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Probe Position Dependence of DNA Dynamics: Comparison of the Time-Resolved Stokes Shift of Groove-Bound to Base-Stacked Probes

Nibedita Pal, Sachin Dev Verma, and Sobhan Sen*

Spectroscopy Laboratory, School of Physical Sciences, Jawaharlal Nehru University, New Delhi 110067, India

Received April 21, 2010; E-mail: sens@mail.jnu.ac.in

Abstract: Time-resolved fluorescence Stokes shift dynamics of a fluorescent probe, 4′,6-diamidino-*2*-phenylindole (DAPI), inside the minor groove of the DNA is measured over five decades in time spans from 100 fs to 10 ns. Two different techniques, fluorescence up-conversion and time correlated single photon counting, are combined to obtain the time-resolved emission spectra of DAPI in DNA over the entire five decades in time. Having the dynamics of groove-bound DAPI in DNA measured over such a broad time window, we are able to convincingly compare our data to earlier time-resolved fluorescence results of a base-stacked probe that replaces a DNA base pair. Results show that the dynamics measured with either the groove-bound or the base-stacked probe are similar in the time span of 100 fs to ∼100 ps but differ substantially from ∼100 ps to 10 ns. Our present data also help to reconcile the previously reported molecular dynamics simulation results and provide important clues that the groove-bound water molecules inside DNA are mainly responsible for the slow dynamics seen in native DNA.

Time-resolved fluorescence Stokes shift (TRFSS) experiments show that the motion of water or other simple liquids are complete within a few picoseconds,[1] but the same experiments with probe molecules inserted into biomolecules find dynamics extending from tens of femtoseconds out to tens of nanoseconds.[2−10] The explanation for these slow dynamics remains controversial.[11] Molecular dynamics (MD) simulations are essential for a full interpretation of these experimental results,[5,12−15] but agreement has been inconsistent on many occasions.[4−9,11−15]

In proteins, the dynamics depend on the probe's position within the protein.[4,5,12] Although DNA has much less structural diversity than proteins, this communication will show that the position of the TRFSS probe within DNA qualitatively changes the observed dynamics. The TRFSS of a probe bound in the minor groove of DNA is measured over five decades in time from 100 fs to 10 ns. The results are compared to earlier measurements by identical methods of a base-stacked probe that replaces a DNA base pair.[6,7] The dynamics measured in either probe position are similar from 100 fs to ∼100 ps but differ substantially from ∼100 ps to 10 ns.

When comparing the results of groove-bound to base-stacked probes, the simplest expectation would be that the groove-bound probe would see a similar, but somewhat smaller, anomalous response, because it is more exposed to normal water than a base-stacked probe. Nonetheless, a qualitative change in the dynamics would not be expected because electrostatic coupling is long-range, and so the probe should interact with all the same elements of DNA solution (i.e., water, ions, and DNA itself) whether the probe is in the groove or in the base stack of DNA. Our data, however, reveal that this argument is not true. In fact, the purpose of our present experiment was to displace minor-groove water by a groove binding probe and see the effect on the dynamics. Such minor-groove water is found to play a vital role in DNA, including the stabilization of anticancer drug intercalation into DNA.[16]

TRFSS experiments capture the dynamics of a system by monitoring the interaction energy of a fluorescent probe with its surroundings.[2−10] Upon optical excitation, the charge distribution in the probe changes. Subsequently, surrounding charges or dipoles (here water, ions, and DNA-proper) move to stabilize the probe by changing the electric field on the probe. Due to the lowering of its energy, the probe's fluorescence spectrum shifts to lower frequencies. Thus, the movement of the surrounding environment is directly reflected in the time-dependent shift of the probe's fluorescence.

Berg and co-workers used a base-stacked probe (a coumarin/abasic-site pair replacing a natural base pair) first to show that DNA dynamics extend into the nanosecond regime[17] and later to show that the TRFSS dynamics follow a single power law from 40 fs to 40 ns.[6,7] Zewail and co-workers reported TRFSS experiments in DNA with both a base-stacked and a groove-bound probe.[8,9] Both results were qualitatively similar, but the measurements did not extend past 100 ps. Ernsting and co-workers have reported high accuracy TRFSS of a base-stacked probe, but their results do not extend to the nanosecond regime either.[18] Pal and co-worker, on the other hand, reported biexponential nanosecond dynamics using groove-bound probes.[10] In an early simulation[14] of a base-stacked probe (a native base), Hynes and Bagchi found slow components in DNA that were assigned to water and ions, but the simulations were not long enough to comment on the nanosecond components.

Using a long (46 ns) simulation of native DNA, Sen et al. found excellent agreement between the experiments with a base-stacked probe and the simulated electric field in the base stack of native DNA.[13] This study also found that the perturbed water, rather than ions or DNA itself, is responsible for the slowest dynamics. On the other hand, Furse and Corcelli simulated DNA with the same groove-bound probe used by Zewail.[15] In apparent contradiction to the study of Sen et al.,[13] they only saw relaxation out to 350 ps.[15] They also found that the moderately slowest dynamics were due to DNA motion, but not water or ion motion.[15] The question arises of whether this contradiction is due to the differences in the interpretation or due to the real differences in the dynamics at different probe positions inside the DNA structure.

The present study used 14-mer duplex DNA (5′-CGCGCAATTGCGCG-3′) hybridized with its complement in sodium phosphate buffer (pH 7) and with DAPI as a minor-groove binding probe (see Supporting Information). In Figure 1, we combine time-resolved decays from fluorescence up-conversion (UPC, 100 fs to 180 ps) and time correlated single-photon counting (TCSPC, 30 ps to 10 ns) experiments to obtain the time-resolved emission spectra of DAPI in DNA. Log-normal fits to the spectra were used to define the shift of each spectrum from the time-zero spectrum of the sample measured in frozen glass. In a glass, all diffusive motions in the sample are frozen, although vibrational and inertial (phonon-

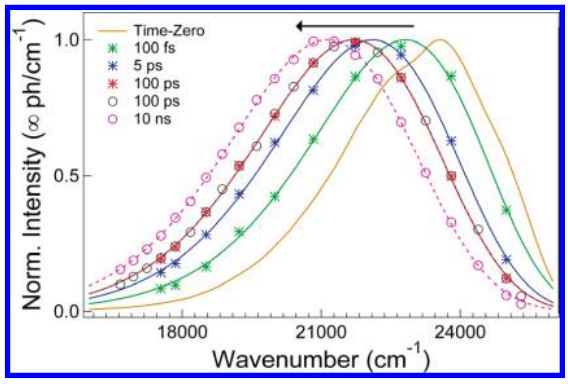

Figure 1. Time resolved emission spectra of DAPI in 14-mer DNA. Scatter points: experimental data (stars, UPC; circles, TCSPC). Line through points: log-normal fits. Solid dark yellow line: time-zero spectrum of DNA-DAPI in glass (see Supporting Information).

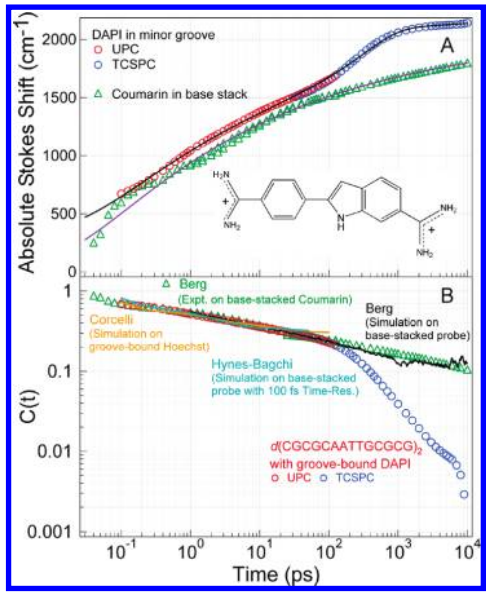

Figure 2. (A) Absolute Stokes shift of DAPI in 14-mer DNA compared to the TRFSS data of base-stacked coumarin. DAPI data (circles: red, UPC; blue, TCSPC) and base-stacked coumarin data (green triangles).[6,7] Solid lines represent fits to the data (see also Supporting Information). DAPI structure is given at the right lower corner. (B) Comparison of solvation correlation function $C(t)$ of groove-bound DAPI (circle) and base-stacked coumarin (triangle). The data are also compared to simulation correlation results (solid lines) reported by Corcelli,[15] Hynes-Bagchi,[14] and Berg.[13]

like) motions persist.[6,7] Thus the reported "absolute" Stokes shifts isolate only diffusive motions.

Figure 2A compares the groove-bound DAPI data with the TRFSS data of base-stacked coumarin.[6,7] Over the initial three decades (100 fs to ~100 ps), the Stokes shifts of groove-bound DAPI and base-stacked coumarin are very similar.[6,7] However, after ~100 ps, the DAPI data deviate from the coumarin data and converge rapidly toward an equilibrium value. Thus, the dynamics measured with the groove-bound probe lack the longest components seen with the base-stacked probe.

The coumarin data were previously fit by a power law modified to cut off the divergence at zero time.[6,7] The same function is unable to model the DAPI data. Instead, we fit (eq 1) the Stokes shift $S(t)$ with the same power law multiplied by a sum of two exponentials to accommodate the convergence in long times.

$$S(t) = S_\infty\left[1 - \left(1 + \frac{t}{t_0}\right)^{-n}\left(a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2}\right)\right] \qquad (1)$$

This empirical fit nicely models the Stokes shift over the entire time window with the parameters $n = 0.146$, $t_0 = 30$ fs, $S_\infty = 2145$ cm^{-1}, $\tau_1 = 460$ ps, $a_1 = 0.77$, $\tau_2 = 6$ ns, $a_2 = 0.10$. Note that the value of the power-law exponent "n" is very similar to the one found with base-stacked coumarin ($n = 0.15$).[6,7]

Figure 2B constructs the solvation correlation function $C(t) = (S_\infty - S(t))/S_\infty$ on a log−log plot from the Stokes shift data in Figure 2A. Figure 2B also compares our DAPI data with previous MD results of Corcelli,[15] Hynes-Bagchi,[14] and Berg.[13] The fitted results to the correlation data of Corcelli[15] and Hynes-Bagchi[14] are plotted here after being shifted vertically upward. This shift is needed to compensate for the inertial components that are found in simulations,[5,13] but not in this study. This plot nicely reconciles the earlier simulation results which accord very well with the DAPI data in short time scales. Both groove-bound and base-stacked probes have been simulated, but a direct comparison in the important nanosecond time range has not been published. However, Corcelli and co-workers are currently simulating base-stacked coumarin and groove-bound hoechst in the nanosecond time scales and find a difference in the dynamics between these probes, similar to the ones seen here in this experiment.[19]

The present result can be explained by recalling the fact that the DNA with a base-stacked probe generally retains its spine-of-hydration, a set of structured water molecules in the groove.[20] Sen et al. suggested that this groove-bound water near a base-stacked probe (a native base) is the major source of the slow dynamics seen in DNA.[13] Very recent simulation by Bagchi and co-workers also show that these groove-bound water molecules are motionally restricted.[21] Binding of a probe within the minor groove of DNA displaces a large amount of these restricted water molecules from the groove near the probe.[15,22,23] The displacement of these groove water molecules would thus lead to the suppression of slow components of the dynamics, as seen in the present experiment.

Many questions remain about the origin of the slow anomalous dynamics in DNA. However, the present study confirms that it is essential to consider the real structural differences in the DNA with a groove-bound and a base-stacked probe when explaining the (slow) dynamics at different probe positions inside DNA. Certainly, many more experimental and simulation studies using other probes and DNA sequences are necessary to further confirm, in general, that the probe positions inside DNA change the observed dynamics, and also to quantify its molecular origin.

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Supporting Information Available: Experimental details, data analysis, additional data, and text. This material is available free of charge via the Internet at http://pubs.acs.org.

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