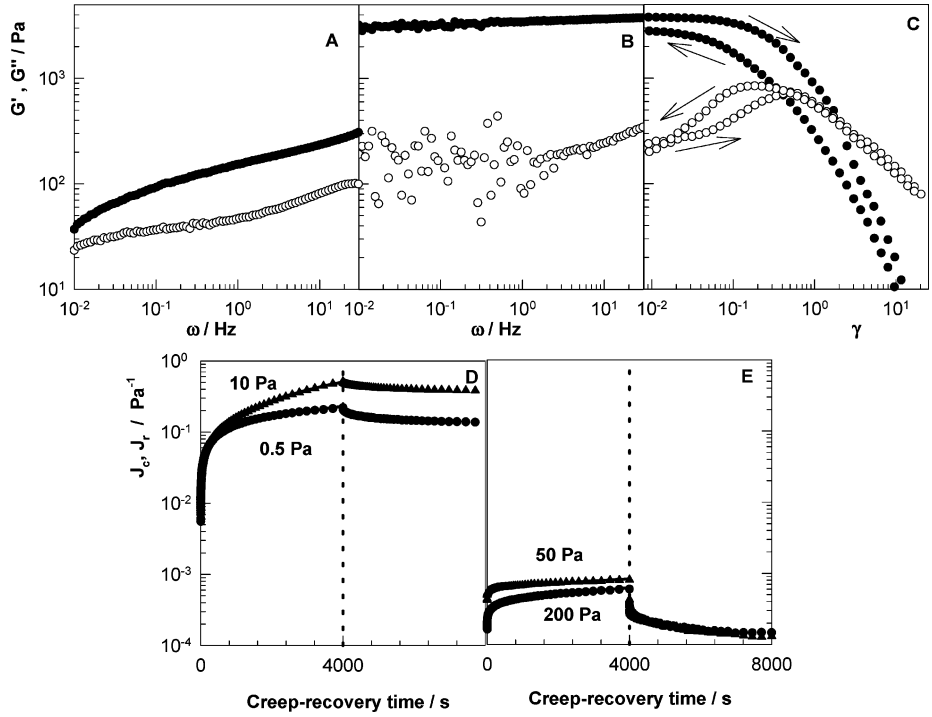

Figure 11. (A, B): G' (●) and G'' (○) of DNA solutions shown as a function of the frequency ω before (A) and after the heating−cooling cycle (B); $\gamma^o = 0.01$. (C) Strain sweep spectra of DNA solutions after the cycle at $\omega = 1$ Hz. Arrows show the directions of up and down sweeps. (D, E) Creep-recovery curves of DNA solutions before (D) and after the heating−cooling cycle (E). Creep $J_c(t)$ and recovery compliances $J_r(t)$ are shown as a function of creep (0−4000 s) and recovery times (4000−8000 s). The applied constant stress τ_0 indicated. DNA = 6.3%; EGDE = 0%; TEMED = 0.44%.

determined by subtracting the residual compliance at the end of the recovery period from the maximum compliance reached at the end of the creep period. J_o of DNA solution before the heating−cooling cycle is much larger, that is, its elastic modulus (J_o^{-1}) is much smaller than that after the cycle. The fitting of the creep data to eq 4 provided satisfactory agreement and are also shown in the figures by the solid curves.

Results in Figure 11C and D reveal that the time-dependent viscoelastic behavior of gels depends on the applied stress τ_0, that is, on the maximum strain γ_{max} reached at the end of the creep period. To compare the characteristics of the gels before and after the cycle, creep-recovery measurements were performed under the condition of a fixed maximum strain γ_{max} by suitably adjusting the applied stress τ_0. The results obtained for $\gamma_{max} = 0.115 \pm 0.005$ indicate that the values of J_o with less than 10% standard deviations are 1.47×10^{-2} and 2.1×10^{-4} Pa^{-1},that is, the plateau moduli are 70 and 4800 Pa before and after the heating−cooling cycle, respectively, in good agreement with the results of the oscillation tests (Figure 11A and B). Because the viscosity η at a given polymer concentration increases with the cross-link density, η of the DNA solution after the cycle was found to be 3 orders of magnitude larger than that before the cycle (1.5×10^7 vs 3.0×10^4 Pa·s). Further, recoverable deformations γ % during creep-recovery tests were 37 and 76% before and after the cycle, respectively, also indicating increasing elastic response and formation of an elastic DNA mesh due to the additional cross-links between the DNA strands.

Phase Transitions in DNA Gels. In this section, the swelling behavior of DNA gels immersed in aqueous 4.0 mM NaBr solutions of PEGs of molecular weight 8000 (PEG-8K) and 20000 g/mol (PEG-20K) was investigated. As reported before by Vasilevskaya et al.,[9] single DNA molecules in aqueous solutions of PEG-8K undergo a first order phase transition at a concentration of 25 w/v % PEG, during which the molecules change on average 3 times their lengths. When transferring the data to DNA gels, one would expect a 27-fold volume change at 25 w/v % PEG. Moreover, in aqueous PEG-20K solutions, single DNA molecules assume re-entrant conformation transitions, during which the molecules first collapse at about 0.3 w/v % PEG but then reswells again at 10 w/v % PEG, as the PEG concentration is continuously increased.[9] Here, we conducted these experiments using macroscopic DNA gel samples prepared at 10% EGDE; thus, the sample length subjected to the swelling tests was increased by a factor of 10^4.

The results are shown in Figure 12 where the swelling ratio V_{rel} of gels is plotted against the PEG concentration in the external solution. Measurements above 60 w/v % PEG cannot be conducted due to the solidification of the mixtures. For PEG-8K (filled symbols), the gel volume gradually decreases as the PEG concentration increases up to 30%, while between 30 and 40% PEG, a 6-fold decrease in the gel volume was observed. At larger PEG contents, the swelling degree only slightly depends on the PEG concentration of the external solution. For PEG-20K (open symbols), although a similar behavior was observable, the range of PEG during which the gel rapidly deswells becomes narrower and occurs between 7.5 and 10% PEG-20K, during which the gel volume changes about five times. Thus, comparison of the present results with those reported before for single DNA molecules shows that (i) DNA gel undergoes 4−5-fold smaller volume shrinkage during the collapse transition compared to the single DNA molecule, (ii) the critical PEG concentration required for this transition is much larger for the DNA gel compared to the DNA molecule, and (iii) a re-entrant swelling transition in aqueous PEG-20K was not observed in DNA gel.

Mayama et al. also observed significant differences in the volume phase transitions of single DNA molecules and DNA gels in aqueous solutions of spermidine.[25] The observed differences may be related to the different contour lengths of

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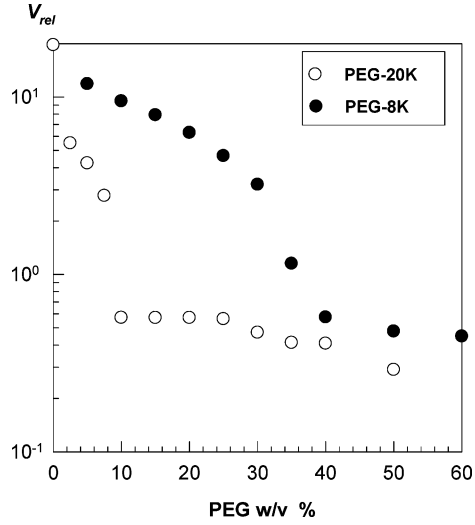

Figure 12. Variation of the swelling ratio V_{rel} of DNA gels with the concentration of PEG of molecular weight 8000 (filled symbols) and 20000 (open symbols) in the external 4.0 mM NaBr solution. Swelling temperature = 21 °C. Gel preparation conditions: temperature = 50 °C, DNA = 6.3%, TEMED = 0.44%, EGDE = 10%.

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single DNA molecules and the network chains. The contour length L of a single ds-DNA molecule used by Vasilevskaya et al. was 56 μm,[9] while, as mentioned in the first section, it is 166 nm for ss-DNA gels subjected to the swelling tests. Thus, the number of Kuhn segments N equal to 560 and 83 for the single DNA molecule and for the DNA network chain, respectively. We should note that these calculations assume that the network chains consist of ss-DNA strands; increasing ds-DNA fragments will significantly decrease the length N and it approaches to unity for pure ds-DNA gels. During the coil−globule transition of polymer chains, the magnitude of the volume change scales as $N^{4/5}$.[25] Thus, calculations for $N = 560$ and 83 show that the volume change during the phase transition in PEG solution should be 4.6-fold smaller for the DNA gel compared to the single DNA molecule, supporting our experimental data. Further, the shift of the critical PEG concentrations toward higher PEG contents indicates that the phase transition of DNA gels requires poorer solvent conditions than that of a single DNA molecule, in accord with the mean-field calculations of Mayama et al.[25] For example, in aqueous PEG-20K, the deswelling transition in DNA molecule occurs at 0.3% PEG compared to 7.5−10% PEG found for DNA gels; thus, the critical concentration shifts about 1 order of magnitude larger DNA concentrations. The reswelling transition in single DNA molecules, which was not observed in DNA gels, occurs at 10% PEG-20K; it seems that, due to the shift in the critical concentrations, the reswelling transition disappears in DNA gels.

Conclusions

DNA hydrogels with a wide range of tunable properties are desirable for applications to make use the characteristics of DNA. In this study, we investigated formation conditions of DNA hydrogels using EGDE cross-linker and TEMED catalyst under various reaction conditions. Rheological measurements indicate that the cross-linking of DNA in semidilute solutions proceeds by alternate gel−sol and sol−gel transitions due to two antagonistic effects of EGDE-TEMED pair: the one destroying the physical bonds (denaturation) and the other creating chemical bonds (cross-linking). UV and fluorescence measurements indicate that, at a DNA concentration of 6.3 w/v %, hydrogels consisting of mainly ss-DNA network chains form, while increasing concentration of DNA at the gel preparation stabilizes its structure so that ds-DNA hydrogels form. The average distance between the effective cross-links in ss-DNA gel is much larger than that in ds-DNA gel making the former gel stable in aqueous solutions. Equilibrium volume swelling ratios of ss-DNA hydrogels indicate that the DNA concentration in gels is about 0.1% and the strands forming the network chains are in a 4-fold extended conformation compared to their conformation after the gel preparation. Creep-recovery tests show that heating a semidilute solution of DNA above the DNA melting temperature (87.5 °C) and subsequently cooling down to 25 °C increases the elastic response of the solution and produces an elastic DNA mesh. DNA hydrogels undergo a volume phase transition in aqueous solutions of PEG's of molecular weights 8000 and 20000 g/mol, whose extent is much smaller than that observed in single DNA molecules.

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Supporting Information Available. Figure S1 showing the mechanical spectra of DNA gels formed under various experimental conditions before and after the heating−cooling cycle. This material is available free of charge via the Internet at http://pubs.acs.org.

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