Ultrafast Internal Conversion of Electronically Excited RNA and DNA Nucleosides in Water

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The DNA and RNA bases absorb ultraviolet (UV) light of wavelengths shorter than 300 nm strongly, and are the dominant UV absorbers in many cells.[1] Not surprisingly, nature has developed a variety of adaptations to control and limit the photochemical damage that might otherwise be initiated by the energy-rich excited states formed by the absorption of UV photons. Enzymatic repair, for example, actively reverses certain kinds of photochemical damage. Equally important safeguards are associated with the intrinsic photophysical properties of the bases themselves. The lowest energy transitions of the bases are located in a spectral region where the UV solar flux is significantly attenuated by the Hartley absorption continuum of ozone. Further protection comes from remarkably efficient relaxation pathways for electronic energy. The existence of these pathways can be inferred from the very low fluorescence quantum yields of the DNA bases at room temperature, which were first reported in 1971.[2] Despite the efforts of many groups, attempts to directly observe the excited-state dynamics of the DNA bases have met with little success in the intervening 3 decades.[3] Here we report the direct observation by femtosecond pump−probe spectroscopy of excited-state absorption of one DNA and three RNA nucleosides in aqueous solution. The results show that the lowest excited singlet state (S_1) decays in under 1 ps for all nucleosides by ultrafast internal conversion to the electronic ground state.

Femtosecond pump−probe experiments were performed using 150 fs UV pump pulses with a center wavelength of 263 nm. This wavelength excites the strong $\pi-\pi^*$ transition of each nucleoside. Probe pulses from 250 to 750 nm were used to measure the absorbance change (ΔA, equivalent to ΔOD) induced by the much more intense pump pulse as a function of delay time. Our femtosecond transient absorption spectrometer, described in more detail elsewhere,[4] can measure absorbance changes as small as 10−20 ppm, and this high sensitivity was essential for detecting the weakly absorbing excited states of the nucleobases. The polarizations of the pump and probe pulses were set to the magic angle, and both pulses were crossed in a liquid jet of the solution under study. The ribonucleosides adenosine (Ado), cytidine (Cyd), and guanosine (Guo) and the DNA nucleoside thymidine (Thd) were obtained from Sigma and used to prepare 0.5−4.0 mM aqueous solutions with ultrapurified water. The pH of these unbuffered solutions was approximately neutral. All measurements were performed at room temperature.

Immediately following the UV pump pulse, weak transient absorption was detected for all nucleosides at visible wavelengths between 450 and 750 nm. The signals decay on a subpicosecond time scale with a time constant that is characteristic of each (vide infra). Results for Ado are shown in Figure 1a. The signals at visible probe wavelengths decay with the same time constant, as

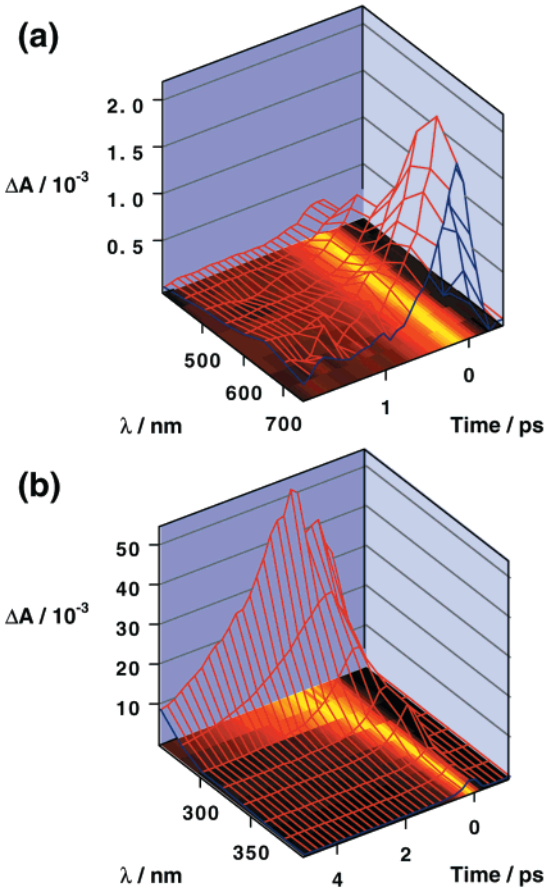

Figure 1. Transient absorbance induced by a 150 fs, 263 nm pump pulse for a 4.0 mM solution of adenosine in water. Transient signals recorded as a function of the time between pump and probe pulses at sixteen separate probe wavelengths are combined in the red wireframe plots for the visible (a) and UV (b) spectral regions. The image beneath each wireframe displays the same data after dividing the transient signal at each probe wavelength by its maximum value. The colormap used in these images displays regions of low and high amplitude by dark red and bright yellow, respectively.

shown by the color contour map underneath the wire mesh. This map was created by dividing the time-resolved signal at each probe wavelength by its maximum value. The parallel (with respect to the wavelength axis) bands of color show at a glance that the signal decays are wavelength-independent. This is consistent with excited-state decay to the ground state, and the visible signals are assigned to S_1 absorption.

The signals in Figure 1a decay to an offset that is approximately constant on the time scale of tens of picoseconds. Similar results were found for the other nucleosides. The offset is due to absorption by solvated electrons produced by two-photon ionization of water molecules, which are randomly distributed throughout the irradiation volume. The subpicosecond decaying signal near $t = 0$ varies linearly with the pump intensity, and is easily masked at higher pump intensities by the offset, which grows quadratically. Signals were recorded at the lowest pump intensities (1−8 GW/cm^2) that produced adequate signal-to-noise ratios for the weak, short-time signals. Even under these conditions a small offset was still present.[5] This is a consequence of the low absorption cross sections of the S_1 states. Assuming that every

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(3) Table 1.2 on p 35 of ref 9 illustrates the lack of consensus on the excited-state lifetimes of the DNA bases.

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(5) The pump intensity used in a previous pump−probe study of thymine, [Reuther, A.; Nikogosyan, D. N.; Laubereau, A. *J. Phys. Chem.* **1996**, *100*, 5570] was too high to observe the excited-state absorption reported here.

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Table 1. S_1 Lifetimes[a] of the Nucleosides in Water at Room Temperature

	adenosine	guanosine	cytidine	thymidine
τ/fs	290	460	540	720

[a] Estimated uncertainty ±40 fs.

photoexcited solute contributes to the excited-state absorption seen at short times, the maximum extinction coefficients of the purine nucleosides, Ado and Guo, are estimated to be $\leq 1000\ M^{-1}\ cm^{-1}$. The extinction coefficients for the pyrimidines, Cyd and Thd, are estimated to be a factor of 3 smaller. These values contrast strongly with a previous estimate of $50\,000\ M^{-1}\ cm^{-1}$ for the thymine S_1 extinction coefficient.[6]

For each nucleoside, transients at 3−6 probe wavelengths from 400 to 800 nm were globally fit[7] to determine the S_1 lifetime. The exponential time constant was adjusted globally, while the amplitude and a constant term (to model the offset) were adjusted independently for each transient. The fitting function was convoluted with a Gaussian instrument response function (fwhm 210 fs). The decay times are summarized in Table 1. While we believe these directly measured values to be the most accurate estimates to date, it is interesting to compare our values with indirect estimates determined from the product of the measured fluorescence quantum yield[8,9] and the estimated radiative lifetime of each compound. For example, Vigny and Duquesne reported values of 0.3, 0.5, 0.8, and 1.0 ps for the nucleotides corresponding to Ado, Guo, Thd, and Cyd, respectively.[10] The good agreement with our results may be fortuitous, especially given the approximations inherent in the estimation of radiative lifetimes.[8] Nevertheless, the prediction that the S_1 lifetimes of the purines are shorter than those of the pyrimidines is in line with our findings and an independent estimate made by an energy transfer method.[11]

After internal conversion, the newly formed molecules in the electronic ground state (S_0) have an excess energy of at least $34\,000\ cm^{-1}$, the average energy gap between the vibrationless levels of S_0 and S_1.[9] This substantial excess energy corresponds to an initial vibrational temperature in excess of 1000 K. This rapid temperature jump causes strong absorption on the red edge of the S_0 spectrum as seen in the signals recorded for Ado at UV probe wavelengths (Figure 1b). Thermal equilibrium is restored by intramolecular vibrational redistribution (IVR) and by the transfer of vibrational energy to the solvent. Hot ground-state absorption provides a direct means of studying the vibrational cooling dynamics of molecules that undergo ultrafast internal conversion.[12]

The decay times of the S_0 red edge absorption depend strongly on probe wavelength, and this behavior is a further hallmark of vibrational cooling. This can be seen in the curvature of the color contour map in Figure 1b. The increasing temporal width of the yellow (maximum amplitude) areas of the color map highlight the progressively slower decays observed at short wavelengths. The time constants that characterize the decay of the hot ground state absorption for Ado increase steadily from 440 fs at 340 nm to 2.0 ps at 270 nm. These decay times are substantially slower than the 290 fs time constant seen at visible wavelengths, indicating that the UV signals cannot be due to a higher-energy transition from S_1.

At the shortest UV probe wavelengths, the signal rise is not instrument-limited, and the maximum occurs at progressively later delay times. The ground-state absorption spectrum thus reshapes and blue-shifts in the first few picoseconds after the pump pulse, consistent with thermalization in S_0. The dramatic thermal broadening of the S_0 absorption spectrum seen at the earliest times after the pump pulse indicates that significant vibrational energy is deposited by internal conversion in the in-plane, ring-stretching modes that couple most strongly to the optical transition.[13]

It was argued previously on the basis of steady-state emission spectroscopy that IVR in S_1 occurs on a subpicosecond time scale.[8] It seems plausible then that the decay of the hot ground-state absorption in ≤ 2 ps is due to unusually rapid intermolecular energy transfer to the solvent. Energy transport to the solvent usually occurs on the 10 picosecond time scale.[14] There is however evidence that hydrogen-bonding liquids can dramatically accelerate intermolecular vibrational cooling.[12d] It is possible that the hydrogen bonding between Watson−Crick base pairs may give rise to similarly high cooling rates in double-stranded DNA.

In summary, the results presented here provide the most detailed view to date of the excited-state dynamics of the nucleobases.[15] Internal conversion occurs on the time scale of 0.3−0.7 ps, converting electronic energy into heat. These remarkable photophysical properties may be a further reason nature entrusted the genetic code to these compounds. Conical intersections are generally required for ultrafast internal conversion dynamics,[16] and we hope that the rich dynamical information revealed by this study will motivate quantum chemical efforts to search for such intersections between the S_1 and S_0 states of the nucleobases. The ability to observe vibrational cooling in S_0, also demonstrated here, suggests that the DNA and RNA bases may be excellent model systems for studying fundamental questions related to energy flow in complex polyatomic molecules. The ability to modify bases individually and in biopolymers using the well-developed tools of synthetic biochemistry could be used to study the microscopic factors that influence vibrational energy flow in very hot molecules to their surroundings.

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Supporting Information Available: Transient absorption signals at 570 nm for the four nucleosides with global fits (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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