A Calorimetric and Spectroscopic Study of DNA at Low Hydration

Sau Lawrence Lee,[†] Pablo G. Debenedetti,*[,†] Jeffrey R. Errington,[‡] Brian A. Pethica,[†] and David J. Moore[§]

Department of Chemical Engineering, Princeton University, Princeton, New Jersey 08544, Department of Chemical Engineering, University at Buffalo, The State University of New York, Buffalo, New York 14260, Unilever Research US, 45 River Road, Edgewater, New Jersey 07020

Received: October 7, 2003

Differential scanning calorimetry (DSC) and infrared spectroscopy (IR) were used to study the thermal behavior of DNA as a function of water content up to 12 wpn (water molecules per nucleotide). The DSC and IR results show cooperativity of thermal DNA denaturation at 12 wpn. In the 3.3 to 7.2 wpn range, an early structural change involving slight disruption of base stacking arises, followed at higher temperature by a denaturation process where the major changes in backbone conformation occur. DNA thermal denaturation in the 3.3 to 12 wpn range is irreversible. However, structural disruptions caused by dehydration are fully reversible. The loss of the DNA ordered conformation upon dehydration leads to a decrease in the endothermic maximum that characterizes the thermal denaturation of DNA. At 3.3 wpn, the DNA loses most of its ordered conformation and undergoes a glasslike transition similar to the transition that denatured DNA experiences during reheating. The calorimetric manifestation of the glass transition is established for denatured DNA at low hydration through the study of water plasticizing effects on T_g and enthalpy relaxation. At 1.0 wpn (the lowest water content achieved in this study), the DSC and IR data do not show any thermal or structural transitions, indicating the fully amorphous character of DNA at such low hydration.

I. Introduction

Nucleic acids are responsible for storage, transmission, and realization of genetic information in cells. They are linear biopolymers made up of nucleotide units. The nucleotide unit consists of a base, a deoxyribose (DNA) or ribose (RNA) sugar, and phosphate. Water is key to the stabilization of the biologically active structures of DNA and RNA. In particular, the stability and conformational integrity of nucleic acids are largely controlled by the interactions with surrounding water molecules.[1−3] Previous studies using X-ray diffraction and infrared and ultraviolet spectroscopy have shown that hydration water is necessary for maintaining the structural integrity of the DNA molecule.[4−7] In the absence of water, the ordered conformation is lost and base stacking is destroyed. Since there is a close correspondence between the biological function of biopolymers and their structure, understanding the relation of water to nucleic acids is of both practical and fundamental importance.

Hydration of nucleic acids can be described generally in terms of two distinct water layers, the primary and secondary hydration shells. For double-helical DNA molecules, about 20 water molecules per nucleotide, which hydrate the phosphate, sugar and base at a relative humidity (RH) of about 80%, form the primary hydration shell, while the more loosely bound water molecules form the secondary hydration shell at a higher RH.[8] In the primary hydration shell, at least 11 to 12 water molecules per nucleotide that are directly bound to the DNA are incapable of crystallization to ice upon cooling to temperatures well below 0 °C.[9,10] The tightly bound water molecules in the primary shell constitute an integral part of the stability of nucleic acid ordered structures.[11] These strongly bound water molecules are difficult to remove, and their removal leads to the collapse of the double helical structure of DNA.[12] Many computational and theoretical studies[13−16] as well as experiments[17,18] have been done to investigate the structural organization of these bound water molecules around a DNA molecule.

DNA has been shown to exist in a variety of secondary structures or conformations. These conformations include the right-handed double-helical A, B, C, and D forms and the left-handed double-helical Z form. On the other hand, RNA is always in the A conformation.[19] One of the important factors in determining the conformation of DNA is the degree of hydration. At high relative humidity (e.g., ≥90%), DNA fibers or films adopt the B conformation at room temperature. As the relative humidity is reduced to a certain threshold, the decrease in hydration leads to the transformation of B-DNA into other conformations (e.g., A or C form), depending on the DNA sequence and the nature of the counterions involved.[20−23] Further decrease in relative humidity introduces a disordered state of DNA which is characterized by a lack of base stacking.[6,7] It is known from IR spectroscopic studies that the structural changes of DNA induced by dehydration are reversible upon rehydration.[4,7] In addition, the type of counterion and the amount bound to DNA molecules can have a significant effect on the conformation of DNA.[21,24,25]

Similar to the conformation of DNA at high relative humidity, DNA in solution also tends to adopt the B-type double helix.[26] Many studies have investigated the thermal stability of double-stranded DNA in aqueous solutions using differential scanning calorimetry (DSC), infrared (IR), Raman, and ultraviolet (UV) spectroscopy. From these studies, it is interesting to note that the melting curve of DNA in an aqueous solution, which shows the transition of the ordered (double-stranded B helix) to the

* Corresponding author: E-mail: pdebene@princeton.edu.
[†] Princeton University.
[‡] The State University of New York.
[§] Unilever Research US.

10.1021/jp0311409 CCC: $27.50 © 2004 American Chemical Society
Published on Web 02/07/2004

disordered state (single-stranded random coil), can be described reasonably well by a two-state transition model.[27,28] The structural changes during the thermal denaturation include disruption of base pairs, unstacking of the bases, and disordering of the deoxyribose-phosphate backbone. The DNA melting process in solution is cooperative, meaning that the structural change during thermal denaturation is abrupt so that no intermediate state exists between the fully native and fully denatured states, and this melting process is usually slowly reversible.[28,29] The studies of melting temperature in DNA solutions as a function of base composition and solvent environment (e.g., salt concentration) also provide valuable information about DNA structures and stabilizing interactions.[30] On the other hand, there are few published works on the thermal stability of nucleic acids at low hydration.[31,32]

Many recent published reports have shown that biopolymers (e.g., proteins and DNA) reveal some glassy characteristics when their water content is low, containing bound water only. The work of Angell and co-workers suggested an interesting analogy between proteins and glasses by analyzing their results in terms of the three canonical characteristics of relaxation in glass-forming systems: non-Arrhenius behavior, nonexponential relaxation, and nonlinearity of the relaxation.[33,34] The work of Sochava et al. and Tsereteli et al. also shows the calorimetric manifestation of a glass transition in many different biopolymers such as proteins and DNA in native, semi-native, and denatured states.[32,35] However, the relationship among the degree of hydration, the secondary structure of biopolymers, and the glass transitions observed in these systems at low hydrations has not been examined in sufficient detail.

As a step toward improving basic understanding of nucleic acid behavior at low water contents, we investigate energetic and structural changes of DNA as a function of temperature and water content (primarily at low hydration levels) using differential scanning calorimetry (DSC) and infrared (IR) spectroscopy. The moisture contents of nucleic acids investigated in this work are between 1.0 and 12 water molecules per nucleotide, the region where no water can be crystallized into ice even on cooling well below 0 °C. Both DSC and IR measurements were performed for DNA at various water contents, primarily in the temperature range from −10 to 160 °C. In this paper, we focus on studying the effects of bound water on the denaturation process of DNA in the solid state at low hydration, establishing general calorimetric characteristics of the glass transition in nucleic acids, and understanding details associated with the changes in structure upon heating. The outline of this paper is as follows. Section II describes the experimental procedures including sample preparation, DSC, and IR measurements. Section III presents the results and their interpretation. Finally, Section IV provides the main conclusions and suggestions for future work.

II. Experimental Methods

The fibrous solid of NaDNA from salmon testes was purchased from Sigma Co. This NaDNA was reported to have a MW of 1.3×10^6 with approximately 2000 bp.[36] The water content as received was measured to be 12 wt % (∼4 wpn), which was determined by Karl Fisher titration using a Mitsubishi CA-02 moisture meter. These samples were used without further purification. The DNA samples were first transferred to vials and 60 mL stainless steel DSC pans without sealing. They were then hydrated by storing them in desiccators over various saturated salt solutions at room temperature. The relative humidities of these saturated salt solutions range from 10% to 80%. The lowest water content was achieved by long exposure to drierite. In all cases (except for the samples in drierite), the hydration of NaDNA fibers was approached from lower values of relative humidity.[37] The samples were equilibrated to constant mass over saturated salt solutions for at least one week to achieve different water contents, ranging from 1 to 12 water molecules per nucleotide.

In addition to the Karl Fisher titration, the water content of NaDNA samples was determined by thermogravimetric analysis (TGA) using a Perkin-Elmer TGA 7. The typical hydrated samples had weights between 10 and 20 mg. During the TGA measurements, a sample was first heated to 160 °C. It was then isothermally held at 160 °C for 30 min to ensure that its weight reached a steady value, indicating that the sample lost all of its water due to evaporation. All measurements were performed under a constant flow (30 mL/min) of dry nitrogen gas. The weight loss of the sample was recorded as a function of time and temperature. The water contents measured by TGA agree with those measured with the Karl Fisher method.

Before carrying out DSC measurements, 30−40 mg of the hydrated samples inside the stainless steel pans were hermetically sealed with an O-ring to prevent any water loss or gain. In most cases, the maximum filling of the DSC pan was achieved. The open space inside a sealed sample pan did not affect our results since the DSC curves, normalized by sample mass, for maximum filling and half filling of the sample pans were indistinguishable within experimental uncertainty. After the DSC scan, the samples were reweighed to ensure that no water loss or gain occurred.

All DSC scans were performed using a Perkin-Elmer Pyris 1 differential scanning calorimeter equipped with a cooling accessory, Intracooler 2P. The DSC instrument was calibrated for temperature and enthalpy using pure indium. Nitrogen was purged through the Pyris 1 housing at 30 mL/min to prevent condensation of water vapor on the measuring surface. An empty sealed stainless steel pan was used as a reference.

For IR spectroscopy studies, thin films of DNA from salmon testes with different water contents were prepared as follows. About 10 mg of the DNA fibers were dissolved in 1 mL of deionized water for at least 24 h to ensure total dissolution. The DNA thin films were obtained by spreading and drying the viscous DNA solutions on AgCl infrared windows in a steady flow of dry nitrogen for at least 6 h. These DNA films were then transferred to desiccators under controlled relative humidities governed by the same saturated salt solutions described previously. Each film was equilibrated for at least a week to achieve a desired hydration level. Prior to IR measurements, the AgCl window with the DNA film was withdrawn from the desiccator and sealed quickly with a gasket of high-vacuum grease to another AgCl window to prevent any water loss. In correlation with the TGA and Karl Fischer titration, the water contents of DNA films were checked by monitoring the O−H absorbance at 3400 cm^{-1} (normalized by the absorbance at 1220−1240 cm^{-1}).[8] Under the same relative humidity, the hydration levels of the DNA film and DNA fiber are identical within experimental error.

IR spectra were recorded using a Nicolet Magna 560 Fourier transform infrared (FTIR) spectrometer equipped with a sample shuttle and a broad-band liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. The sealed DNA sample was mounted in a temperature-controlled holder (Harrick, Ossining, NY). For the current experiments, the temperature of DNA films was controlled and monitored between 20 and 130 °C using a Harrick temperature controller. Spectra were generated by co-

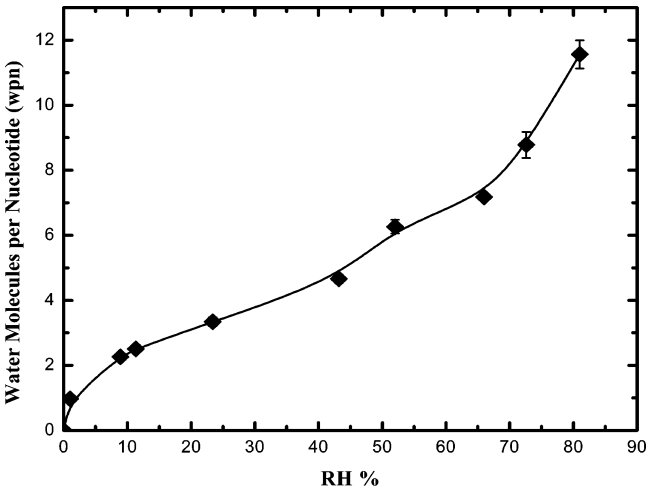

Figure 1. Water content (water molecules per nucleotide, wpn) as a function of relative humidity for NaDNA at 25 °C, as measured by thermal gravimetric analysis (TGA).

addition of 124 interferograms collected at a 4 cm^{-1} resolution in about 60 s, while the spectrometer was under a continuous dry air purge. If necessary, a spectrum of water vapor was subtracted from the sample spectrum.

III. Results and Discussion

(a) Differential Scanning Calorimetry (DSC). Figure 1 shows the water content in terms of water molecules per nucleotide (wpn) as a function of relative humidity (rh) for NaDNA fibers at 25 °C, as measured by TGA. Similar results were obtained using Karl Fisher titration. Comparison of the adsorption curve of NaDNA with the results of Falk et al.[37] and Lee et al.[38] shows good agreement. In this study, zero water content is never achieved. The lowest water content of DNA samples achieved in this study is ca. 1.0 wpn as shown in Figure 1. This was done by equilibrating DNA fibers over drierite (∼0 rh) for at least 1 week (note that the rh over drierite is not precisely known and the rh value corresponding to this point has been arbitrarily assigned in Figure 1). This result is consistent with the finding of Tao et al.,[39] showing that not all bound water molecules can be removed by exposing DNA samples over drierite.

Figure 2 shows a typical DSC scan of DNA at low hydration in the temperature range from 20 to 250 °C. Two salient features are observed in this figure for DNA at 12 wpn: an endothermic maximum at about 90 °C and an exothermic minimum around 200 °C. The endothermic maximum at first heating corresponds to the denaturation (or melting) of DNA as seen in solution. The exothermic minimum is due to pyrolysis of the DNA fibers since the sample becomes blackened after heating above 200 °C, as observed in a previous study by Marlowe et al.[12] Because the DNA is greatly damaged at 200 °C, this exothermic transition is not discussed further in this paper. Other than Figure 2, no DSC and IR results reported here were made at temperatures above 160 °C.

To distinguish the general features of thermal denaturation from those associated with the glass transition of solid DNA, the following DSC-scan steps were used. A DNA sample was first heated from an initial temperature to at least 140 °C. This is henceforth referred to as the first DSC scan. The sample was then immediately cooled to its initial temperature and held isothermally for 30 min. After the completion of the isothermal hold, the sample was reheated to at least 140 °C, a procedure referred to henceforth as the second DSC scan. These heating

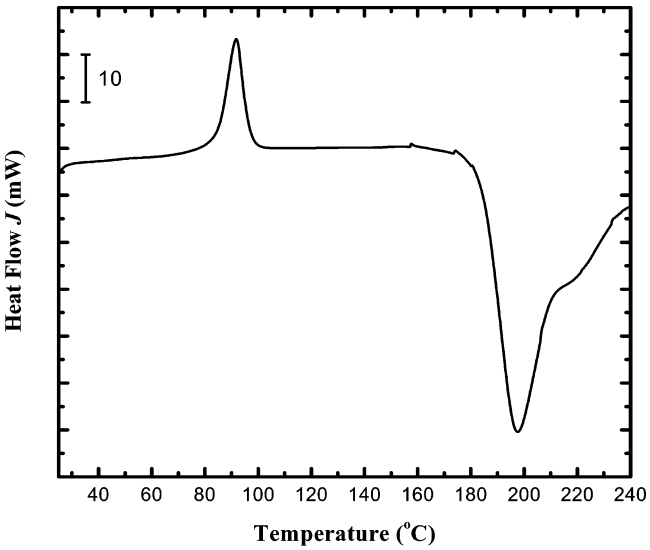

Figure 2. DSC scan (10 °C/min) of DNA at 12 wpn. The first endothermic maximum is due to the denaturation process, while the second exothermic minimum is due to pyrolysis.

and cooling scans were performed at a scan rate of 10 °C/min. Since the water content of DNA is known to have a significant effect on the denaturation and glass transition temperatures, the initial low-temperature limit of these DSC scans for each sample was adjusted to allow us to obtain a temperature range within which the entirety of the transitions observed in the first and second DSC scans are captured. In the present study, the initial temperature of the DSC-scan steps decreases with increasing water content. Similar DSC scan steps up to 95 °C were used for a 30 mg/mL DNA solution, which was prepared by dissolving DNA fibers in deionized water for at least 24 h. Figures 3, 4a, and 4b show the first and second DSC scans for DNA samples at various water contents (30 mg/mL solution and 1.0 to 12 wpn). Similar to a previous study,[27] a broad endothermic maximum, which corresponds to DNA melting, is observed around 80 °C for the first DSC scan of DNA in solution (Figure 3). The partial reappearance of such maximum in the second DSC scan after 30-minute isothermal hold indicates the reversible character of thermal denaturation of DNA in solution.

The first DSC scans of DNA with 1.0, 3.3, 4.7, 6.3, 7.2, 8.8, and 12 wpn are shown in Figure 4a. Similar to the DSC heating curve for DNA in solution (Figure 3), a rather sharp endothermic maximum near 90 °C, corresponding to DNA denaturation, is observed in the first DSC scan at 12 wpn. When the water content of DNA decreases from 12 to 6.3 wpn, this endothermic maximum (indicated by vertical arrows in Figure 4a) is broadened and gradually reduced in magnitude. Its temperature also decreases. As the hydration level of DNA samples is further reduced (4.7−3.3 wpn), the endothermic maximum is replaced by a step increase in the heat flow (J), while the temperature at which this increase in J occurs increases with decreasing water content. For the DNA with 1.0 wpn, no transition is found because at this hydration the DNA is fully disordered (see also IR results below), a condition characterized by absence of ordered base stacking and pairing.[6,40] At water contents from 3.3 to 8.8 wpn, an additional small maximum is observed at lower temperatures around 50−60 °C (indicated by a horizontal arrow in Figure 4a). A similar maximum has also been observed in the first scan of proteins and calf thymus DNA at low hydration levels in previous studies.[31,35] This feature is sharp for water contents in the range 4.7 to 7.2 wpn, and it eventually

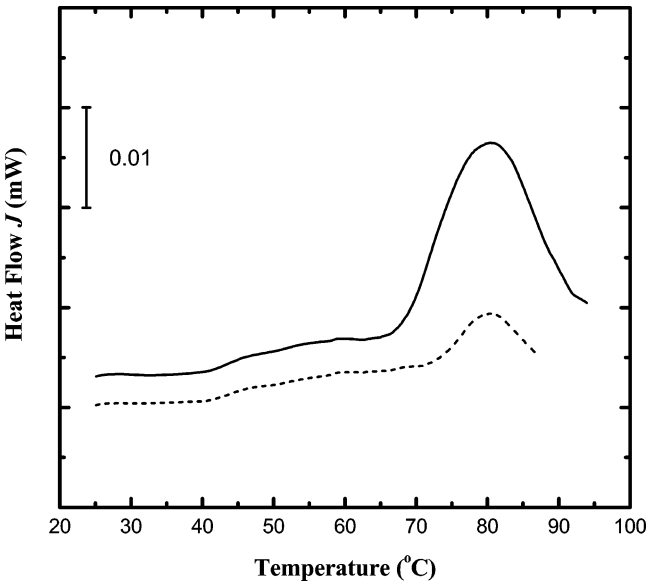

Figure 3. The first and second DSC scans of 30 mg/mL DNA in solution (10 °C/min scan rate). The DNA was first heated to 95 °C (solid line). It was immediately cooled to room temperature and held isothermally for 30 min. The sample was then heated again to 90 °C (dashed line). This second scan is shifted for clarity.

vanishes at 1.0 and 12 wpn (Figure 4a). The molecular details associated with DNA thermal denaturation at low hydration, as well as the origin of this additional maximum, will be discussed below in conjunction with the IR data.

In contrast to the reversible character of DNA melting in solution indicated by the partial recovery of the endothermic maximum (Figure 3), the thermal denaturation of DNA in the solid state is not reversible, illustrated by the complete absence of the endothermic maxima in the second scan (Figures 4b and 4c). Unlike the first DSC scans, all second DSC scans for denatured DNA are characterized by a single step increase in the heat flow (J) which corresponds to an increase in the heat capacity (C_p), except for the DSC scan of DNA at 1.0 wpn. The irreversible character of thermal DNA denaturation at low hydration levels is not simply caused by the much higher temperature on the first heating. An additional DSC experiment shows that the major endothermic maximum did not reappear in the second DSC scan for DNA with 7.2 wpn even though the sample was only heated to 98 °C during the first DSC scan. At the low hydration levels of 3.3 wpn, the first and second DSC scans become similar to each other. The resemblance of the first and second DSC scans, as well as the decrease in the intensity of the endothermic maximum (DNA denaturation) with water content, are caused by the structural change induced by dehydration. Spectroscopic data will be presented later in this section to support this argument.

Important features of the C_p increase observed in the second scans for the denatured DNA at low hydrations resemble those observed in the glass transition of synthetic amorphous polymers,[41,42] which will be discussed below. By analogy, the temperature at which the C_p increase occurs, indicated by an arrow in Figure 4b, is referred as to the glass transition temperature (T_g) in this study. This onset temperature decreases with increasing water content (Figure 5). Thus, bound water, which is essential for maintaining DNA structural and functional integrity, serves as a plasticizer of denatured DNA by depressing its T_g. In addition, as the water content reaches 12 wpn, the increases in C_p becomes more diffuse. A similar effect was observed in DSC studies of protein−water systems by Angell and co-workers[33] and by Miyazaki et al.[43,44] In Figure 5, the T_g for the DNA at 1.0 wpn is estimated to be above 200 °C by a simple extrapolation. Since the DNA will undergo pyrolysis before it reaches such a high temperature, no glass transition is found. Therefore, both first and second DSC scans for DNA at 1.0 wpn are simply characterized by a straight line (see Figure 4c).

The effect of scan rate (Q) on the T_g of denatured DNA at water contents of 2.5, 3.3, 4.7, and 6.3 wpn was also investigated. By increasing Q from 2 to 30 °C/min, the T_g of these denatured DNA samples increases by about 9−12 °C. In addition, anneal and scan studies were performed. The thermally denatured DNA samples that contain 4.7 and 6.3 wpn were annealed at room temperature for different times and were then heated to at least 140 °C. At a water content of 6.3 wpn, annealing of denatured DNA samples at 25° ($<T_g$) lead to an overshoot in the C_p increase (an additional endothermic maximum) during reheating as illustrated in Figure 6a. The temperature and intensity of this overshoot increase with increasing annealing time. This result is consistent with previous studies[45] and is known as enthalpy relaxation, which has also been observed in many glass-forming, protein−water and polymeric systems.[32,46,47] However, as the water content of DNA decreases, the intensity of the additional maximum is greatly reduced. For instance, at the water content of 4.7 wpn, only a small annealing prepeak developed despite annealing the DNA sample at 25 °C for a month (Figure 6b), indicating that only minor and slow enthalpy relaxation occurs during annealing at 25 °C at such low hydration. The first DSC scans for the DNA at 6.3 and 4.7 wpn are inserted in Figures 6a and 6b, respectively, for comparison with the DSC scans of the annealed DNA samples. The dissimilarity of the first DSC scan and those of the annealed samples (thermally denatured DNA) indicates that the DNA denaturation at low hydrations is thermally irreversible after 18−30 days at 25 °C. It is important to emphasize that the enthalpy relaxation phenomena observed in this study are quite different from those reported in the previous work of Rüdisser et al.[48,49] In their anneal-and-scan DSC studies, the DNA samples were not thermally denatured prior to the annealing step.

(b) Infrared Spectroscopy (IR). IR spectroscopy was used to probe the structural changes that accompany DNA denaturation at low hydration (1.0, 3.3, 7.2, and 12 wpn) during the first DSC scan. The IR spectra of DNA exhibit many characteristic bands that are sensitive to both dehydration and thermal disordering. In the following discussion, we focus on specific bands and spectral regions that characterize base stacking and pairing (1750−1550 cm^{-1}) and backbone conformation (1055−1065 cm^{-1} and 1080−1090 cm^{-1}).[50] Some of these bands have been previously observed to undergo significant changes upon DNA melting in solution.[51,52] Since the absorbance of the band around 1220 cm^{-1} due to the PO_2^- antisymmetric stretching vibration is largely invariant to temperature, it is used as a standard for intensity normalization in this study.

NaDNA from salmon testes equilibrated over RH above 95% or in solution adopts the B-form helix at 25 °C with characteristic IR bands at 835, 1086 (PO_2^- symmetric stretching vibration), 1223 (PO_2^- antisymmetric stretching vibration), and 1715 cm^{-1} (in-plane C═O and C═N stretching vibration of bases). An example is shown in Figure 7a. For the DNA with 12 wpn (at 88% rh), the A-form helix dominates, with characteristic IR bands at 861, 1089 (PO_2^- symmetric stretching vibration), 1234 (PO_2^- antisymmetric stretching vibration), and 1709 cm^{-1} (in-plane C═O and C═N stretching vibration of

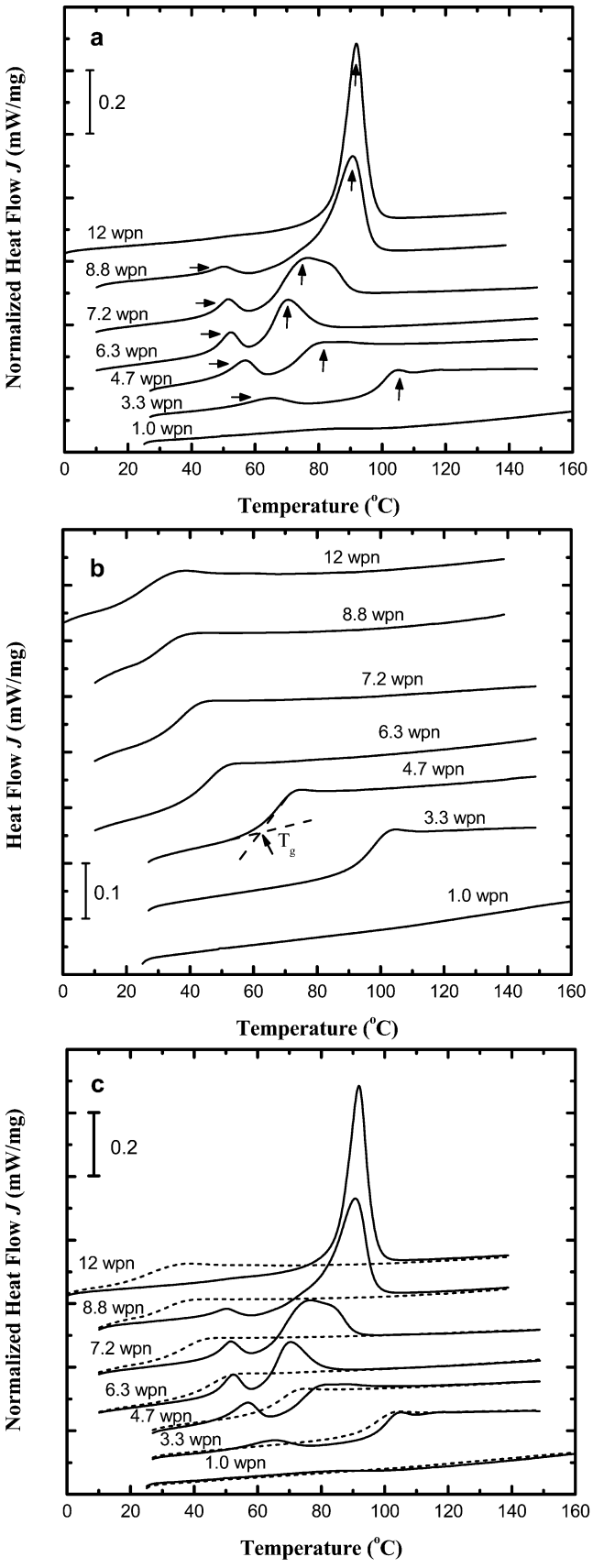

Figure 4. (a) First scans of DNA at various water contents ranging from 1.0 to 12 wpn. (b) Second scans of DNA at various water contents ranging from 1.0 to 12 wpn. (c) Comparison of the first and second scans of DNA at various water contents ranging from 1.0 to 12 wpn. The DSC scans were performed at a scan rate of 10 °C/min, and all heating curves in (a)−(c) are normalized by the sample mass.

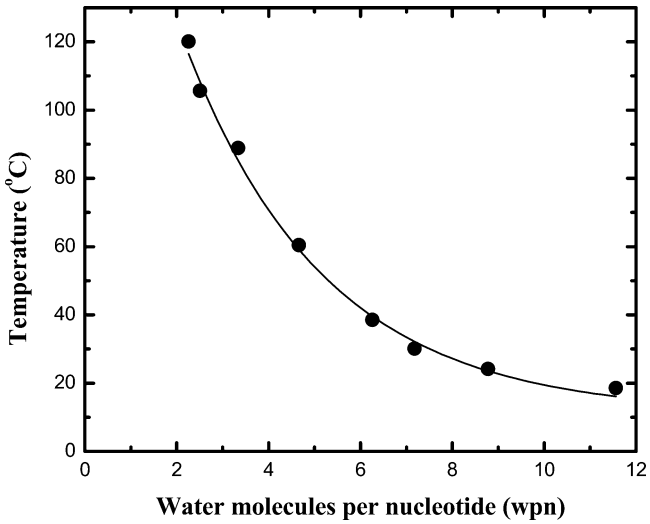

Figure 5. Dependence of glass transition temperature (T_g) of denatured DNA on water content. The determination of T_g is based on a scan rate of 10 °C /min.

bases). As the water content is further reduced, DNA starts to lose its ordered double-helix structure resulting in a decrease in the absorbance of the band around 1709−1715 cm^{-1}.[50] In contrast to the IR spectrum of DNA in solution (Figure 7a) and that of DNA with 12 wpn (Figure 7b), the peak around 1709−1715 cm^{-1} is not observed at water contents of 1.0, 3.3, and 7.2 wpn, as shown in Figures 7c, 7d, and 7e, indicating a loss of base interactions and at least partial denaturation of the DNA double-helix. Furthermore, none of these low-water IR spectra shows a set of characteristic IR bands that describes a specific helical structure of DNA. Unlike the thermal irreversibility of DNA denaturation at low hydrations, the IR spectral changes induced by dehydration are fully reversible upon rehydration. This is consistent with a previous study.[4]

Significant temperature-induced IR spectral changes are observed for DNA with 3.3, 7.2, and 12 wpn. In contrast, the DNA with 1.0 wpn reveals negligible spectral changes upon heating. As temperature increases, a significant change in absorbance is observed in the bands that arise from the PO_2^- symmetric stretching vibration (1085−1090 cm^{-1}) and the C−O stretching vibration of the phosphodiester (1060−1065 cm^{-1}). The temperature effects on the IR spectra within the 1000−1150 cm^{-1} region (backbone conformation) for DNA at 12, 7.2, and 1.0 wpn are shown in Figures 8a, 8b, and 8c, respectively. For DNA at 12 wpn, the absorbance of the 1089 and 1052 cm^{-1} bands decreases with temperature. As the temperature increases beyond 100 °C, the peaks associated with these two bands vanish, and a small and broad peak around 1064 cm^{-1} appears. For DNA at 7.2 wpn, as temperature increases, the absorbance of the 1087 cm^{-1} band decreases while the 1062 cm^{-1} band, which manifests itself only at temperatures above 40 °C, increases in its absorbance. Similar qualitative changes are observed in this region for DNA at 3.3 wpn (the IR spectrum for DNA with 3.3 wpn is not shown here). On the other hand, the general features of the IR spectrum within the 1000−1150 cm^{-1} region for DNA at 1.0 wpn are insensitive to temperature.

To investigate these backbone conformation spectral changes of DNA in a more quantitative manner, the absorbance of the band around 1085−1090 cm^{-1} (PO_2^- symmetric stretching vibration), normalized by the 1220 cm^{-1} band intensity, is plotted as a function of temperature for DNA at 1.0, 3.3, 7.2, and 12 wpn (Figure 9). Analogous to a melting curve of DNA in solution obtained by UV spectroscopy, the thermal profile

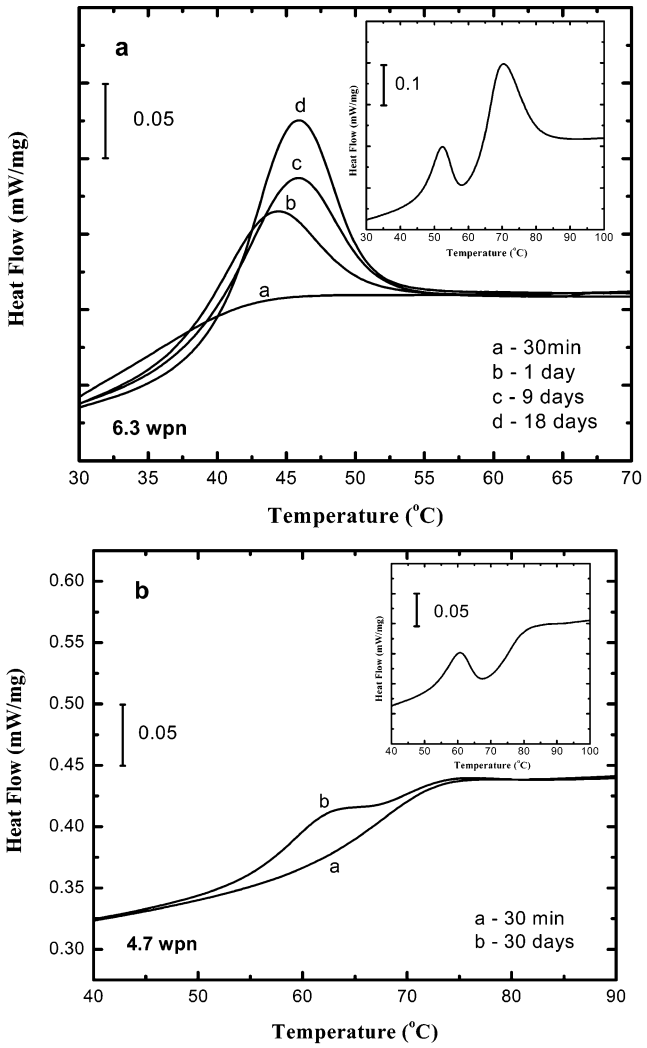

Figure 6. (a) DSC scans of thermally denatured DNA at 6.3 wpn, annealed at 25 °C for different times. (b) DSC scans of thermally denatured DNA at 4.7 wpn annealed at 25 °C for different times. The corresponding first DSC scans for DNA are shown as inserts, demonstrating the irreversible character of DNA denaturation at low hydration. All DSC scans are normalized by the sample mass.

for the phosphate vibrations of DNA at 12 wpn shows a sharp decrease in absorbance in the temperature range from 80 to 110 °C (cooperative transition). A similar transition is observed for DNA at 7.2 wpn between 50 and 80 °C, but the decrease in absorbance is much smaller. These are the respective temperature ranges where the large endothermic maximum is observed in the first DSC scan for DNA at 12 and 7.2 wpn. Therefore, the normalized absorbance of this IR band is a good measure of thermal denaturation of DNA. The marked change in the absorbance of the IR band around 1085−1090 cm^{-1} in these temperature ranges can be attributed to conformational changes in deoxyribose-phosphate backbone upon disruption of the DNA helical structure. For DNA at 3.3 wpn, the decrease in absorbance is significantly broadened, and it occurs at a higher temperature range. On the other hand, there is no visible transition in this spectral parameter over the temperature range studied for the 1.0 wpn DNA sample. This IR result is consistent with the DSC measurements, since the first DSC scan for DNA at 1.0 wpn does not show any thermal transitions (Figures 4a). The absence of any significant changes in both DSC scans and IR spectrum suggests that the DNA at 1.0 wpn is already fully disordered.

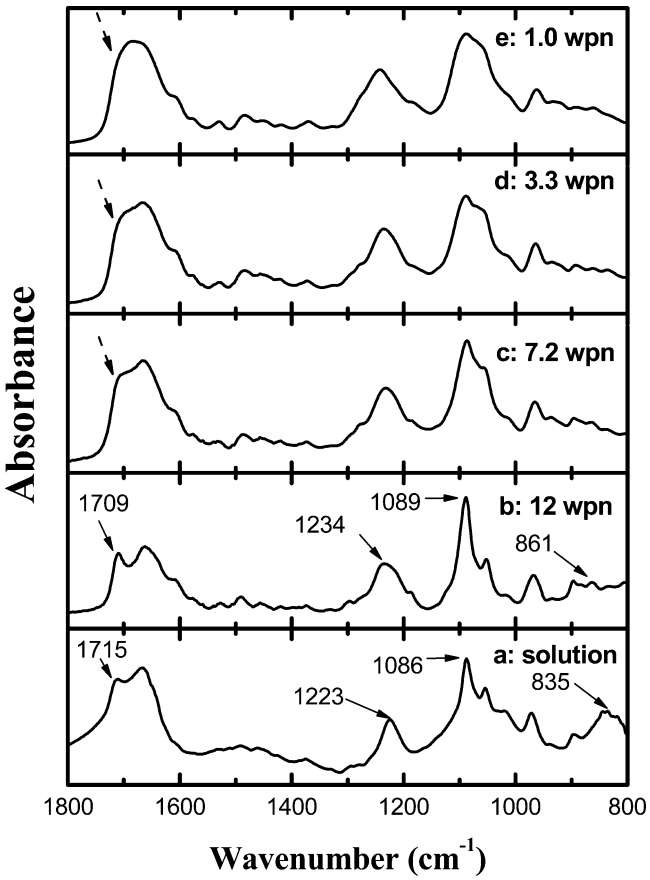

Figure 7. IR spectra of DNA containing different amounts of bound water at 25 °C prior to any thermal treatment. (a) 30 mg/mL solution, (b) 12 wpn, (c) 7.2 wpn, (d) 3.3 wpn, (e) 1.0 wpn.

From 12 to 7.2 wpn, the sharp transition indicated by the decrease in the absorbance of the IR band around 1085−1090 cm^{-1}, is progressively smeared and eventually vanishes at 3.3 wpn (Figure 9), corresponding to the DSC data where the intensity of the main endothermic maximum decreases from 12 to 4.7 wpn and is eventually replaced by an increase in J at 3.3 wpn (Figure 4a). This observation and the IR spectra shown in Figures 7b, 7c, 7d suggest that dehydration causes DNA to become progressively disordered. Since dehydration leads to the loss of ordered structure accompanied by the depletion of bound water, less heat is thus required for DNA denaturation. Therefore, the magnitude of the endothermic maximum of DNA decreases with decreasing water content (Figure 4a). At low enough hydration levels (e.g., 3.3 wpn) but still above 1.0 wpn, the amorphous character of DNA dominates and causes the nucleic acid to undergo a transition similar to the glass transition observed in the second DSC scan (Figure 4c).

The IR bands in the 1550−1750 cm^{-1} spectral region, which are assigned to various double-bond in-plane vibrations of the bases (e.g., C═O, C═N, and C═C), and are therefore sensitive to base stacking and pairing, also undergo significant changes in their absorbance as temperature increases. For DNA at 12 wpn (Figure 10a), the abrupt increase in absorbance of the band within the 1550−1750 cm^{-1} region shows that the structural changes in base stacking and pairing occur at the temperature at which the endothermic maximum is observed during the first DSC scans. The disappearance of the peak at 1709 cm^{-1} in this temperature range further suggests that these changes in base stacking and pairing are associated with the loss of A-form helical structure upon DNA denaturation. Since the backbone conformation-related changes in the absorbance of the IR band

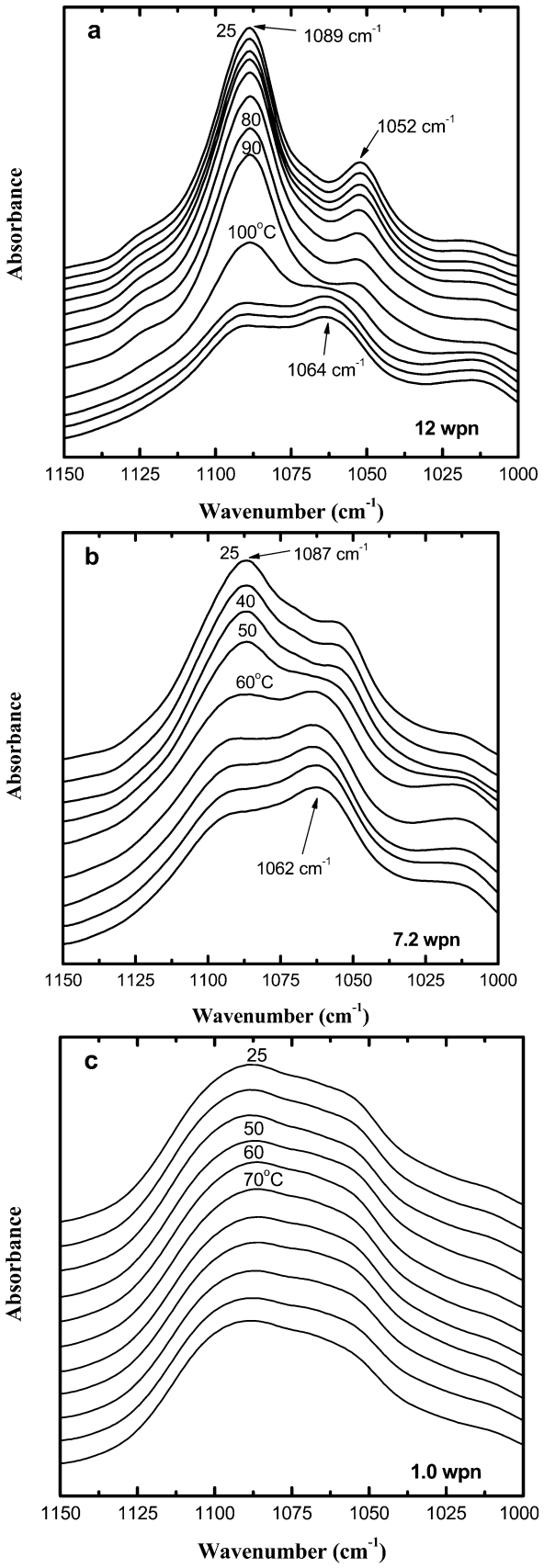

Figure 8. Temperature effects on IR spectra of DNA within the 1000−1150 cm^{-1} region at different water contents. The temperatures of each successive pair of spectra differ by 10 °C, except for spectra at 25 and 30 °C. (a) 12 wpn, (b) 7.2 wpn, (c) 1.0 wpn.

around 1085−1090 cm^{-1} also occur in a similar temperature range, the DNA denaturation at 12 wpn is a cooperative process.

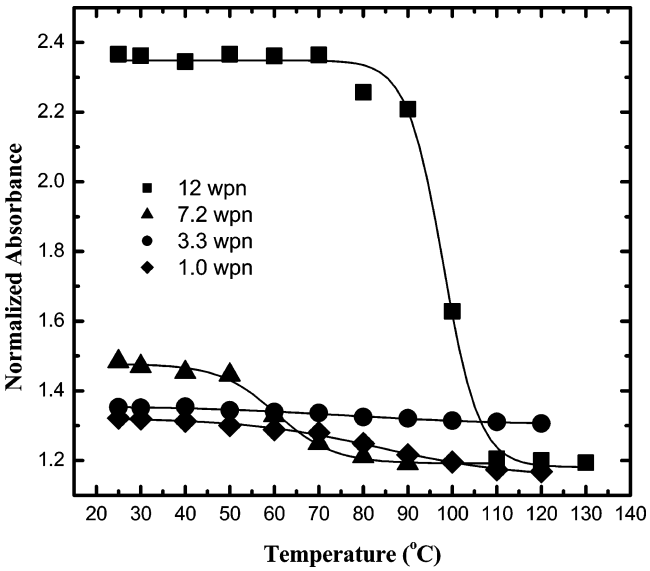

Figure 9. Thermal profiles of DNA at 1.0, 3.3, 7.2, and 12 wpn obtained by monitoring the absorbance of the symmetric phosphate stretching vibration band (1085−1090 cm^{-1}) band, normalized by the 1220 cm^{-1} band intensity, as a function of temperature.

On the other hand, for DNA with 7.2 and 3.3 wpn, this initially abrupt change in absorbance of the band within the base stacking-sensitive IR region starts to occur at the temperature where the first maximum in the first DSC scan occurs. This significant change in the absorbance continues approximately until the temperature of the second maximum, as observed in the first DSC scan, is reached. This is shown in Figures 10b and 10c. The magnitude of these changes in absorbance within the 1550−1750 cm^{-1} region decreases with decreasing water content since the samples with low water content lack any appreciable amount of ordered structure. Thus, the DNA at 1.0 wpn, which is fully disordered, shows no significant changes in its IR spectrum as a function of temperature (Figure 10d).

On the basis of the IR data shown in Figures 10b and 10c, it follows that the structural perturbations of DNA at the low hydration levels of 3.3 and 7.2 wpn, which is already in a semi-denatured state, happen before undergoing thermal denaturation (major endothermic maximum observed at higher temperature in the first DSC scan). In the first DSC scan, these structural perturbations are indicated by a small and broad endothermic maximum. To further understand the origin of this small maximum, three different DSC scans were performed. The first heating was interrupted before DNA denaturation occurs. As shown in Figure 11, this first DSC scan completely removes the small endothermic maximum, and the removal of such maximum does not have any effect on the major thermal denaturation in the second DSC scan. In addition, the IR spectrum after this first DSC scan retains its general features at the final temperature where the first heating is ended although the DNA sample is cooled back to room temperature. This thermal irreversibility of DNA at low hydration will be discussed further at the end of this section. After thermally denaturing the DNA, the glass transition is observed in the third DSC scan. As water content increases to 12 wpn and decreases to 1.0 wpn, such first endothermic maximum vanishes. Thus, the corresponding spectral signatures should not be associated with major structural changes such as the separation of double-stranded DNA. They are likely due to the slight structural perturbation of base stacking, indicated by the absorbance changes in the 1550−1750 cm^{-1} region.

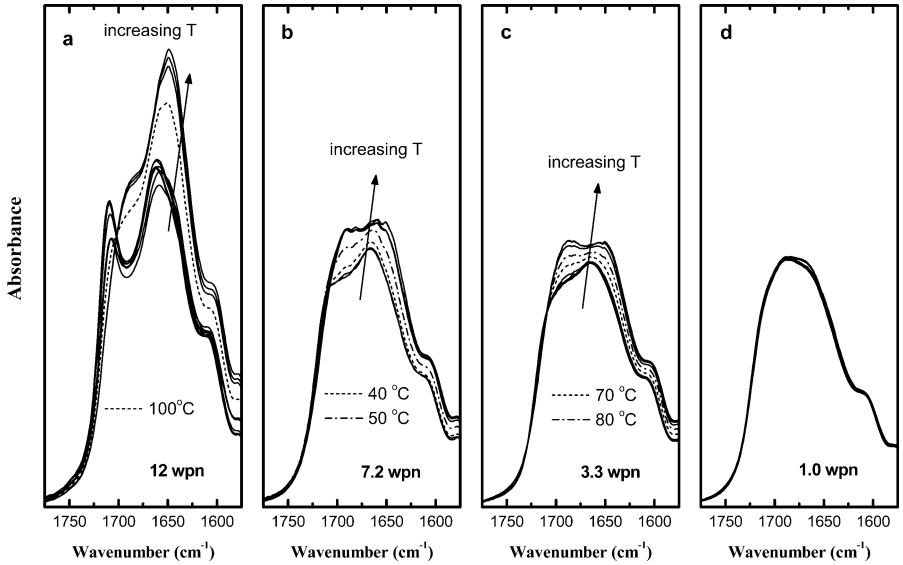

Figure 10. Temperature effects on IR spectra of DNA within the 1550−1800 cm^{-1} region at different water contents. (a) 12 wpn, (b) 7.2 wpn, (c) 3.3 wpn, (d) 1.0 wpn. The sample at each hydration was heated from 30 to 110 °C; spectra are shown at 10 °C intervals.

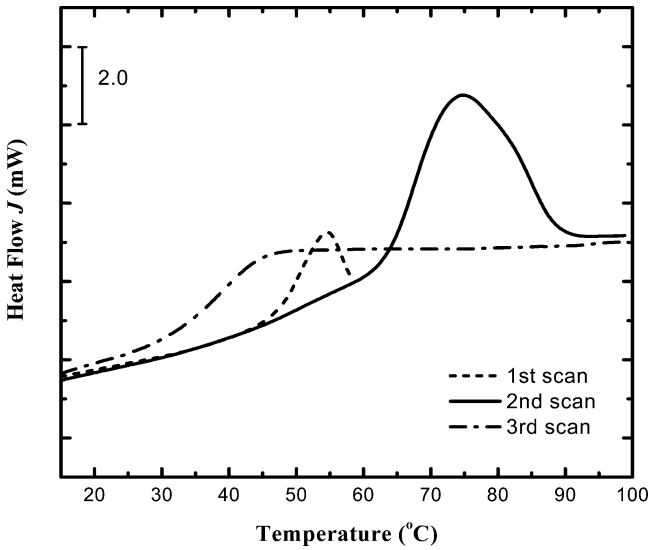

Figure 11. DSC scans (10 °C/min) of DNA at 7.2 wpn. The first heating was interrupted or stopped before denaturation occurred. The DNA was immediately cooled to 25 °C and then reheated instantly to 120 °C (no isothermal hold at 25 °C). Note that the first scan removes the endothermic peak at 55 °C. Comparison of second and third scans shows the irreversibility of thermal denaturation.

Additional IR spectra of DNA at 1.0, 3.3, and 7.2 wpn were taken from 30 to 130 °C after the DNA samples were heated once. The IR spectra at all temperatures during the second heating closely resemble those at high temperatures during the first heating. For instance, the intensity of the 1062 cm^{-1} band for DNA with 3.3 and 7.2 wpn never decreases to its original value (data are not shown here). This finding confirms the irreversible nature of thermal denaturation of DNA at low hydration. This irreversibility is not due to the loss of hydration water, since the intensity of 3400 cm^{-1} region, a convenient measure of DNA hydration, recovers its original value upon cooling.

IV. Conclusions

DSC and IR spectroscopy were used to investigate the thermal properties and thermal denaturation of salmon testes NaDNA at low water contents, corresponding to bound water only (1.0 to 12 wpn). Similar to DNA melting in solution, the DSC data show that the thermal denaturation at 12 wpn is characterized by a sharp endothermic maximum. The magnitude of this peak decreases with decreasing water content and eventually vanishes at 3.3 wpn due to the loss of ordered structure upon dehydration, as confirmed by the IR data. As a result, the first and second DSC scans become similar, and both scans are characterized by a step increase in *J*. Calorimetric manifestation of glass transition is established for denatured DNA by studying the plasticizing effect of water on T_g and enthalpy relaxation. In conjunction with the DSC data, the IR spectra show the cooperativity of thermal denaturation at 12 wpn. As the hydration level decreases (3.3 and 7.2 wpn), an early structural perturbation involving slight disruption of base stacking and pairing, corresponding to the small endothermic maximum in the first DSC scan, is observed. It is followed at higher temperature by a major denaturation process associated with the changes in a backbone conformation. The IR data confirm the irreversible character of DNA denaturation at low water contents, which is not simply due to the loss of bound water. For fully disordered, 1.0 wpn DNA, the DSC and IR results do not show any thermal or structural transitions, respectively.

On the basis of the present DSC and IR results, it will be interesting to extend our study to other DNAs, such as calf thymus DNA and polynucleotides, over a range of water contents from dehydrated samples up to concentrated solutions. This will allow the investigation of the effects of different DNA conformations at various water contents (other than A and B form helical structures) and DNA composition on the thermal stability of DNA. In addition, it will also be interesting to investigate the counterion effects on the thermal properties of DNA at low hydration levels.

Acknowledgment. P.G.D. gratefully acknowledges the support of Unilever Research US and thanks Professors J. L. Carey and D. W. Wood for helpful discussions.

References and Notes

(1) Texter, J. *Prog. Biophys. Mol. Biol.* **1978**, *33*, 83.
(2) Westhof, E. *Annu. Rev. Biophys. Biophys. Chem.* **1988**, *17*, 125.
(3) Saenger, W. *Annu. Rev. Biophys. Biophys. Chem.* **1987**, *16*, 93.
(4) Falk, M.; Hartman, K. A., Jr.; Lord, R. C. *J. Am. Chem. Soc.* **1963**, *85*, 391.

(5) Lindsay, S. M.; Lee, S. A.; Powell, J. W.; Weidlich, T.; DeMarco, C.; Lewen, G. D.; Tao, N. J.; Rupprecht, A. *Biopolymers* **1988**, *27*, 1015.
(6) Sclavi, B.; Peticolas, W. L.; Powell, J. W. *Biopolymers* **1994**, *34*, 1105.
(7) Zehfus, M. H.; Johnson, W. C., Jr. *Biopolymers* **1984**, *23*, 1269.
(8) Falk, M.; Hartman, K. A., Jr.; Lord, R. C. *J. Am. Chem. Soc.* **1963**, *85*, 387.
(9) Falk, M.; Poole, A. G.; Goymour, C. G. *Can. J. Chem.* **1970**, *48*, 1536.
(10) Mrevlishvili, G. M.; Carvalho, A. P. S. M. C.; Ribeiro da Silva, M. A. V.; Mdzinarashvili, T. D.; Razmadze, G. Z.; Tarielashvili, T. Z. *J. Therm. Anal. Cal.* **2001**, *66*, 133.
(11) Chandrasekaran, R.; Radha, A.; Park, H.-S. *Acta Crystallogr.* **1995**, *D51*, 1025.
(12) Marlowe, R. L.; Lukan, A. M.; Lee, S. A.; Anthony, L.; Chandrasekaran, R.; Rupprecht, A. *J. Biomol. Struct. Dyn.* **1996**, *14*, 373.
(13) Hummer, G.; Garcia, A. E.; Soumpasis, D. M. *Biophys. J.* **1995**, *68*, 1639.
(14) Chuprina, V. P.; Heinemann, U.; Nurislamov, A. A.; Zielenkiewicz, P.; Dickerson, R. E.; Saenger, W. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 593.
(15) Madhumalar, A.; Bansal, M. *Biophys. J.* **2003**, *85*, 1805.
(16) Makarov, V.; Pettitt, B. M.; Feig, M. *Acc. Chem. Res.* **2002**, *35*, 376.
(17) Michalarias, I.; Beta, I.; Ford, R.; Ruffle, S.; Li, J.-C. *Appl. Phys. A* **2002**, *74 [Suppl.]*, 1242.
(18) Leporc, S.; Mauffret, O.; El Antri, S.; Convert, O.; Lescot, E.; Tevanian, G.; Fermandjian, S. *J. Biomol. Struct. Dyn.* **1998**, *16*, 639.
(19) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(20) Wolf, B.; Hanlon, S. *Biochemistry* **1975**, *14*, 1661.
(21) Lavalle, N.; Lee, S. A.; Rupprecht, A. *Biopolymers* **1990**, *30*.
(22) Weidlich, T.; Lindsay, S. M.; Rupprecht, A. *Phys. Rev. Lett.* **1988**, *61*, 1674.
(23) Kang, H.; Johnson, W. C., Jr. *Biochemistry* **1994**, *33*, 8330.
(24) Yuan, H.; Quintana, J.; Dickerson, R. E. *Biochemistry* **1992**, *31*, 8009.
(25) Lipanov, A.; Kopka, M. L.; Kaczor-Grzeskowiak, M.; Quintana, J.; Dickerson, R. E. *Biochemistry* **1993**, *32*, 1373.
(26) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry I*; W. H. Freeman and Company: New York, 1980.
(27) Duguid, J. G.; Bloomfield, V. A.; Benevides, J. M.; Thomas, G. J., Jr. *Biophys. J.* **1996**, *71*, 3350.
(28) Charkrabarti, M. C.; Schwarz, F. P. *Nucleic Acids Res.* **1999**, *27*, 4081.
(29) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry III*; W. H. Freeman and Company: New York, 1980.
(30) Freifelder, D. *Molecular Biology*; Jones and Bartlett Publishers: Boston, 1983.
(31) Liu, Y.; Tan, F. *J. Thermal Anal.* **1995**, *45*, 35.
(32) Tsereteli, G. I.; Belopolskaya, T. V.; Grunina, N. A.; Vaveliouk, O. L. *J. Therm. Anal. Cal.* **2000**, *62*, 89.
(33) Green, J. L.; Fan, J.; Angell, C. A. *J. Phys. Chem.* **1994**, *98*, 13780.
(34) Angell, C. A. *Science* **1995**, *267*, 1924.
(35) Sochava, I. V.; Smirnova, O. I. *Food Hydrocolloids* **1993**, 513.
(36) Tanaka, K.; Okahata, Y. *J. Am. Chem. Soc.* **1996**, *118*, 10679.
(37) Falk, M.; Hartman, K. A., Jr.; Lord, R. C. *J. Am. Chem. Soc.* **1962**, *84*, 3843.
(38) Lee, S. A.; Lindsay, S. M.; Powell, J. W.; Weidlich, T.; Tao, N. J.; Lewen, G. D.; Rupprecht, A. *Biopolymers* **1987**, *26*, 1637.
(39) Tao, N. J.; Lindsay, S. M.; Rupprecht, A. *Biopolymers* **1989**, *28*, 1019.
(40) Starikov, E. B. *Phys. Rep.* **1997**, *284*, 1.
(41) Sperling, L. H. *Introduction to Physical Polymer Science*; John Wiley & Sons: New York, 1992.
(42) Fried, J. R. *Polymer Science and Technology*; Prentice Hall: New York, 1995.
(43) Miyazaki, Y.; Matsuo, T.; Suga, H. *Chem. Phys. Lett.* **1993**, *213*, 303.
(44) Miyazaki, Y.; Matsuo, T.; Suga, H. *J. Phys. Chem. B* **2000**, *104*, 8044.
(45) Belopolskaya, T. V.; Tsereteli, G. I.; Grunina, N. A.; Vaveliouk, O. L. *J. Therm. Anal. Cal.* **2000**, *62*, 75.
(46) Berens, A. R.; Hodge, I. M. *Macromolecules* **1982**, *15*, 756.
(47) Hodge, I. M. *Macromolecules* **1983**, *16*, 898.
(48) Rüdisser, S.; Hallbrucker, A.; Mayer, E. *J. Phys. Chem.* **1996**, *100*, 458.
(49) Rüdisser, S.; Hallbrucker, A.; Mayer, E. *J. Phys. Chem. B* **1997**, *101*, 266.
(50) Taillandier, E.; Liquier, J.; Taboury, J. A. In *Advances in Infrared and Raman Spectroscopy*; Clark, R. J. H., Hester, R. E., Eds.; Wiley Heyden: New York, 1985; Vol. 12, Chapter 2.
(51) Mohammadi, S.; Klement, R.; Shchyolkina, A. K.; Liquier, J.; Jovin, T. M.; Taillandier, E. *Biochemistry* **1998**, *37*, 16529.
(52) Dagneaux, C.; Liquier, J.; Taillandier, E. *Biochemistry* **1995**, *34*, 14815.