Dielectric Response of Suspended Nucleotides at Terahertz Frequencies

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***Abstract*—Terahertz time domain spectroscopy (THz-TDS) was used to determine the dielectric response of hydrated nucleotides at different concentrations. A Debye model with two relaxation times was used to fit the data for each concentration. We found that the hydrogen-network structure of the hydrated purine nucleotides is more stable than that of the pyrimadines. A suspension model was used to separate the dielectric response of the hydrated nucleotide from that of bulk water, and from this analysis the thickness of the hydration layer is determined.**

I. Introduction And Background

BIOMOLECULES are often large complex structures, and interesting vibrational and possibly electronic excitations may occur in these macrostructures at low frequencies. The situation is further complicated by the fact that the properties of many biomolecules are strongly influenced by the surrounding environment. For example, the presence of a hydration shell can be crucial to much of the biomolecule's functionality, such as hybridization in DNA. The amount of water associated with the hydration layer surrounding DNA and the range of its effect are not fully understood.

Important information about the excitations in biomolecules and the characteristics of the hydration layer can be obtained from measurements of the dielectric response at terahertz frequencies. To investigate the dielectric response of these systems, a time-domain spectrometer (THz-TDS), which operates in the terahertz frequency range, was used. The instrument was designed to measure the optical response of biomolecules in solutions, similar to their native environment, using a transmission configuration. The coherent nature of the measurement allows us to directly determine both real and imaginary components of the response, without a Kramers-Kronig analysis, over a semicontinuous frequency range with high signal to noise.

II. THEORY

It is possible to model our system as a nucleotide with a hydration layer, suspended in bulk water. Using the suspension model, the dielectric response of the nucleotide with the hydration layer is separated from bulk water using the equation [1]

$$\sqrt{\varepsilon_m} = n\sqrt{\varepsilon_b} + (1-n)\sqrt{\varepsilon_w}$$

where ε_m is the dielectric response of the mixture, ε_b represents the response for the biomolecule with its hydration shells and ε_w is the response for bulk water. The volume fraction of hydrated nucleotides is denoted by n. The dielectric response of bulk water and the mixture were measured directly, and the volume fraction is treated as a fit parameter. With this information, the dielectric response of the fully hydrated nucleotide was determined.

Polar liquids often display Debye relaxation type behavior, which is given by the following expression [2]

$$\hat{\varepsilon}(\omega) = \varepsilon_\infty + \sum_{j=1}^{n} \frac{\varepsilon_j - \varepsilon_{j+1}}{\left[1 + \left(i\omega\tau_j\right)^{1-\alpha_j}\right]^{\beta_j}}.$$

ω is the angular frequency, ε_j are the static dielectric constants for each relaxation mode, ε_∞ is the high frequency dielectric, and τ_j are the relaxation times for each mode. α_j and β_j are parameters from the Cole-Davidson and Cole-Cole models. For our analysis, we consider only two Debye relaxation modes and assume $\alpha_j = 0$ and $\beta_j = 1$ [3].

III. EXPERIMENTAL SET-UP

The THz-TDS was optimized to collect data in the range from 50 GHz to 1500 GHz. The THz frequency radiation was generated by pumping a biased GaAs photoconductive antenna with a mode-locked Ti: sapphire laser operating at a wavelength of 800 nm. This terahertz radiation was directed through the sample and refocused on a detection system with off-axis parabolic mirrors.

The detection was accomplished with free-space electro-optic sampling [4] where part of the pump for the photoconductive antenna is used to probe the detector. The orientation of the linearly polarized probe beam is changed by the THz field as they concurrently travel through a ZnTe crystal detector. After emerging from the ZnTe crystal, the probe beam traverses a quarter-wave plate, converting it to an elliptically polarized beam. Finally, the beam passes through a Brewster-angle analyzer, which separates the beam into s- and p-wave components. The relative intensity of these two beams, which is measured by a pair of balanced diodes, is proportional to the intensity of the THz radiation field.

The nucleotides in this experiment were hydrated with HPLC water at various concentrations. Nucleotide samples were placed betwixt two pieces of glass that were separated with a Teflon gasket of known thickness. The dielectric response of the two pieces of glass is known, and with this information, it is possible to distinguish between the contributions from the sample and sample holder.

IV. RESULTS

Our measurements were performed on concentrated solutions of pyrimidine and purine nucleotides at a temperature of 20°C. Understanding the response of these biomolecules is a prerequisite to similar ongoing research on various forms of DNA. The measured real and imaginary dielectric constants of deoxycytidylic acid (dCMP) are shown in Fig. 1 as a function of frequency from 0.05 THz to 1.0 THz. The responses for deoxyadenylic acid (dAMP), deoxyguanylic acid (dGMP) and deoxythymidylic acid (dTMP) are

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qualitatively the same. For each nucleotide, the frequency dependent dielectric constant can be described by the Debye model discussed above. The relaxation time τ_1 is interpreted as the time it takes the hydrogen network associated with the nucleotide-hydration layer structure to break and reform [5].

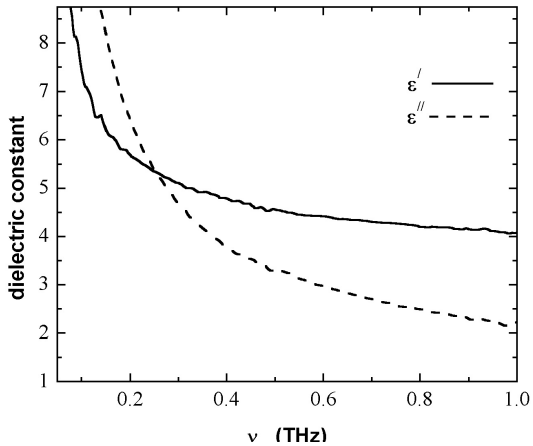

Fig. 1 Real and imaginary parts of the dielectric constant as a function of frequency for 1.36 mM dCMP.

The concentration dependence of τ_1 is plotted in Fig. 2 for the four nucleotides. Initially when the concentration of purine nucleotides (dGMP and dAMP) increases, the relaxation time also increases, implying the hydrogen network is more stable (i.e., the hydration layer surrounding the biomolecule is stronger, as in proteins).

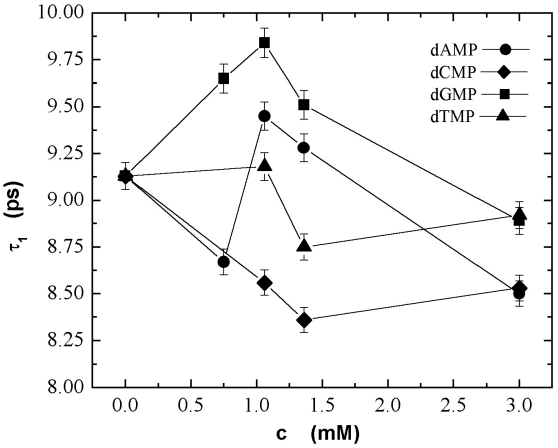

Fig. 2 Concentration dependence of the relaxation time for the first Debye mode for dAMP, dCMP, dGMP and dTMP.

The 0.75 mM concentration of dAMP is low because the reduced dielectric response of the nucleotide relative to bulk water is stronger than the increased contribution from the hydrogen network. For pyrimidines, the opposite occurs, indicating a more fragile hydrogen-network structure (i.e., like the hydration layer surrounding an ion). It is evident that purines with the greatest number (and strength) of hydrogen bonds have the largest increment of all four of the nucleotides, while pyrimidines have the least. The implication here is that we are at a transition from ion-type behavior to large-biomolecule behavior. Eventually, the relaxation times for all the nucleotides level out at a value below that for water. This occurs because the hydration layer around each nucleotide is compromised at higher nucleotide concentrations.

Using a suspension model, the dielectric response of the hydrated nucleotides can be separated from that of bulk water, and we find that the static dielectric constants and relaxation times for the hydrated nucleotides are reduced. It is possible to estimate the hydration layer from concentration-dependence studies, and this analysis suggests that the nucleotides affect the water to the fourth shell; this is consistent with other work on short chained DNA structures [6].

V. CONCLUSION

We have demonstrated that it is possible to investigate hydrated biomolecular dynamics in the presence of a large bulk-water background using a suspension model. Once the dynamics of the fully hydrated nucleotide has been found, two pieces of information follow. For hydrated nucleotides, the dielectric response at THz frequencies can be understood in terms of two Debye relaxation modes. The concentration dependence of the relaxation time for one of these modes indicates that the hydrogen network for the purines is more stable than the pyrimidines with respect to bulk water. This can be interpreted as purine nucleotides are H-bond forming materials, like proteins, while pyrimidines nucleotides are H-bond destroying materials, like ions. It seems that the nucleotides form a transition betwixt structure building type materials and structure breaking type materials. It is also possible to determine that there are approximately four shells of water molecules in the hydration layer surrounding each nucleotide.

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