Concentration-dependent effects on fully hydrated DNA at terahertz frequencies

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Abstract Using terahertz time-domain spectroscopy (THz-TDS), the frequency-dependent dielectric constant of deoxyribonucleic acid (DNA) in solution was measured. The response of the buffer solution is dominated by two Debye modes in this frequency range, and, from an analysis of the concentration dependence, the presence of the DNA increases the main relaxation time and dielectric constant. This reflects the fact that the water in the hydration layer is more tightly bound under the influence of the DNA molecule in comparison to bulk water. This dynamical slowing down with increasing DNA concentration is similar to what is observed with purine nucleotides, but opposite to the behavior of pyrimidine nucleotides. In addition, a suspension model was used with the concentration-dependent data to isolate the dielectric response of the hydrated DNA molecule. The data for the hydrated DNA molecule is still dominated by a Debye response. It is also possible to determine the thickness of the hydration layer, and the DNA molecule influences the surrounding water out to 16 or 17 Å, which corresponds to about six effective hydration layers.

Keywords Terahertz · DNA · Hydration · Viscosity · Relaxation

1 Introduction

In order for deoxyribonucleic acid (DNA) to function properly, it must be in the hydrated state. For example, DNA conformation is associated with the hydration of the phosphate groups [1], and DNA recognition of a protein can be mediated directly through water molecules at the protein–DNA interface without direct contact between the two biomolecules [2–5]. The objective of this work is to investigate this DNA–water interaction using terahertz time domain spectroscopy (THz-TDS). There has been recent work in the terahertz range with respect to hydrated biomolecules involving proteins [7, 8] and DNA [9–12], and studies in non-hydrated DNA [13]. The THz-TDS can provide broadband coherent spectral information on this system, meaning both the real and imaginary parts of the dielectric response are determined without the use of a Kramers–Kronig relationship. It is well suited for this particular task because these measurements cover a frequency range where large molecule vibrational modes are expected

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to exist, and the dominant Debye response of bulk water also falls in this range, allowing us to investigate the influence of hydration on DNA molecular dynamics. This will be done by observing the change in the Debye response with increasing DNA concentration.

2 Experimental procedure

A schematic diagram of the THz-TDS system is shown in Fig. 1. A Ti:sapphire laser, which is pumped by a 5-W vanadate laser, produces 800-nm pulses with a width less than 100 fs. After a 90/10 beam splitter, the 800-nm pump beam from the Ti:sapphire laser is directed through a delay stage to a GaAs photoconductive antenna with a ~ 200 -V bias. This produces broadband THz radiation that is collected with an off-axis parabolic (OAP) mirror and directed as a parallel beam through the sample cell. After the sample, another OAP is used to refocus the transmitted THz beam onto a (110) ZnTe crystal, which is used as a free space electro-optic sampling (FSEOS) detector. A polarized probe beam from the Ti:sapphire laser is also focused on the ZnTe detector where the birefringence, induced by the THz field, modulates the phase difference in the two orthogonal components of the probe beam. This beam then passes through a $\lambda/4$ plate where the two polarization components are separated with a second polarizer (Analyzer), and the imbalance is measured with a pair of balanced diodes. This technique relies on the Pockels effect where the signal is proportional to the amplitude of the THz field. The probe beam gates the detector, and by changing the time delay of this beam, it is possible to measure the time profile of the THz beam.

DNA samples were purchased from Sigma-Aldrich (D-1626), and eight different concentrations (0.5, 0.8, 1.0, 1.2, 3.0, 5.0, 10.0, and 20.0 mg/ml) were prepared using a Tris-EDTA 100 $\times$ buffer concentrate from Sigma-Aldrich (T9285) with high-purity low-conductivity water (HPLC) from Fischer Scientific. To remove naturally occurring impurities, the samples were processed using gel filtration, yet this did not have a noticeable effect on the data. A transmission configuration was used where the hydrated samples were sealed between two 2-mm-thick glass plates with spacers having different thicknesses. Details of the measurement

Fig. 1 The experimental setup used for THz-TDS

technique and data analysis, which determine the complex frequency-dependent dielectric response of the sample, are published elsewhere [14].

DC conductivity measurements on all eight concentrations, the buffer solution, and the HPLC water were performed using a Corning Checkmate II instrument. Viscosity was measured with three different Cannon-Fenske Routine glass viscometers purchased from Cannon Instrument Company: size 50 for the range from 0.8 to 4 cSt, size 350 for the range from 100 to 500 cSt, and size 700 for the range from 20,000 to 100,000 cSt.

3 Analysis based on the Debye model

Hydrated DNA was studied before in the THz and IR frequency range, yet these measurements were performed on pressed pellets or thin films at significantly lower hydration levels [15–17]. Our samples are much closer to the hydration levels associated with DNA's natural environment. Water molecules have dipole moments, and the electromagnetic response of a dipole is best described with a Debye relaxation model [18]. Liquid water is a weak hydrogen-bonded network of molecules, which is easily disrupted by electromagnetic fields. Previous experiments on water find two Debye relaxation modes in the THz frequency range [14, 19]. The lower-frequency mode describes the dynamics associated with the reestablishment of this network [20, 21], and the higher-frequency mode represents rotational relaxation of the molecular dipoles within the network [22]. We expect that the parameters of this model will change as the dynamics are altered by the hydration of DNA molecules in solution. Vibrational excitations of the DNA molecule are usually described with a Lorentz oscillator model. One expects these types of excitations to exist in the THz frequency range, but, if present, they are obscured in our measurement by the large Debye contributions from the water molecules. Yet, one can see the contributions the DNA makes on the surrounding hydration water.

Measurements were taken of eight concentrations of DNA ranging from 0.5 to 20.0 mg/ml, as well as for the buffer solution and HPLC water. The HPLC water data fit listed in Table 1 is described in Ref. [7]. The samples were held at a temperature of 20 ± 0.1 °C during which more than a hundred scans were taken over several days for each concentration. After reducing the measurements to express the real and imaginary

Table 1 Table of the Debye parameters for each concentration of DNA

Solution	ε_1	τ_1 (ps)	ε_2	τ_2 (fs)	ε_∞
HPLC H_2O	76.0 (0.9)	9.49 (0.06)	3.29 (0.05)	231 (6)	5.5 (0.1)
Buffer	72.1 (0.9)	9.43 (0.06)	3.21 (0.05)	222 (6)	5.4 (0.1)
0.5 mg/ml DNA	78.9 (1.0)	9.46 (0.06)	3.41 (0.05)	229 (6)	5.7 (0.1)
0.8 mg/ml DNA	82.3 (1.0)	9.46 (0.06)	3.51 (0.05)	227 (6)	5.6 (0.2)
1.0 mg/ml DNA	86.8 (1.0)	9.55 (0.06)	3.58 (0.06)	234 (6)	5.9 (0.1)
1.2 mg/ml DNA	84.4 (1.0)	9.53 (0.06)	3.47 (0.05)	234 (6)	5.7 (0.1)
3.0 mg/ml DNA	84.1 (1.0)	9.40 (0.06)	3.51 (0.06)	225 (6)	5.6 (0.1)
5.0 mg/ml DNA	82.1 (1.0)	9.48 (0.06)	3.40 (0.04)	219 (6)	5.6 (0.1)
10.0 mg/ml DNA	81.6 (1.0)	9.52 (0.06)	3.41 (0.04)	213 (6)	5.8 (0.1)
20.0 mg/ml DNA	80.4 (1.0)	9.68 (0.06)	3.57 (0.05)	259 (7)	6.0 (0.1)

parts of the dielectric response, the data sets were analyzed in terms of a Debye relaxation model over the frequency range from 100 to 1,100 GHz. The complex dielectric constant for the standard Debye model with two relaxation modes is given by

$$\varepsilon(\omega) = \varepsilon_{\infty} + \frac{\varepsilon_1 - \varepsilon_2}{1 + i\omega\tau_1} + \frac{\varepsilon_2 - \varepsilon_{\infty}}{1 + i\omega\tau_2} \quad (1)$$

where ω is the angular frequency, ε_{∞} is the high-frequency dielectric constant, ε_1 and ε_2 determine the strengths of the modes and τ_1 and τ_2 are the relaxation times for each of these modes. Figure 2 shows a sample two Debye relaxation fit and corresponding data set.

The parameters from this fitting are listed in Table 1 for each concentration, and some interesting trends are observed in the data. Initially, the presence of the DNA causes a dynamical slowing down of the complex hydrogen-bonded network in the hydration layer surrounding DNA. The static dielectric constant shows a noticeable variation with concentration, as indicated in Fig. 3 where the arrow denotes the response for the pure buffer solution (i.e., zero concentration). The relaxation time for the first Debye mode is plotted as a function of concentration in Fig. 4. At low concentrations, there are some small variations in the relaxation time, with possibly a local maximum at 1 mg/ml, but these changes are stagnate and slightly below being statistically significant. Previous research has shown before that the relaxation times do not deviate much from water [11]. Eventually, as the concentration is increased, there is a small statistically significant increase in the relaxation time, which occurs even at concentrations where the static dielectric constant starts to decrease again. With regard to the relaxation time τ_2 , we see a small significant decrease and a significant rebound at 20 mg/ml. The speeding up would follow from the fact that more water molecules are being attached to the higher concentration of DNA. The ultimate slow down of (τ_2) would be most likely due to the high viscosity of the DNA solution at higher concentrations: a more in-depth discussion will follow.

Increments of the relaxation time and the static dielectric constant have been observed with increasing concentrations of other biomolecules such as amino acids, proteins, and purines [14, 23–29]. Biomolecules in solution generally establish more complex hydrogen bonded networks than bulk water and, as a result, water reorientation dynamics are slowed down. Theoretical work on DNA has shown that there is a dynamic equilibrium between the bound and free water molecules [30], and the bound water molecules have a much longer reorientational relaxation time than that of bulk water [31]. The situation is actually more

Fig. 2 Sample Debye fit and data for 1 mg/ml DNA

Fig. 3 The static dielectric constant as a function of the logarithm of the DNA concentration. The arrow represents the pure buffer solution data point

complex in that the influence of the DNA molecule on the hydration-shell water varies with proximity to the molecule, and this is reflected in a distribution of reorientational relaxation times [32]. From the initial increase, it appears DNA encourages a more complex hydrogen-bonded network in the surrounding water (i.e., the hydration layer) than the bulk. Increases in the static dielectric constant can also occur when the ionic content of the solution is increased; however, free-ionic transport is not a factor in our measurements because the dc conductivity was measured and found to be $1\text{--}2\ \mu\text{S}/\text{cm}$. For typical ionic relaxation times, conductivity this small would affect the dielectric constant by a factor much less than one, which is negligible compared to our measured dielectric constants.

Above a concentration of $1\ \text{mg}/\text{ml}$, the static dielectric constant starts to decrease. This probably indicates a fundamental change in the dynamics, possibly associated with hydrogen bond shearing from neighboring hydrated DNA molecules, instead of a softening of the hydrogen-bonded network because the relaxation time continues to increase.

It is possible to compare this result to what is found for solutions of the individual nucleotides [14, 29]. Depending on whether the nucleotide is a purine or pyrimidine, the static dielectric constant initially increases or decreases with increasing nucleotide concentration, respectively. For purines, this network is more complex than for pyrimidines; so the mobility of hydrated purine is less than that of pyrimidine, and this is reflected by the different

Fig. 4 The relaxation times for the first Debye mode versus concentration. The arrow represents the zero concentration pure buffer solution

qualitative behavior in the static dielectric constant. DNA has a phosphate backbone on both sides instead of on just one side. Since the phosphates are known to have a higher propensity for hydrogen bonding than the bases [33], it is reasonable that the concentration dependence of the static dielectric constant for DNA would be similar to that of purine in that this structure would build more complex hydrogen-bonded structures relative to the buffer solution.

One expects changes in the viscosity to be reflected in the relaxation time. Unlike nucleotides, the viscosity of DNA increases exponentially with concentration as shown in Fig. 5. Even though the relaxation time does increase with concentration, an insignificant change is observed over the same range in concentration where the viscosity increases by three orders of magnitude. It is well known that highly viscous solutions can exhibit non-linear behavior with respect to dielectric relaxation times [34]; there are regimes where relaxation time is independent of bulk viscosity [35], which is the case with our DNA solutions. Both the main relaxation time that is associated with the reestablishment of the hydrogen bond network [20, 21] and the secondary relaxation time that describes the free rotational relaxation time of the molecular dipoles within the network [22] are slowed at high concentrations and high viscosities. The secondary relaxation time seems to be more sharply altered than the main relaxation time. This implies that viscosity has more of an effect on the rotation time of the molecular dipoles than the reestablishment of the H-bond network.

4 Thickness of the hydration layer

The system that we are investigating has two main components: the hydrated biomolecule, which includes both the hydration water and the biomolecule solute, and the bulk water molecules with the buffer ions. All concentrations of DNA that were used (0.5, 0.8, and 1.0 mg/ml) in the suspension model have bulk water present, and we are well below the concentration limit (2 g of DNA/g of water) [36] where bulk water should disappear and which would indicate a lack of hydration layer overlap. This ensures that the DNA molecules are over 32 Å (16 Å per DNA molecule). While the analysis with the Debye model examines the response of the system as a whole, it is possible to separate the contributions using a suspension model. This model has been shown to be fairly accurate with dilute solutions [14, 29, 37–39]. The

Fig. 5 The viscosities of DNA and the nucleotide dGMP versus concentration. While the viscosity of the nucleotide changes very little, it increases exponentially for DNA. We were unable to measure the viscosity of DNA beyond 13 mg/ml

Fig. 6 The final calculated ε_b , which includes data for all used concentrations as well as two sample fits for 0.8 mg/ml

suspension model describes the frequency-dependent dielectric response of the mixture ε_m with the equation

$$\sqrt{\varepsilon_m} = \nu_b \sqrt{\varepsilon_b} + (1 - \nu_b) \sqrt{\varepsilon_s} \quad (2)$$

where ε_b and ε_s are frequency-dependent dielectric constants for the DNA molecule with its hydration layer and the bulk buffer solution, respectively, and ν_b is the volume fraction of the hydrated biomolecule. After measuring ε_m for different concentrations, it is possible to determine ν_b as a fit parameter, using the measured value for ε_s , and then we solve for ε_b . This can be done, given the knowledge of the differences in the concentrations that were measured, by solving with linear algebra at all frequencies. From this volume fraction, it is possible to determine the number of hydration layers which are influenced by DNA, and our fits have an error between 3 and 4%. Figure 6 shows ε_b , the dielectric response of the DNA and the hydration shells, for all concentrations as well as two sample fits.

The analysis using the suspension model was only consistent for frequencies above 200 GHz, and from this analysis the dielectric constant of the hydrated biomolecule ε_b is

Fig. 7 With the suspension model, it is possible to separate the contributions to the dielectric response into that from the DNA molecule with its surrounding hydration shell and that from the bulk buffer solution. The real part of the dielectric constant for these two contributions is plotted as a function of frequency

Fig. 8 Graph depicting the dielectric response of DNA and the hydration layer, along with the range of error of the calculation

plotted as a function of frequency in Fig. 7, where Fig. 8 shows the quality of the fit. Also shown in the figure is the measured dielectric constant for the buffer solution, ϵ_s , that was used in the analysis. Clearly, the response for the hydrated DNA is still dominated by a Debye relaxation, most likely from a significant amount of water in the hydration shell. From the volume fraction determined by fitting the data at concentrations below ~ 1 mg/ml, the hydration layer is estimated to average six molecules thick, implying that DNA molecules influence the surrounding water out to approximately 16 or 17 Å at low concentrations, considering that a water molecule with bond takes up a diameter of 3 Å. For concentrations above 1 mg/ml the volume fraction starts to decrease, indicating that interference or overlap is occurring between neighboring DNA molecules; the distance between DNA could be different at higher concentrations. This measurement of the hydration layer thickness matches well with experiments and simulations reporting that DNA affects the surrounding water from 15 to 20 Å [40–41]. The influence of a biomolecule on the surrounding water environment is much greater with DNA than with nucleotides [14] where the influence only extends to four layers. This is reasonable because the phosphate backbone and sugar ring in DNA are highly charged and have a higher affinity for water than the exposed bases of the nucleotides [41]. This is also reflected in the fact that DNA is a stronger acid than the nucleotides.

5 Conclusions

The dielectric response of fully hydrated native DNA is dominated by Debye modes associated with water molecules in the hydration layer and the bulk buffer solution. The static dielectric constant associated with the lower-frequency mode initially increases as a result of the DNA molecules. This response is similar to that observed for purine nucleotides, and implies that native DNA molecules encourage the formation of more complex hydrogen-bonded networks in the surrounding solution in comparison to bulk water. Some small variations in the relaxation time are observed at low concentrations, though these changes are on the edge of being statistically significant. Eventually, at higher concentrations a marked increase in relaxation time is observed. While this slowing down of the dynamic response is consistent with the increase in viscosity, very large changes in the viscosity occur even before a measurable change in the relaxation time is observed. Contrary to this behavior, virtually no

changes were reported in the relaxation times for the nucleotides, consistent with the much smaller concentration dependence of the viscosity.

Using the suspension model, the dielectric response of the hydrated DNA molecule was separated from that of the bulk buffer solution. This deconvolution of the data with the suspension model is only consistent at frequencies above ~200 GHz. Even without the contribution of the bulk solution, the response is dominated by a large Debye mode, probably from the water molecules in the hydration layer. From the concentration dependence, the volume fraction yields a hydration layer thickness of approximately six water molecules, or, stated another way, the DNA molecule influences the surrounding water out to approximately 16 Å. At concentrations above ~1 mg/ml, the volume fraction starts to decrease, probably a result of interactions with neighboring hydrated DNA molecules.

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