Rheological Behavior of Responsive DNA Hydrogels

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ABSTRACT: Dynamic rheological measurements performed during the solution cross-linking of DNA (about 2000 base pairs long) at a concentration of 9.3% w/v show formation of strong to weak DNA hydrogels depending on the concentration of the cross-linker ethylene glycol diglycidyl ether (EGDE). At a cross-linker content of 10 wt % or above (with respect to DNA), the elastic modulus G' of DNA hydrogels is more than 2 orders of magnitude larger than the viscous modulus G'', and both moduli are essentially independent of frequency over the range $10^{-2}-10^{1}$ Hz. The value obtained for G' (10^{3} Pa) is of the same order of magnitude as the elastic modulus for chemical gels or cross-linked biopolymer gels. At lower cross-linker contents (below 10 wt %), weak DNA hydrogels exhibiting frequency-dependent moduli were obtained. Thermal behavior of DNA gels and DNA solutions was investigated by heating the samples above the DNA melting temperature (87.5 °C) and subsequently cooling down to 25 °C. At high cross-linker contents, no significant changes in the dynamic moduli were observed. At low cross-linker contents, however, a significant increase in the dynamic moduli was observed during both heating and cooling. The results were explained with the partial dissociation of the double helix into flexible single strand fragments during heating so that the number of entanglements increases. On cooling back, the dissociated strands cannot reorganize to form the initial double-stranded conformation so that the hydrogen bonds formed act as physical junction zones in addition to the chemical cross-links formed by EGDE. The heating−cooling cycles of DNA solutions produce physical gels exhibiting an elastic modulus in the order of megapascals. Thermoreversible DNA hydrogels were also obtained due to the transition between semidilute and dilute regimes of the same DNA solution depending on the conformation of the DNA chains.

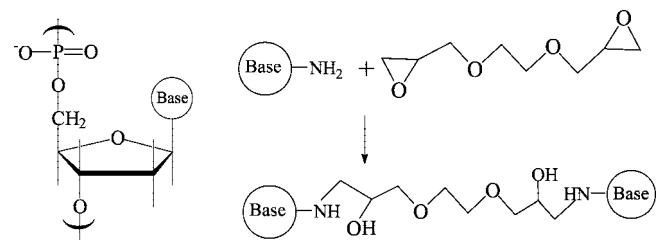
Introduction

Deoxyribonucleic acid (DNA) serves as the carrier of genetic information in living organisms and is composed of building blocks called nucleotides consisting of deoxyribose sugar, a phosphate group, and four bases—adenine (A), thymine (T), guanine (G), and cytosine (C) (Scheme 1A). DNA has a double-helical conformation in its native state which is stabilized by hydrogen bonds between A and T and between G and C.[1] When a DNA solution is subjected to high temperatures (80−90 °C), the hydrogen bonds holding the two strands together break and the double helix dissociates into two single 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. DNA denaturation was extensively investigated in the past few decades as it gives useful information on DNA regarding its structure and stability. Typically, melting curves of DNA are obtained by monitoring the UV absorption at 260 nm.[2−4] The disruption of base stacking decreases the electronic interaction between the bases so that it becomes easier for an electron to absorb a photon. Thus, denaturation leads to the hyperchromic effect, i.e., the increased absorption of light.

At concentrations below the critical overlap concentration, DNA form viscous structures in aqueous solutions, while at high concentrations, DNA molecules overlap and entangle to form a weak gel.[5] Only a few reports exist in the literature on the viscoelastic properties of DNA solutions. The linear viscoelastic moduli of DNA solutions have been measured by Mason et al. in the concentration range 0.1−1.0% w/v.[5] It was shown that the solutions in saline buffer behave as an entanglement network of semiflexible coils exhibiting an elastic modulus and a crossover frequency that vary with concentration according to known scaling laws.[5,6] Sun et al. showed that heating of semidilute solutions of DNA (0.5% w/v) leads to a decrease of the elastic modulus, indicating that the rigid-rod-like DNA molecules lost their rigidity due to the dissociation of the two strands.[6,7] Thus, the semiflexible ds-DNA consisting of fragments of about 150 base pairs behaving as rigid segments becomes flexible on heating so that the viscosity of the solution decreases. The breakup of the DNA base pairs begins at a temperature as low as 50 °C, which is well below the chain melting temperature (87 °C).[6]

DNA hydrogel is a network of chemically cross-linked DNA strands swollen in aqueous solutions. 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. DNA hydrogels were recently prepared starting from branched DNA molecules via ligase-mediated reactions.[8] 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).[9] EGDE has been widely used for cross-linking of polysaccharides, proteins, and organic molecules.[10] EGDE contains epoxide groups on both ends that can react with nucleophiles, including amino groups, sulfhydryls, and hydroxyls. Since the amino groups on the nucleotide bases react with the EGDE, heat-resistant interstrand cross-links form during the cross-linking reactions, which lead to the formation of a three-dimensional DNA

Scheme 1. Nucleotide Repeat Unit of DNA (A) and the Cross-Linking Reaction of the Amino Groups on the Nucleotide Bases with EGDE Leading to Chemical DNA Hydrogels (B)



(A) **(B)**

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network (Scheme 1B). Indeed, it was shown that the elastic modulus of DNA gels is 1−2 orders of magnitude larger than the viscous modulus and is almost frequency-independent.[11] Tanaka et al. showed that the DNA gel formed by EGDE cross-linker exhibits a discrete volume phase transition in water−acetone mixture induced by a change in solvent composition.[9] The gel also exhibits a continuous collapse transition in response to Ca^{2+} ions in the surrounding aqueous NaCl solution[12] or to polyamines and cationic dyes.[13] In a series of papers, Costa et al. investigated the swelling behavior of DNA gels under various conditions. It was shown that the DNA gels are responsive systems exhibiting drastic volume changes in response to the external stimuli, such as the concentrations of inorganic salts, polyamines, cationic macromolecules, or surfactants.[14−16]

In the present work, we monitored the gelation reactions of DNA and EGDE in aqueous solutions by classical rheometry using oscillatory deformation tests. To our knowledge, no rheological investigation has yet been conducted for this kind of reactions. Gelation reactions were carried out at a DNA concentration of 9.3 wt % in 4.0 mM sodium bromide solution at pH = 10. In order to follow the gradual formation of the three-dimensional DNA network, gelation reactions were carried out in the presence of various amounts of the chemical cross-linker EGDE. The complex shear modulus G^* measured can be resolved into its real and imaginary components, i.e.

$$G^* = G' + iG'' \tag{1}$$

where the elastic (or storage) modulus G' is a measure of the reversibly stored deformation energy and the viscous (or loss) modulus G'' represents a measure of the irreversibly dissipated energy during one cycle. Here, we show that strong DNA gels with a loss factor $\tan\delta = G''/G'$ below 10^{-2} could be prepared at large EGDE contents. These gels were stable even at temperatures above the melting temperature of DNA. It was also of inherent interest to investigate the change in the viscoelastic properties of weak DNA gels in response to temperature changes between below and above the DNA melting temperature. UV measurements were also coupled with the elasticity results to explain the experimental observations. As will be seen below, physical junction zones can be generated in DNA solutions by the heating and cooling cycles which lead to the formation of viscoelastic gels with an elastic modulus in the order of megapascals.

Experimental Part

Materials. DNA hydrogels were made from DNA sodium salt from salmon testes (Sigma). According to the manufacturer, the % G−C content of the 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 ∼2000 base pairs. The cross-linker ethylene glycol diglycidyl ether (EGDE, Fluka), *N,N,N′,N′*-tetramethylethylenediamine (TEMED, Merck), and sodium bromide (NaBr, Merck) were used as received.

Cross-Linking Reactions. DNA was first dissolved in 4.0 mM NaBr at 35 °C for 2 days. DNA solution was mixed with various amounts of EGDE, and pH was adjusted to 10 using TEMED. The solution was then transferred between the parallel plates of rheometer. DNA concentration at cross-linking was 9.3% w/v. The cross-linker (EGDE) content of the reaction solution was expressed as

$$\text{EGDE \%} = \frac{\text{mass of pure EGDE}}{\text{mass of DNA}} \times 10^2 \tag{2}$$

Since 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.

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 $\gamma = 0.01$ were selected to ensure that the oscillatory deformation is within the linear regime. The reactions were monitored in the rheometer at 50 °C up to a reaction time of 2 h, following 1 h at 25 °C. Thereafter, frequency- and strain-sweep tests (both up and down) at $\gamma = 0.01$ and $\omega = 1$ Hz, respectively, were carried out. Thermal behavior of DNA gels and DNA solutions 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 as well as 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 conducting the free-radical cross-linking copolymerization of acrylamide and *N,N′*-methylenebis(acrylamide) in aqueous solutions within the rheometer.[17] The gels formed between the parallel plates was heated to 90 °C within 20 min and, after keeping at 90 °C for 10 min, cooled down to 25 °C within 1 h. No changes in the dynamic moduli of gels were observed after this procedure indicating that the evaporation during the measurements is negligible.

Hyperchromicity Measurements. For the hyperchromicity measurements, DNA gels were prepared in an oven under the same experimental conditions as in the rheological experiments. The gels and the solutions were then subjected to the heating and cooling cycles in the oven, as described above. Before the measurements, the samples were diluted to a concentration of 26 mg/L. The degree of denaturation was estimated from the optical absorbance at 260 nm measured with a T80 UV−vis spectrophotometer. The results were presented as the normalized absorbance with respect to that measured at 25 °C. Thermal denaturation and renaturation curves of dilute DNA solutions (26 mg/L) in 4.0 mM NaBr were obtained by recording the optical absorbance at 260 nm while the solutions were heated and cooled in the spectrophotometer chamber by circulating water at controlled temperature.

Results and Discussion

ds-DNA was cross-linked in aqueous solutions at pH = 10 using EGDE as a cross-linker. The DNA concentration in the solution was set to 9.3% w/v, which is well above its critical overlap concentration (0.043% w/v, see below). The reactions were carried out isothermally between the parallel plates of the rheometer at 50 °C for 2 h, following at 25 °C for 1 h. For comparison, DNA solutions without the EGDE cross-linker were also heated under the same experimental conditions. We discuss the results of our experiments in three subsections. In the first subsection, the characteristic features of the cross-linking reactions and the viscoelastic properties of the resulting DNA gels are discussed. In the second and last subsections, the thermal behavior of gels and solutions of DNA in terms of their temperature responsivity against the temperature changes between below and above the melting temperature is discussed and experimental observations are interpreted.

Formation of DNA Gels. Dynamic rheological measurements were performed at a fixed frequency ω and amplitude γ during the cross-linking process in order to follow the gradual formation of the three-dimensional DNA network. A typical gelation profile of DNA solutions at a cross-linker content of

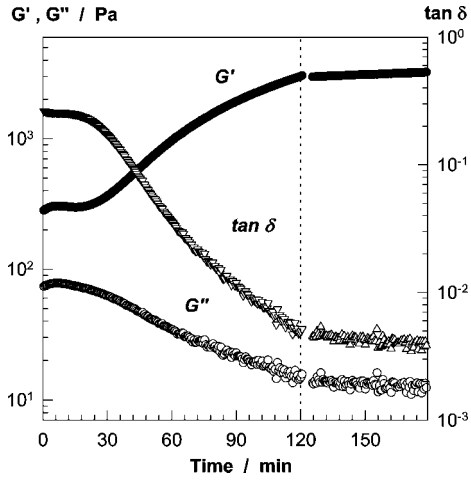

Figure 1. Elastic modulus G', the viscous modulus G'', and the loss factor tan δ during the cross-linking of ds-DNA in the presence of 10% EGDE. The dotted vertical line represents the transition from the isothermal reaction period at 50 °C to that at 25 °C. $\omega = 1$ Hz, $\gamma = 0.01$.

10% is shown in Figure 1, where the elastic modulus G', the viscous modulus G'', and the loss factor tan δ, which is the ratio of G'' to G', measured at 1 Hz are shown as a function of the reaction time. The temperature was set to 50 °C up to 2 h, shown in the figure by the vertical dotted line, while it was 25 °C at longer times. The semilogarithmic plot was chosen for clearer representation of the changes in the dynamic moduli of the reaction system at short reaction times. Before the start of the gelation reactions, DNA solution exhibits an elastic modulus G' of about 300 Pa and a viscous modulus G'' of around 80 Pa. The cross-linking of DNA is characterized by an initial lag phase of about 30 min, during which both moduli remain almost unchanged. The appearance of the lag phase may be related to the formation of soluble DNA aggregates in the reaction system. The lag phase is followed by a log phase during which G' rapidly increases while G'' decreases, suggesting the occurrence of the cross-linking reactions between the DNA aggregates formed during the initial reaction period. Thus, the number of elastically effective DNA chain portions with both ends cross-linked increases while the number of free strands decreases as a result of cross-linking. During the second isothermal reaction period at 25 °C, both moduli keep almost unchanged, indicating that the cross-linking reactions stop by reducing the temperature.

Figure 2 shows the variations of the dynamic moduli and the loss factor tan δ during the cross-linking reaction DNA at various cross-linker contents between 1 and 49%. The higher the cross-linker (EGDE) content, the larger is the elastic modulus of gels and the smaller is their viscous modulus. For example, with increasing the cross-linker concentration from 2.5 to 25%, that is, with increasing molar ratio of epoxide groups in EGDE to the guanine base in DNA from 0.45 to 4.5, G' increases from 500 to 3000 Pa while G'' decreases from 90 to 10 Pa. In the presence of a very excess amount of EGDE, a decrease in the elastic modulus was always observed probably due to the occurrence of side reactions. The quantity tan δ represents the ratio of dissipated energy to stored energy during one deformation cycle. Figure 2 also shows that, with increasing cross-linker concentration, tan δ decreases; at EGDE contents above 5%, tan δ becomes less than 0.01, so that they are strong gels with negligible viscous properties.

The UV absorption at 260 nm was almost unchanged after the cross-linking reactions, indicating that the gels formed under the experimental condition consist of mainly ds-DNA chains,[18] in accord with the fluorescence measurements of Costa et al.[15] After the gelation reactions, frequency-sweep tests at $\gamma = 0.01$ as well as strain sweep tests at $\omega = 1$ Hz were carried out at 25 °C over the frequency range 0.01−40 Hz and over the strain range 0.01−1.2. The results of the measurements are shown in Figure 3 together with those obtained from the un-cross-linked DNA solution. Mechanical spectra of DNA gels with a high cross-linker content (25% EGDE or 4.5 mol of epoxide groups per mol of guanine) show that G' is more than 2 orders of magnitude larger than the viscous modulus G''. Both G' and G'' are essentially independent of frequency over the range 10^{-2}−10^{1} s^{-1}, which indicates the dominant viscoelastic relaxations of the network are at lower frequencies; that is, the relaxation time, τ, of the DNA network is long. Such rheological behavior matches of the characteristics of a strong gel. The value obtained for G' (10^3 Pa) is of the same order of magnitude as the elastic modulus for chemical gels such as poly(acrylamide) hydrogels or cross-linked biopolymer gels including actin, agarose, and lysozyme.[19] Further, strong DNA gels exhibit strain independence over the range of 10^{-2}−10^{0} strain, and one obtains reversible strain sweep spectra, indicating that the cross-links in strong DNA gels are stable under the experimental conditions. At lower cross-linker contents, so-called weak gels were obtained exhibiting frequency-dependent moduli. Strain sweep tests conducted from low to high strains and from reverse direction show a plateau below 10% strain; at larger strain G' decreases but reversibly, indicating that no fracture occurs even when a large strain was applied.

Since 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[20,21] $G = (\rho/\bar{M}_c)RT\nu_2^0$, 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 in their usual meanings. Assuming complete conversion of DNA to the cross-linked material ($\rho\nu_2^0 = 93$ kg/m^3), calculations show that $\bar{M}_c$ equals 164, 72, and 63 kg/mol for EGDE contents 5, 10, and 25%, respectively. Since the distance between the base pairs in ds-DNA is 0.34 nm, the result implies that, above 5% EGDE, the average distance between the effective cross-link points in the gel network is less than the persistence length of ds-DNA (50 nm); that is, the rigid segment of ds-DNA is connected each other using EGDE bridges.

Thermal Behavior. Thermal behavior of DNA gels and DNA solutions was investigated by heating the samples 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. For dilute solutions of DNA (26 mg/L) in 4.0 mM NaBr, this heating procedure resulted in almost complete melting of ds-DNA, i.e., formation of ∼95% ss-DNA fragments (Figure S1). On cooling back to 25 °C, the amount of ss-DNA fragments decreased to 60% (Figure S1). In our experiments, the changes in the dynamic moduli of gels and solutions were monitored during the course of the heating and cooling cycle as a function of temperature and time. At EGDE contents of 10% or above, no significant changes in the moduli of gels were observed. For example, Figure 4 shows the mechanical spectra of gels with 10−49% EGDE before and after the heating−cooling cycle. Both moduli as well as the loss factor of strong DNA gels slightly increase after the cycle. One may expect that the cross-linker EGDE remained unreacted in the gel after the cross-linking process may further react during the heating step so that G' increases. Further, melting of some DNA fragments may produce dangling chains on the gel network leading to the increase in the viscous moduli of gels after the cycle. Thus, the gels at high cross-linker contents are largely stable to changes in the temperature.

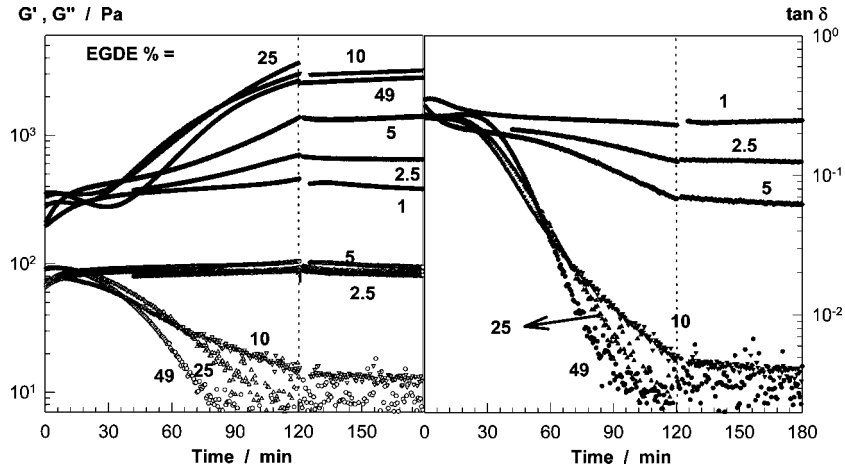

Figure 2. Elastic modulus G' (filled symbols), the viscous modulus G'' (open symbols), and the loss factor tan δ during the cross-linking of ds-DNA in the presence of various amount of EGDE indicated. The dotted vertical line represents the transition from the isothermal reaction period at 50 °C to that at 25 °C. $\omega = 1$ Hz, $\gamma = 0.01$.

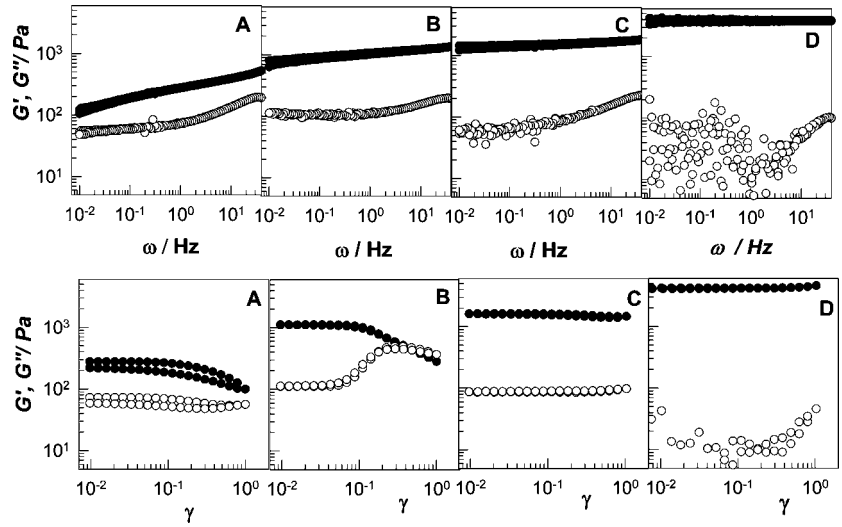

Figure 3. G' (filled symbols) and G'' (open symbols) of DNA gels and DNA solution at 25 °C shown as a function of the frequency ω at $\gamma = 0.01$ (upper graphs) and strain γ at $\omega = 1$ Hz (lower graphs) measured just after the gelation reactions. Sweep tests were conducted in up and down directions. All up and down sweeps overlapped except the strain sweep curves of the un-cross-linked sample. EGDE = 0 (A), 3.0 (B), 5 (C), and 25% (D). The DNA gels were made within the parallel plates of the rheometer except the gel with 3.0% EGDM which was made in an oven at 50 °C.

However, significant variations in the dynamic moduli of the samples were observed at low cross-linker contents. In Figure 5, the variations of G' and G'' of the samples with 0−2.5% EGDE are shown during the course of the heating and cooling periods. During the heating period and particularly above 70 °C, the moduli first slightly decrease and then increase as the heating time at 90 °C is increased. The increase in both moduli at 90 °C is significant for the un-cross-linked sample. Further, a dramatic increase in the elastic and viscous moduli was also observed during cooling of the samples back to 25 °C. For example, the un-cross-linked sample of DNA exhibits an elastic modulus of 65 kPa after the heating−cooling cycle compared to its initial value of 0.2 kPa. At the same time, the viscous modulus of this sample also increases from 0.06 to 11 kPa during the cycle. More than 2 orders of magnitude increase of both moduli suggest occurrence of conformational changes in the DNA chains during the heating and cooling periods.

Figure 5 also shows that, as the cross-linker content is increased, the extent of the increase of both moduli decreases. The larger the cross-linker content, the smaller the dynamic moduli of gels after the heating−cooling cycle. Let G' and G'_T be the elastic moduli of gels before and after the heating−cooling cycle; the fractional increase of the modulus $f_{G'}$ can be calculated as $f_{G'} = G'_T/G' - 1$. In Figure 6A, $f_{G'}$ is plotted as a function of the initial modulus G', which is proportional to the number of effective cross-links in DNA gels. It is seen that the formation of chemical cross-links between the DNA strands significantly suppresses the increase of the modulus of gels during the heating−cooling cycle. In Figure 6B, the loss factor tan δ of the gels are shown before and after the heating−cooling cycle plotted against the initial modulus G'. At high cross-link densities, tan δ slightly increases. This is, as mentioned above, due to the formation of dangling chains by the partial melting of ds-DNA in strong gels, which increases their viscous properties. In contrast, however, at low cross-link densities, tan δ slightly decreases, indicating that the elastic component of the complex modulus increases more than its viscous component. After the heating−cooling cycle, frequency and strain sweep tests were repeated at 25 °C. The results are shown in Figure S2 for the DNA solution and for DNA gels with low cross-link densities both before and after the cycles. The general trend is that, although both moduli dramatically increase, they exhibit increasing extent of frequency and strain dependences after the cycle.

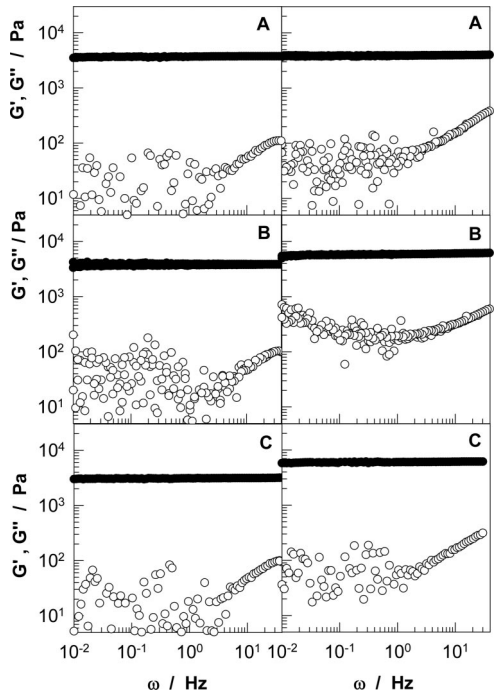

Figure 4. G' (filled symbols) and G'' (open symbols) of DNA gels at 25 °C shown as a function of the frequency ω before (left) and after the heating−cooling cycle (right). $\gamma = 0.01$. EGDE = 10 (A), 25 (B), and 49% (C).

To explain the observations, hyperchromicity measurements were performed to get information concerning the conformation changes of DNA during the heating and cooling periods. For this purpose, gels and solution of DNA with a concentration of 9.3% were heated to 90 °C with a rate of 3.25 °C/min, and after 10 min, they were quenched in an ice bath to fix the conformation of the DNA molecules they assumed at 90 °C. UV measurements were conducted after dilution of the samples to a concentration of 26 mg/L. The results are collected in Table 1. The normalized absorbance was found to be 1.072 for the un-cross-linked sample, indicating that about 18% of the DNA segments melt to form ss-DNA regions. The fraction of ss-DNA fragments decreases from 18 to 6% as the EGDE content is increased from 0 to 2.5%. It should be noted that the measurements on gels with higher cross-linker content cannot be conducted due to the fact that they cannot be diluted to a concentration of 26 mg/L. After cooling the samples at 90 °C back to 25 °C with a rate of 1.1 °C/min, comparison of the UV absorptions at 260 nm reveals that the degree of denaturation remains at 7%, and it further decreases as the cross-linker content is increased (Table 1). Thus, the hydrogen bonds that were partially broken during heating form again during the cooling period both in gels and in the solution. It should be mentioned that the complete melting of DNA at 90 °C cannot be achieved due to the high DNA concentration. If the solutions were first diluted with 4.0 mM NaBr to a concentration of 26 mg/L before the heating procedure, the melting of DNA was almost complete at 90 °C (Figure S1). The reason why the double-helix molecules do not dissociate completely even at 90 °C is due to the high concentration of the DNA counterions. 9.3% w/v DNA solution is 0.3 M in phosphate groups carrying a net negative charge or, due to the condition of electroneutrality, 0.3 M in counterions. Such a high counterion concentration in the gel or in the solution increases the stability of ds-DNA, as was observed by adding salt to dilute DNA solutions.[23]

The dramatic increase of both moduli during heating and cooling periods can thus be related with the partial denaturation and renaturation of DNA chains in the hydrogels, respectively. The following scenario may explain the results of our observations (Figure 7). The hydrogen bonds holding the two strands together break on heating so that the double helix partially dissociates into two single-strand fragments. Since ss-DNA is a flexible polymer compared to semiflexible ds-DNA, formation of flexible strand portions would increase the number of entanglements so that the moduli of the samples increase. On cooling back to 25 °C, due to the high DNA concentration of the system, the strands, which were partially melted during the heating period, cannot reorganize to form the initial double-stranded conformation. Hence, the hydrogen bonds are formed during cooling between strands belonging to different ds-DNA molecules. These portions of the strands forming double-stranded regions act as physical junction zones in addition to the chemical cross-links formed by EGDE so that the elastic modulus increases. Very large elastic and viscous moduli of the final gels also reveal formation of a poorly connected three-dimensional DNA structure with the presence of mobile aggregates of DNA. Further, since EGDE decreases the extent of denaturation of DNA, lesser number of H-bonds is disrupted during heating so that lesser number of physical cross-links forms during the cooling step. As a consequence, the fractional increase in the modulus decreases on raising the cross-linker content.

The results also show that the partial denaturation−renaturation cycle of the DNA solution produces a physical gel exhibiting unusual large dynamic moduli. To increase further the moduli of DNA solutions, the duration of the isothermal heating period at 90 °C was increased from 10 to 25 min. This new heating−cooling procedure increased G' and G'' to 115 and 32 kPa, respectively, compared to the values of 65 and 11 kPa found by experiments with 10 min heating time at 90 °C (Figure S3). DNA solutions were also subjected to two successive heating−cooling cycles. During the second heating period, melting of the physical cross-links decreased both moduli, but then, they increase rapidly due to the increasing number of entanglements between ss-DNA fragments as well as due to the formation of physical cross-links (Figure S3). A value of elastic modulus of close to 0.5 MPa was obtained after the second cycle, indicating that a physical gel based on the DNA exhibiting an elastic modulus in the order of MPa can easily be prepared by successive heating−cooling cycles.

Previous works showed that the semidilute solutions of ds-DNA in the concentration range 0.1−1.0% w/v becomes less helical and more flexible on heating so that the viscosity of the solution significantly decreases.[6,7] However, the present work conducted at a DNA concentration of 9.3% w/v shows opposite behavior, except that a slight decrease in the moduli was observable between 70 and 90 °C (Figure 5). To explain the reason for this contradiction, DNA solutions of various concentrations were subjected to the heating−cooling cycles. The results are shown in Figure 8, where G', G'', and tan δ are plotted against the temperature and time for three different DNA concentrations. It is seen that the slight decrease of the dynamic moduli above 70 °C, as was observed at 9.3% w/v DNA, becomes more pronounced as the DNA concentration is decreased. At or below 6% w/v DNA, G'' exceeds G' above 70 °C; i.e., the loss factor tan δ becomes larger than unity, indicating liquidlike response of the system due to the melting of DNA chains. However, on cooling back to 25 °C, tan δ decreases again below unity, indicating reformation of the viscoelastic gel. Thus, depending on concentration, dynamic moduli of DNA solutions decrease or increase during melting at 90 °C. Figure 8 also shows that thermoreversible DNA hydrogels can be obtained at or below 6% w/v DNA concentrations without a chemical cross-linker. These gels can be melted and re-formed simply by the heating and cooling cycles.

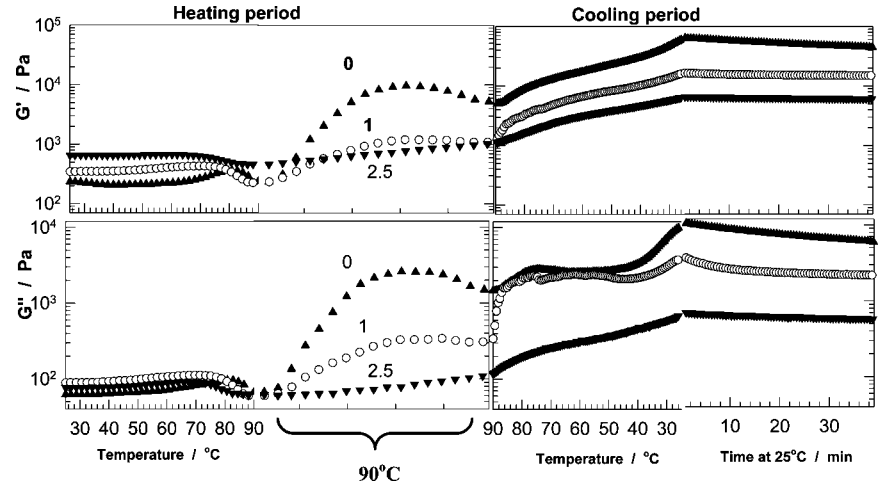

Figure 5. Viscoelastic behavior of gels and solutions of DNA during the heating−cooling cycle. $\omega = 1$ Hz, $\gamma = 0.01$. EGDE = 0 (▲), 1.0 (○), and 2.5% (▼).

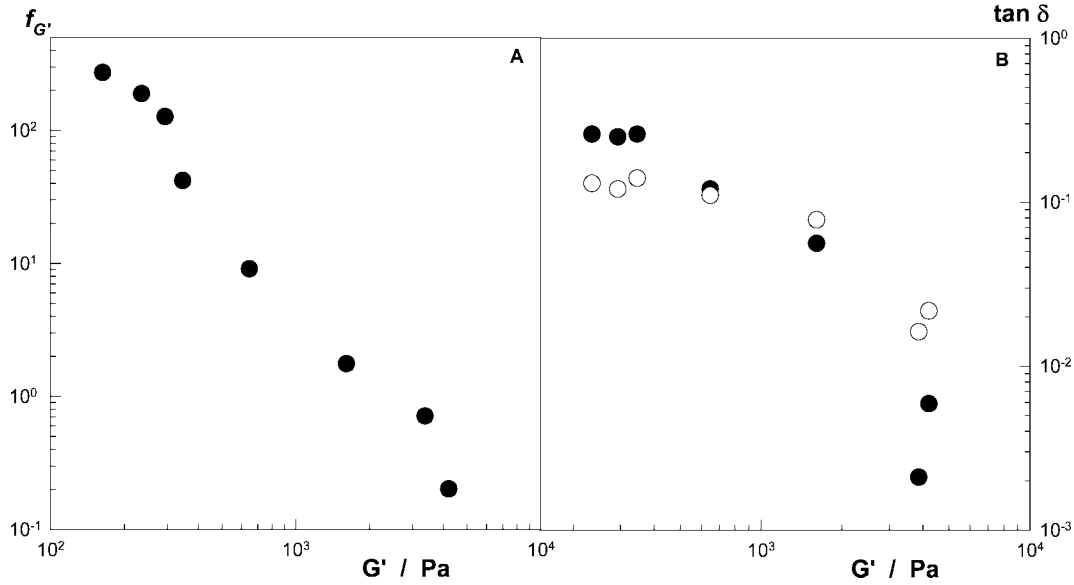

Figure 6. (A) Fractional increase of the elastic modulus of DNA gels after the heating−cooling cycle ($f_{G'}$) shown as a function of their initial modulus G'. (B) Loss factor tan δ of DNA gels and DNA solutions before (filled symbols) and after the heating−cooling cycle (open symbols) shown as a function of G'.

Table 1. UV Absorbance at 260 nm Relative to That Measured at 25 °C (A_{rel}) and the Amount of ss-DNA Fragments (ss-DNA %) for Various Cross-Linker Contents[a]

	at 90 °C		at 25 °C	
EGDE %	A_{rel}	ss-DNA %	A_{rel}	ss-DNA %
0	1.072	18	1.027	7
1	1.053	13	1.018	4
2.5	1.023	6	1.007	2

[a] The samples were first heated to 90°C at a rate of 3.25°C/min, and after 10 min, they were quenched in an ice bath to fix the DNA conformation at 90°C. The results are shown in the second and third columns. The results in the fourth and last columns were obtained by slowly cooling the samples back to 25°C at a rate of 1.1°C/min. The relative standard deviations for ss-DNA % were less than 7%.

Critical Overlap Concentration of DNA. The results presented in the previous section can be explained by the change of the critical overlap concentration of DNA depending on its conformation. In comparison with ds-DNA, ss-DNA is more flexible since its persistence length p is about 1 nm while that of ds-DNA is around 50 nm.[24−26] Since the contour length L of DNA used in this work with 2000 base pairs (bp) is much larger than the persistence length p, the DNA molecules can be considered as ideal random walks of rigid segments, so that its radius of gyration R_g in dilute solutions can be calculated using the equation[5,26,27]

$$R_g = (Lp/3)^{0.5} \tag{3}$$

Since one bp corresponds to 0.34 nm of length along the ds-DNA chain, its contour length L equals 680 nm. For ss-DNA chains, the distance between the bases is 0.58 nm,[25,28] which gives $L = 1180$ nm. Using these values together with eq 3, R_g was estimated as 106 and 20 nm for ds- and ss-DNA, respectively. The critical concentration c^* that the DNA chains start to overlap can be calculated as[29]

$$c^* = \frac{\bar{M}_w/N_A}{\left(\frac{4}{3}\pi R_g^3\right)} \times 10^2 \quad \text{(in \% w/v)} \tag{4}$$

where $\bar{M}_w$ is the molecular weight of DNA and N_A is the Avogadro's number. ds-DNA has a molecular weight of 2000 bp or 1.3×10^6 g/mol, while ss-DNA formed by its dissociation would have $\bar{M}_w = 0.65 \times 10^6$ g/mol, which lead to $c^* = 0.043$

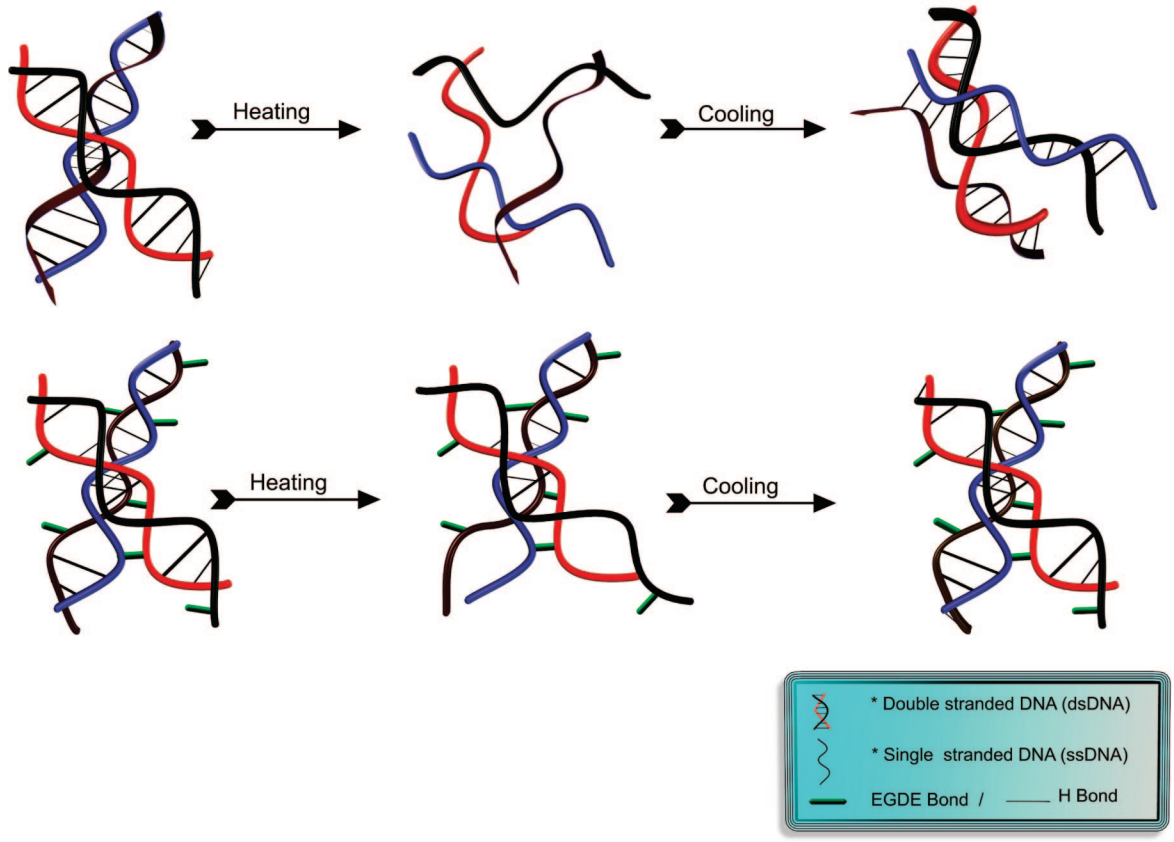

Figure 7. Cartoon demonstrating conformational changes of DNA in solutions (up) and in gels (down) during the heating−cooling cycle.

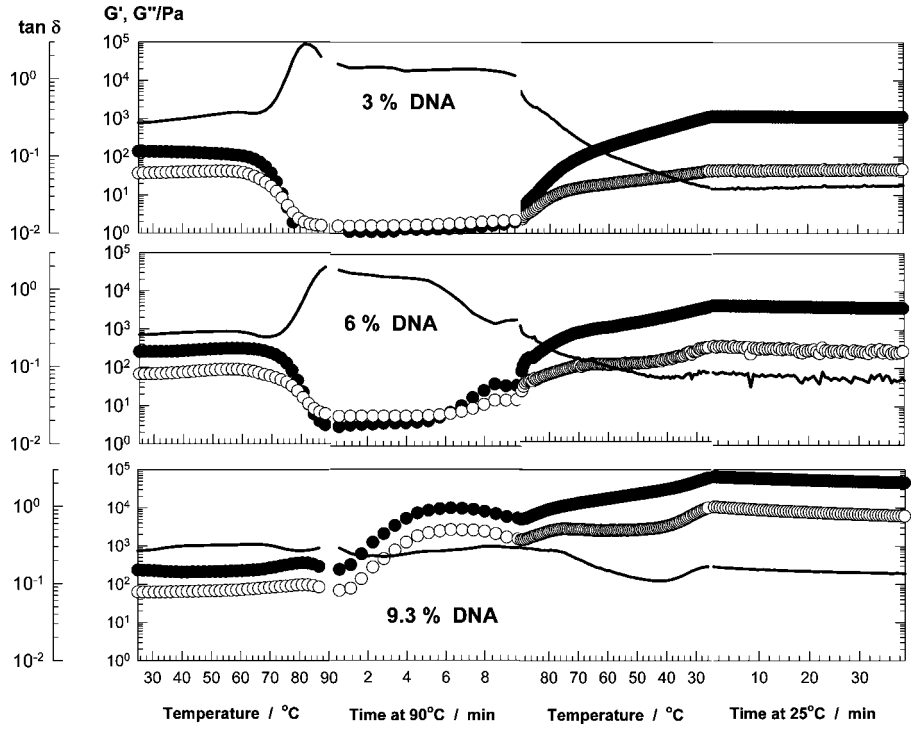

Figure 8. G' (filled symbols), G'' (open symbols), and tan δ (solid curves) of DNA solutions during the heating−cooling cycle at $\gamma = 0.01$ and $\omega = 1$ Hz. DNA concentrations (in % w/v) are indicated.

and 3.2% w/v for ds- and ss-DNA, respectively. Thus, a ds-DNA solution having a concentration between 0.043% and 3.2% w/v will undergo a transition between semidilute and dilute regimes during heating and cooling cycles. It should be mentioned that c^* may not define the true entanglement concentration c_e at which the onset of the plateau elastic modulus is observed.[5] c_e is considerable larger than the overlap concentration c^*. According to Figure 8, c_e for ss-DNA should occur between 6% and 9.3% w/v DNA compared to $c^* = 3.2\%$ w/v.

Thus, the underlying physical picture leading to the observed phenomenon in Figure 8 is two opposite effects of DNA melting on the viscoelastic behavior of DNA solutions. If the DNA

concentration c is above the critical entanglement concentration c_e for ss-DNA chains, the solution remains in the semidilute regime during the course of the melting process. Thus, melting of ds-DNA increases the dynamic moduli due to the increasing number of entanglements in the semidilute solution of flexible ss-DNA chains. Opposing this, however, if c is below c_e, melting of semidilute ds-DNA solution produces a dilute ss-DNA solution so that both moduli drastically decreases. Thus, DNA solutions at concentrations 6% w/v or below form thermally reversible gels due to the transition between semidilute and dilute regimes depending on the conformation of DNA chains. Moreover, since increasing number of entanglements between ss-DNA chains at high temperature also increases the probability of hydrogen bonding between the base pairs, larger dynamic moduli were obtained on cooling of solutions of higher DNA concentration. As seen in Figure 8, cooling the solutions back to 25 °C produces viscoelastic gels exhibiting elastic moduli of 1 and 65 kPa for the DNA concentrations 3% and 9% w/v, respectively. As a final note, the results in Figure 8 imply that the heating−cooling cycles of DNA solutions offer a simple technique for the preparation of thermally reversible pure DNA hydrogels. The network elastic modulus of the gels can be reliably and precisely tuned over more than three decades by the heating and cooling cycles.

Conclusions

Dynamic rheological measurements were performed during the cross-linking process of DNA in the presence of EGDE cross-linker in order to follow the gradual formation of the three-dimensional DNA network. The cross-linking of DNA is characterized by an initial lag phase of about 30 min, during which the dynamic moduli remain almost unchanged. The lag phase is followed by a log phase during which the elastic modulus G' rapidly increases while the viscous modulus G'' decreases. Mechanical spectra of DNA gels with a high cross-linker content show that G' is more than 2 orders of magnitude larger than the viscous modulus G'', and both moduli are essentially independent of frequency over the range $10^{-2}-10^{1}$ Hz. At lower cross-linker contents, so-called weak gels were obtained exhibiting frequency-dependent moduli.

Thermal behavior of DNA gels and DNA solutions was investigated by heating the samples above the DNA melting temperature and subsequently cooling down to 25 °C. At high cross-linker contents, no significant changes in the moduli of gels were observed, indicating that these gels are largely stable to changes in the temperature. At low cross-linker contents, however, a significant increase in the dynamic moduli of the samples was observed during the heating and cooling periods. The larger the cross-linker content, the smaller the dynamic moduli of gels after the heating−cooling cycle, indicating that the chemical cross-links between the DNA strands significantly suppresses the increase of the modulus of gels during the heating−cooling cycle. The results were explained with the partial dissociation of the double helix into single-strand fragments during the heating period, which increases the number of entanglements so that the moduli of the samples increase on heating. On cooling back to 25 °C, the dissociated strands cannot reorganize to form the initial double-stranded conformation so that the hydrogen bonds are formed between strands belonging to different ds-DNA molecules. These portions of the strands forming double-stranded regions act as physical junction zones in addition to the chemical cross-links formed by EGDE so that the elastic modulus increases. The results also show that the heating−cooling cycles of DNA solutions produces a physical gel exhibiting an elastic modulus on the order of megapascals. Thermoreversible DNA hydrogels were also obtained at or below 6% w/v DNA concentrations without a chemical cross-linker due to the transition of the system between semidilute and dilute regimes depending on the conformation of DNA chains.

Supporting Information Available: Figure S1 showing the thermal denaturation and renaturation curves of a dilute DNA solution (26 mg/L), Figure S2 showing the mechanical spectra of DNA gels at 25 °C before and after the heating−cooling cycle, and Figure S3 showing the viscoelastic behavior of DNA solutions (9.3% w/v) during first and second heating and cooling cycles with an isothermal heating period at 90 °C of 25 min. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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(18) Samples taken before and after the gelation reactions were diluted to a concentration of 26 mg/L, and their UV absorbencies at 260 nm were recorded. The rise of the optical absorbance, normalized with respect to that measured before the reaction, was less than 1.008 for EGDE contents between 0 and 2.5%. Assuming that the DNA strands melt completely, a rise of the normalized absorbance to 1.4 is expected.[22] The results thus suggest that the solutions and gels contain less than 2% ss-DNA fragments after the reactions. At higher EGDE contents, UV measurements cannot be conducted due to the insolubility of the samples in water.
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