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Sodium-Ion Binding to DNA: Detection by Ultrafast Time-Resolved Stokes-Shift Spectroscopy

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Because DNA is a polyelectrolyte, its counterions can have profound effects on the DNA itself.[1,2] Recent debate has centered on the possible role of "bound" counterions.[2−7] The existence and prevalence of bound ions is unresolved, as is the exact position of the important binding sites. Do ions bind in the minor groove, in the major groove, at single phosphate oxygens, or between phosphate groups? Also at issue is whether bound counterions are a significant determinant of DNA properties, or if they are a secondary effect resulting from preexisting features of DNA structure.[8] Current evidence for bound counterions has come primarily from X-ray crystallography, NMR, and computer simulation. Each method highlights different aspects of ion binding, because each technique has different criteria for classifying an ion as bound. These criteria include various combinations of spatial position, high local density or long residence time. This communication shows that time-resolved Stokes-shift (TRSS) spectroscopy supports the existence of bound sodium ions and provides a new and different perspective on their properties and their effects on the DNA double helix.

The application of TRSS spectroscopy to DNA has been described in detail previously.[9−11] A coumarin-102 deoxyriboside[12] is incorporated into a synthetic oligonucleotide as a replacement for a base pair. The fluorescence spectrum of the coumarin-102 group is sensitive to the polarity of its environment. Upon optical excitation, the dipole moment of the coumarin-102 group increases. Groups in the environment that bear charges, dipoles, or more complex charge distributions move to stabilize the coumarin-102 dipole by increasing the electric field at its position. As the energy of the coumarin-102 excited state is lowered, its fluorescence spectrum shifts to lower frequencies. Thus, the movement within the DNA is directly reflected in the time-dependent evolution of the fluorescence spectrum.

Measurements were made on the 17-mer 5′-GCATGCGC(cou)-CGCGTACG-3′ hybridized with its complement. (cou = coumarin, which is paired with an abasic-site analogue in the complement.) We have reported results on this duplex with sodium counterions elsewhere.[10,11] In the current studies, additional measurements were made on the same sequence with tetrabutylammonium (TBA) counterions.

The mean frequencies and second central moments (i.e., standard deviations) of the spectra are shown in Figure 1 for both the Na^+ and TBA samples. The logarithmic kinetics of the mean frequencies, i.e., the data forming a straight line on a log−time plot, is a highly unusual characteristic of DNA, which we have discussed before.[10]

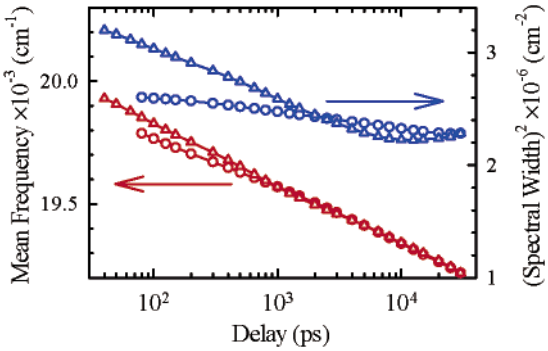

Figure 1. Time evolution of the mean frequency (red, left) and second central moment (blue, right) of the emission spectra of the coumarin in DNA with sodium (triangles) and tetrabutylammonium (circles) counterions. The Na^+ results are matched to TBA at 30 ns (see Supporting Information).

Standard theory predicts that the emission spectra will shift in frequency without changing shape. Thus, the standard method of analyzing TRSS experiments calls for reducing the time-resolved emission spectra to their mean frequencies. Comparing the mean frequencies in Figure 1, there is only a small difference between the samples with TBA or Na^+ counterions, even though TBA has a 2.5-times lower specific conductance (i.e., slower diffusion rate) than Na^+. Diffusion of free counterions is not the origin of the unusual logarithmic kinetics nor the primary determinant of its rate.

Unexpectedly, there is a significant difference in the evolution of the spectral shape as measured by the second central moments (Figure 1). This effect is seen more clearly by plotting the entire emission spectrum after removing the frequency shift with time. For the TBA sample, the shift for each time $\nu_{TBA}(t)$ and the amplitude were each adjusted to form a common spectral shape. Figure 2a shows that the spectra from different times do overlap to form a common shape, especially near the peak and on the low-frequency side of the spectrum. The constancy of the spectral shape is consistent with standard theory, which is based on linear coupling of the optical transition to the local electric field. On the high-frequency tail, there is a small, but systematic narrowing of the spectrum with time. This small effect can be explained without major new assumptions. For example, the narrowing could be attributed to a small quadratic coupling[13] in addition to the dominant linear coupling.

Figure 2a also shows the steady-state emission spectrum of the same oligonucleotide in a 3:1 glycerol:water glass at 195 K. In this sample, all diffusive motion is frozen, so that this spectrum should be a good model for the very early ("zero-time") spectrum in aqueous solution. This is true. The frozen spectrum has the same overall spectral shape as the time-resolved spectra, and its high-frequency edge is slightly broader than the earliest measurement at 80 ps.

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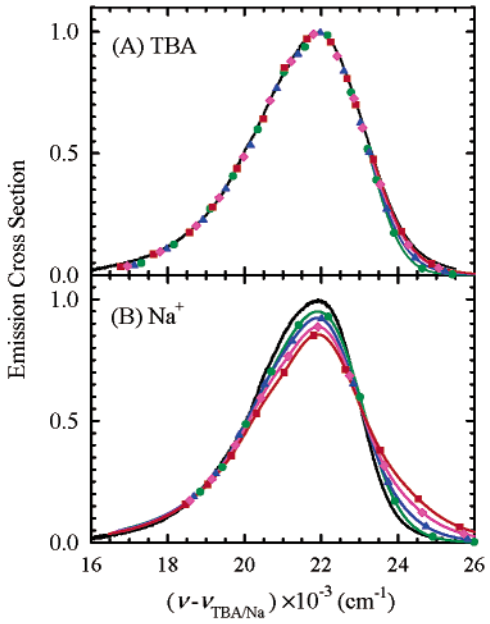

Figure 2. Emission spectra shifted in frequency to show changes in shape: 80 ps (red squares), 650 ps (purple diamonds), 5 ns (blue triangles), 40 ns (green circles), steady-state glass (black solid curve, unshifted). (A) Tetrabutylammonium counterions. (B) Sodium counterions.

When the counterion is changed from TBA to Na^+, the emission shape changes more dramatically with time. Figure 2b shows spectra that have been shifted by a frequency $\nu_{Na}(t)$ to match their peaks and multiplied in amplitude to create constant areas under the spectra.

At early times, the spectrum has a very broad high-frequency edge that narrows at long times. The final spectral shape is nearly identical to that with TBA. This strong and unexpected effect indicates a specific interaction of the Na^+ ions with the oligonucleotide. We suggest that the TBA cannot undergo this interaction because its bulky alkyl groups prevent close approach of the TBA to sites on the DNA.

It is reasonable to regard these strong specific interactions of the Na^+ as "binding" to the DNA. Moreover, the TRSS results show that these bound counterions are important for the chemical properties of DNA. Spectral shifts of coumarins correlate well with standard measures of polarity. Polarity has major effects on chemistry, due to the ability of local electric fields to facilitate charge transfer or to stabilize charge separation in transition states. Although TRSS measurements cannot directly comment on the structural nature of counterion binding, they do reflect the chemical effect of the binding.

The ion-dependent spectral narrowing could be caused by either of two mechanisms. In the first, the oligonucleotides with bound counterions could have a high transition frequency and the loss of the high-frequency tail would represent unbinding kinetics in the excited state of the coumarin. In this case, the high-frequency tail would already exist in the equilibrium ground state and would also be present in the zero-time emission spectrum.

Alternatively, ion binding could have little effect on the transition frequency, but could slow the dynamics of the DNA. In this case, the spectrum would be narrow in the ground state and in the zero-time emission spectrum. The oligonucleotides without bound ions would shift to lower frequencies more rapidly than those with bound ions. The spectral peak is dominated by the unbound oligonucleotides. When spectra are shifted to match their peaks, the frame of reference follows the unbound dynamics. During the early (and unobserved) part of the dynamics, the spectrum of the bound oligonucleotides would appear to lag behind at higher frequencies. At intermediate times, the spectrum would show a high-frequency tail. At long times, the bound oligonucleotides would catch up to the unbound ones, and the spectrum would narrow.

Figure 2b shows that with Na^+ counterions, the spectrum of the frozen sample is much narrower than the spectra at early times. If the frozen sample is again a good model for the zero-time spectrum, the second mechanism is indicated. The ion-dependent dynamics of the high-frequency tail result from a slowing of the overall DNA dynamics when counterions are bound.

The spectra in Figure 2 are evenly spaced in logarithmic time, and the magnitude of spectral change is approximately uniformly distributed over these times. Roughly speaking, the ion-dependent dynamics follow the same logarithmic kinetics that the ion-independent dynamics do. The dynamics are spread over a broad range of picosecond and nanosecond time scales. The lifetimes of the binding sites must be as long or longer than the observed ion-dependent spectral dynamics.

A caveat to these results is that the coumarin probe may affect the nature of the binding sites. If the binding is relatively distant from the coumarin, e.g., at the phosphate oxygens, the current results may be transferable to unmodified DNA with semiquantitative accuracy. If the binding sites are very close to the coumarin, e.g., at the base of the grooves, the results may be only qualitatively representative of the phenomena in unmodified DNA. In either case, relative comparisons based on modifying either the DNA or the counterion will be more transferable than absolute numbers.

In conclusion, TRSS show the existence of bound counterions through easily observed spectral changes. TRSS experiments provide a new perspective on these bound ions that emphasizes their chemical and dynamical effects on DNA.

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Note Added after ASAP: In the version published 9/6/2003, the current addresses of the first two authors were transposed. The final version published on the Web 9/15/2003 and the print version are correct.

Supporting Information Available: Details of experiment and analysis, comparison of TBA and Na^+ spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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