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Dipole Relaxation Losses in DNA

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ABSTRACT

The electrodynamic response of DNA in the millimeter wave range is investigated. By performing measurements under a wide range of humidity conditions and comparing the response of single-stranded DNA and double-stranded DNA, we show that the appreciable ac conductivity of DNA is not due to photon-assisted hopping between localized states but is instead due to dissipation from dipole motion in the surrounding water helix. Such a result, where the conductivity is due to the constrained motion of overdamped dipoles, reconciles the vanishing dc conductivity of DNA with the considerable ac response.

The electrical conductivity of DNA has been a topic of much recent interest and controversy.[1] Measurements from different groups have reached a variety of conclusions about the nature of charge transport along the double helix. DNA has been reported to be metallic,[2] semiconducting,[3] insulating,[4,5] and even a proximity-effect-induced superconductor.[6] However, questions have been raised in many of these papers with regard to the role played by electrical contacts, length effects, and the manner in which electrostatic damage, mechanical deformation by substrate−molecule interaction, residual salt concentrations, and other contaminants may have affected these results. Some recent measurements, where care was taken to both establish a direct chemical bond between λ-DNA and Au electrodes and also control the excess ion concentration, have given compelling evidence that the dc resistivity of the DNA double helix over long length scales ($<10\ \mu$m) is very high indeed ($\rho > 10^6\ \Omega\cdot$cm).[7] These results were consistent with earlier work that found flat $I-V$ characteristics and vanishingly small conductances[5] but contrast with other studies that found a substantial dc conductance that was interpreted in terms of small polaron hopping.[8] Although we will revisit this subject below, dc measurements that show DNA to be a good insulator are also in apparent contradiction with recent contactless ac measurements that have shown appreciable conductivity at microwave and far-infrared frequencies,[9,10] the magnitude of which approaches that of a well-doped semiconductor.[11]

Previously, the ac conductivity in DNA was found to be well parametrized as a power law in ω.[9,10] Such a dependence can be a general hallmark of ac conductivity in disordered systems with photon-assisted hopping between random localized states[12] and led to the reasonable interpretation that intrinsic disorder, counterion fluctuations, and possibly other sources created a small number of electronic states on the base pair sequences in which charge conduction could occur. However, such a scenario would lead to thermally activated hopping conduction between localized states and is thus inconsistent with a very low dc conductivity.[7] A number of outstanding issues arise: Are there localized regions along the helix where a continuous conducting path is not present but ac hopping between localized states over distances of a few base pairs can still occur? Are there sensitive length dependencies in the DNA strands? Are there differences between the samples of various groups? Are there perhaps different charge-conduction mechanisms that play a role at finite frequency?

To resolve some of these matters, we have performed ac conductivity experiments in the millimeter wave range under a wide range of humidity conditions. We show that the appreciable ac conductivity of DNA in the microwave and far-infrared regimes should not be viewed as some sort of hopping between localized states and is instead likely due to dissipation in the dipole response of the water molecules in the surrounding hydration layer. Our data can be well described by a Debye-like relaxation of water molecules in the surrounding water helix. At low humidities, the response is well modeled by considering the rotation of single water molecules in the structural water layer. As the number of water molecules per base pair increases, dissipation due to the collective motion of water dipoles increases until eventually the conductivity resembles that of bulk water. By measuring both single-stranded (ssDNA) and double-stranded DNA (dsDNA) over a wide range of humidities, we are able to show that, at least in principle, all of the ac conductivity of DNA can be assigned to relaxation losses of water dipoles. This finding can be taken to support those measurements that find a vanishingly small dc conductivity and indicate that DNA is a poor candidate for a molecular wire.

Double-stranded DNA films were obtained by vacuum drying a 7 mM PBS solution containing 20 mg/mL of sodium

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salt DNA extracted from calf thymus and salmon testes (Sigma D1501 and D1626). The results were found to be independent of the use of calf or salmon DNA. Our choice of these concentrations deserves further explanation. It is well known that at a given temperature the double-helical conformation of DNA can exist in solution only within a certain concentration of positive ions. Excess salt cannot be removed by vacuum drying, so large amounts of residual salt in films could introduce significant errors in conductivity due to both the ionic conduction of the salt itself and its additional hydration during humidity changes. Melting-temperature calculations[13,14] for long native pieces of DNA with C−G content around 40% show that a 2−10 mM concentration of sodium cations is enough to stabilize the double helix at room temperature. Films were prepared with differing salt amounts, and it was found that as long as the excess salt mass fraction is kept between 2 and 5% the final results were not significantly affected. To improve the DNA/salt mass ratio, we used as high a concentration of DNA as possible, but 20 mg/mL appears to be the limit. Higher concentrations make it difficult for DNA fibers to dissolve, and the solution becomes too viscous, which prevents the production of the flat uniform films that are of paramount importance for the quasi-optical resonant technique. Single-stranded DNA films were prepared from the same original solution as the double-stranded ones, with preliminary heating to 95 °C for 30 min and fast cooling to 4 °C. The dry films were 20−30 μm thick and were made on top of 1-mm sapphire windows. Immediately after solution deposition, the sapphire substrates were vacuum centrifuged at 500*g*. This expels any air trapped inside the viscous solution; otherwise, the evaporation process causes the formation of air bubbles that destroy the film uniformity. In both dsDNA and ssDNA cases, the conformational state was checked by fluorescence microscope measurements. Additionally, some ssDNA films were also made by depositing 20 mg/mL DNA solution in distilled water or PBS buffer at a constant 95 °C and then keeping the film well above the denaturing temperature until dry. Such films should be entirely constituted of ssDNA; no experimental difference was found between these films and those prepared in the alternative manner.

The ac conductivity was measured in the millimeter spectral range. This difficult-to-access frequency regime is particularly relevant because it spans the approximate expected time frame for relaxation processes in room-temperature liquids (1−10 ps). Importantly, it is also below the energy range where one expects to have appreciable structural excitations. Backward wave oscillators (BWO) in a quasi-optical setup (100 Ghz−1 THz) were employed as coherent sources in a transmission configuration. The technique and analysis are well established.[15] For plane waves incident normally on a slab of material, transmission resonances occur when the slab is an integer number of half wavelengths. Thus, using an ∼1-mm sapphire disk as a substrate, resonances occurred approximately every 50 GHz. Having analyzed the transmission through the sapphire alone prior to mounting the sample, we found that the optical properties of the substrate were well characterized. Using a two-layer transmission model, each resonance can be analyzed to extract the optical properties of the DNA film, allowing for a 1.5-cm^{-1} resolution of the spectra.

Samples were measured at room temperature at several fixed humidity levels that were maintained by the inclusion of a saturated salt solution in the hermetically sealed measurement environment.[16] The changes in thickness and mass of the DNA films at different humidities were tracked by separate measurements within a controlled environment in a glovebox.

The total number of water molecules per nucleotide A can be correlated to the relative humidity x ($x = 0-1$) through the so-called Branauer−Emmett−Teller (BET) equation:[17]

$$A = \frac{BCx}{(1-x)(1-x+Cx)} \tag{1}$$

The constant B is the maximum number of water molecules in the first-layer sites. According to the statistical formulation of the BET equation by Hill,[18] mobile water molecules within the double helix can be characterized as two types. The first are ones within the initial hydration layer, which are directly attached to DNA and have a characteristic binding energy ϵ_1. Water molecules of the second and all other layers can be approximated as having a binding energy ϵ_L. To a good approximation, this ϵ_L can be taken to be that of bulk water. These parameters enter into the BET equation through the expression for C, which equals $De^{\{(\epsilon_1 - \epsilon_L)/kT\}}$ where D is related to the partition function of water. Also, we should note that there is, in actuality, a structural 0th layer of water molecules containing 2.5−3 water molecules per nucleotide that cannot be removed from the helix under typical conditions.[19]

That it is reasonable that the mobile water layers of DNA can be modeled by distinguishing two different sets of water parameters was first established by Falk et al.'s[16] use of the BET equation to describe the hydration of sodium and lithium DNA salts of calf thymus and salmon testes. They found good agreement between experimental data and theory with constants $B = 2.2$ and $C = 20$. We performed a similar hydration study of our dsDNA and ssDNA films; as shown in Figure 1, the hydration of our films is perfectly consistent with Falk's result. Note that there is no appreciable difference in the hydration between dsDNA and ssDNA.

In Figure 2, we present data for the extracted $\sigma_1(\omega)$ values of both dsDNA and ssDNA thin films. One can see that in both cases the conductivity is an increasing function of frequency. Because the conductivity is also an increasing function of humidity, one may wish to try to separate the relative contributions of charge motion along the DNA backbone or between base pairs from that of the surrounding water molecules.

First, one can consider that there should be two main effects of hydration in our dsDNA films. There is the hydration itself, where water molecules are added in layers to the double helix; this is well described by the BET equation.[17] Additionally, the conformational state of dsDNA

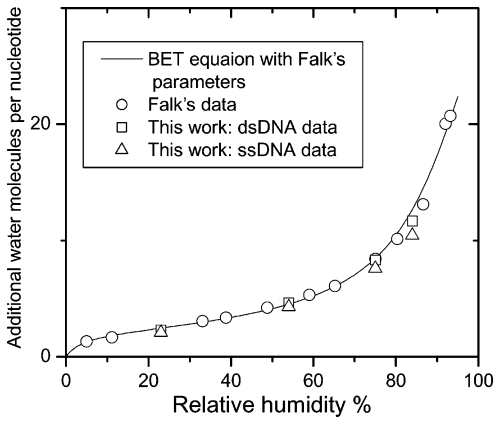

Figure 1. Absorption of water molecules per nucleotide as a function of humidity. The data represented by the open circles is taken from Falk et al.[15]

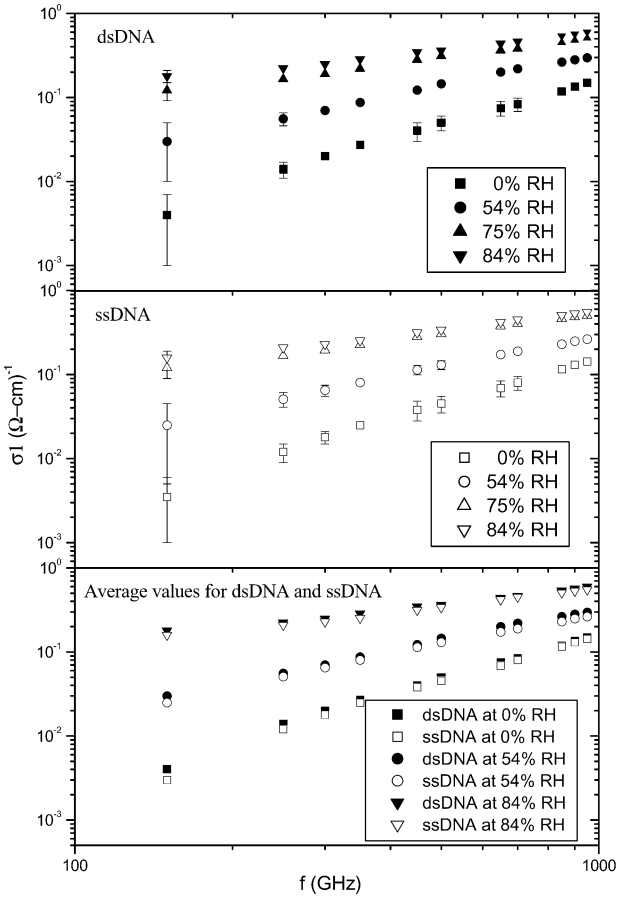

Figure 2. Frequency dependence of the conductivity of calf thymus DNA at different relative humidity levels. (Top) Double-stranded DNA. (Middle) Single-stranded DNA. (Bottom) Comparison of conductivity between single- and double-stranded DNA.

also changes as a function of humidity. For example, sodium salt calf thymus DNA is in a B-like disordered form at humidities from 0 to 40%, above which it transfers to the A form and finally to a well ordered B-form at humidities higher than 80%.[20,21] Additional water molecules certainly contribute to the increase in conductivity, but at high humidities, there is the possibility that some of the conduction might be due to an increase in electron transfer along the dsDNA helix in the ordered B form. However, because such

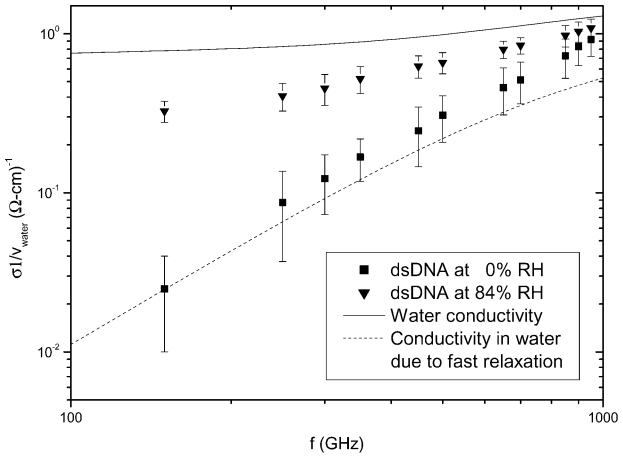

Figure 3. Conductivity of dsDNA and ssDNA films normalized by the volume fraction of all water molecules (structural plus hydration layer). For clarity, only 0 and 84% humidities are shown. The solid line represents the conductivity of pure water as modeled by the biexponential Debye model using the parameters of Ronne et al. The dashed line shows just the contribution from single water molecule relaxation.

an effect would be much reduced in disordered and denaturalized ssDNA films and because Figure 2c shows that to within the experimental uncertainty the conductivities of dsDNA and ssDNA in the millimeter wave range are identical, it is most natural to suggest that water is the major contribution to the ac conductivity. From this comparison of dsDNA and ssDNA, we find no evidence for charge conduction along the DNA backbone.

In Figure 3, we plot the conductivity σ_1 of the DNA films normalized by the expected volume fraction of water molecules including both the hydration layers plus the structural water. Although this normalization reduces the spread in the thin-film conductivity at the lowest frequencies, it does not reduce it to zero, showing that if the largest contribution to the conductivity comes from water then the character of its contribution changes as a function of humidity.

The complex dielectric constant of bulk water has been shown to be well described by a biexponential Debye relaxation model,[22−24] where the first relaxation process,[22] characterized by a time scale of $\tau_D = 8.5$ ps, corresponds to the collective motion of tetrahedral water clusters and the second corresponds to faster single-molecular rotations[25] with a time scale of $\tau_F = 170$ fs. For bulk water, the contribution of each relaxation process is determined by the static dielectric constant $\epsilon_S(T) = 88e^{-0.0046T[^\circ C]}$, $\epsilon_1 = 5.2$, and the dielectric constant at high frequencies $\epsilon_\infty = 3.3$.

$$\hat{\epsilon}(w) = \epsilon_\infty + \frac{\epsilon_S - \epsilon_1}{1 + i\omega\tau_D} + \frac{\epsilon_1 - \epsilon_\infty}{1 + i\omega\tau_F} \qquad (2)$$

We can use eq 2 to gain insight into the conduction processes occurring in the water layers. For high hydration levels, where multiple water layers exist around the dipole helix, the relaxation losses of the water layer may approach those of bulk water. We compare the above equation using

the independently known values[22] for τ_D, ϵ_S, τ_F, and ϵ_1 to the experimental data normalized to the expected volume fraction of the water. As seen in Figure 3, the conductivity of well-hydrated DNA is seen to approach that of bulk water.

As the number of water layers decreases, one expects that the contribution of cluster relaxation processes decreases. It is reasonable that this first term of eq 2, which is due to the collective motion of water clusters, cannot contribute at low humidity because the structural water is not tetrahedrally coordinated. Remarkably, the 0% humidity conductivity is approximately described by a model that includes only the fast single-molecular rotation of bulk water. This is interesting because such behavior is contrary to that of many systems that find longer average relaxation times in thin adsorbed gas layers than in the corresponding bulk systems.[26]

In Figure 3, along with the experimental data at two representative humidity levels, the two theoretical curves for 0 and 100% humidity are plotted. With the only two assumptions being that at 0% humidity the sole relaxational losses come from singly coordinated water molecules in the structural water layer and that it is only at higher humidity levels where the collective losses can gradually play a greater role, the theoretical curves provide a very good bound to the data over almost all of the measured frequency range.

The only appreciable discrepancy between theory and experiment is the high-frequency data at low humidity, where the biexponential Debye model underestimates the conductivity. This may be due to a number of reasons. At very low relative humidities, it is possible for the ionic phosphate groups on the DNA backbone to form stable dihydrates that may make their own contribution to relaxation losses through their additional degree of freedom.[16] Alternatively, it may be that at higher frequencies for low-hydration samples the weak restoring force from charge−dipole interaction in the structural water layer becomes more significant and our biexponential Debye model is less applicable. We should also note that one advantage of working in the millimeter spectral range is the known weak contribution of ionic conduction in this regime.[27] The motion of the surrounding relatively large mass counterions becomes appreciable only at lower frequencies.[28]

In conclusion, we have found that the considerable ac conductivity of DNA can be largely ascribed to relaxational losses of the surrounding water dipoles. The ac conductivities of ssDNA and dsDNA were found to be identical to within the experimental error, indicating that there is essentially no charge conduction along the DNA backbone itself. The conclusion that the observed conductivity is derived from the water layer is supported by the fact that, over much of the range, it can be well described by a biexponential Debye model, where the only free parameter is the relative contributions of single water molecule and tetrahedral water cluster relaxation modes. Our result also implies that a previous interpretation of ac conductivity[9] in terms of the photon-assisted hopping of electrons between localized states is not likely to be the correct one. This has implications on the interpretation of measurements, such as that by Yoo et al.[8] that compared the activation energy inferred from their polaron hopping fits to the activation energy arrived at from ref 9.

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