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Intrinsic Lifetimes of the Excited State of DNA and RNA Bases

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The four bases of DNA are the chromophores of DNA. When a DNA is irradiated by ultraviolet light, the bases are electronically excited and should, in principle, become prone to photochemical reactions that lead to mutagenesis and carcinogenesis.[1−4] Such lethal consequences are largely avoided, however, because the excited bases are believed to have a lifetime so short that they relax to their ground state before a photochemical reaction takes place. It has been suggested that the ultrashort lifetime of the photoexcited DNA bases is what led Nature to adopt the four nucleobases as the durable carriers of genetic information.[5] Consider, for example, the isomers, 2-aminopurine and adenine (6-aminopurine). It appears that thermodynamics of base pairing played little role in the selection of the latter as a nucleobase because, for instance, these two bases have comparable association constants for pairing with uracil.[6] It is the lifetime of the excited state of these bases that is widely different between them: tens of ns for 2-aminopurine versus 1 ps for adenine.[5] The excited-state lifetimes of DNA bases have been measured to be generally very short (of the order of 1 ps or even less) in aqueous solution,[7−14] but it has never been tested whether these short lifetimes are an intrinsic property of these molecules or a fortuitous result of their interaction with solvents. Since the solution has often proved to be a vastly different medium from the structurally confined biological environment devoid of strong dielectric effect, a short lifetime in a labile solution may not mean the same in an in vivo organism unless it is intrinsic to the molecule. Here we show explicitly for the first time, by measuring the lifetime for an isolated molecule, that the ultrashort lifetime of all nucleobases is their genuine molecular property, resulting from an extremely facile internal conversion. The lifetime should therefore be largely independent of the medium, that is whether in vacuo, in solution, or in vivo.

Details of our experimental setup for femtosecond pump−probe ionization have been published elsewhere.[15] In short, we used a third harmonic pulse of a Ti:sapphire laser to pump the molecule to its electronically excited state, and then after a given time delay, we probed the population of the excited state by multiphoton ionization using the fundamental light. An effusive beam condition was employed throughout this experiment because dissociative ionization of clusters generated by supersonic expansion often yields misleading conclusions about the genuine property of a molecule.[16] The powder sample of DNA bases was heated in a metal or Teflon oven to 170 °C (250 °C for guanine) to attain a vapor pressure of a few mTorr and effused into the interaction region. The sample was purchased from Aldrich Chemical Co. and used without further purification.

Figure 1 shows the pump−probe transient of adenine at the pump wavelength of 267 nm. The experiment was performed at a high level of probe power where one of the transitions was saturated to achieve a sufficiently large signal-to-noise ratio. Although three photons of probe pulse were needed to ionize the excited state of adenine, the dependence of the transient ion signal on the probe power was quadratic, indicating the saturation. Since the sum of the energy of one pump photon and one probe photon matches the energy of a highly excited state with an absorption band near 200 nm,[17] this resonant state may be saturated first in the ionization step. The dependence of the transient ion signal on the pump power remained linear throughout the experiment.

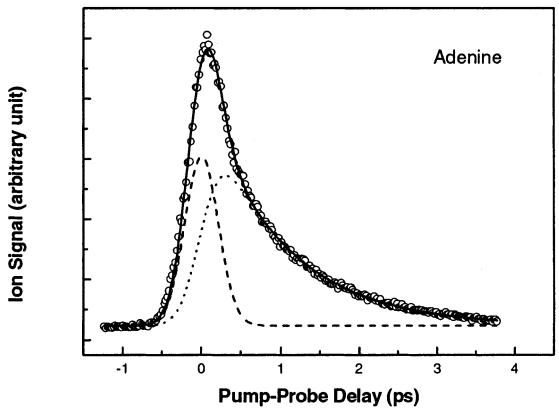

Figure 1. Pump−probe transient ionization signal of adenine in the gas phase at the pump wavelength centered at 267 nm. Hollow circles: experimental data; solid line: theoretical fit to the data; dashed line: Gaussian component of the fit; dotted line: exponential component of the fit. The Gaussian component results from coherent absorption of the pump and probe pulses. The averaged exponential decay time is 1.0 ps, which is the intrinsic lifetime of the excited state of adenine.

The transient has a large spike around the time zero, followed by a decay that drops completely to the background level, in contrast to the decay curve of an earlier picosecond study.[18] It could be well fitted into the sum of a Gaussian function (dashed line in Figure 1) and a single exponential decay (dotted line) convoluted by the cross-correlation of the lasers. The time constant of the exponential decay was 1.0 ps. The ratio of the Gaussian component to the exponential component was increased as the probe power was increased, but the decay time of the exponential component remained constant. The most likely explanation for the Gaussian component is coherent absorption of the pump and probe photons, especially when their intensities are high. For example, when the pump and the probe pulses coincide at zero delay time, excitation to the aforementioned resonant state at 200 nm is possible by the $(1 + 1')$ excitation with one pump and one probe photons, in addition to the excitation to the first excited state by a single pump photon. Both kinds of excitation contribute to the ion signal. When the pump and probe pulses do not overlap in time, however, the former type of excitation can no longer occur. The ion signal in this case is due only to those molecules that have been excited to the first excited state by the pump pulse alone. Therefore, the Gaussian component is strongly centered around time zero. The

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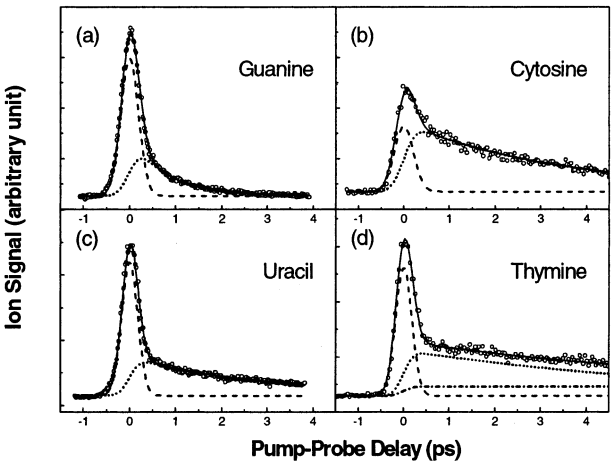

Figure 2. Pump−probe transient ionization signal of (a) guanine, (b) cytosine, (c) uracil, and (d) thymine in the gas phase at the pump wavelength centered at 267 nm. Same notations are used as in Figure 1. The averaged exponential decay times are 0.8 ps for guanine, 3.2 ps for cytosine, and 2.4 ps for uracil. The transient for thymine had a double exponential character, with a short component of 6.4 ps and a long component of longer than 100 ps due to a triplet state, which is represented by the dash−dotted line.

coherent nature of the Gaussian component would also predict decrease of its relative intensity upon lowering the probe power, as experimentally verified.

The fact that the transient ion signal reaches the background level in a few picoseconds after the excitation means that the excited state decays to a dark state that cannot be ionized with the probe laser in that time scale. Thus, the single-exponential decay reflects the excited-state lifetime of adenine. The identity of the dark state is not clear from this experiment alone, but it has been suggested that adenine decays to the electronic ground state by internal conversion,[8−14] possibly through a conical intersection.[11] An ultrafast decay channel was proposed for adenine by theoretical studies,[5,19] which is not present in 2-aminopurine. On the other hand, we cannot completely rule out the possibility of a triplet state acting as a dark state,[20,21] although intersystem crossing is generally known to occur on a much slower time scale.

The coupling between electronic states of adenine can be explained by the proximity effect.[22] In nitrogen-heterocyclic and aromatic carbonyl compounds with nonbonding electrons, adjacent nπ^* and $\pi\pi^*$ states are readily mixed by out-of-plane vibrational modes. A strong vibronic coupling between these states pushes the energy of the lower excited state down toward the ground electronic state, leading to a crossing of the potential energy surfaces of the two states and an efficient decay of the excited state to the ground state through internal conversion.[5,19]

One possible generic mechanism for the ultrafast relaxation of adenine can be suggested as follows. Because the band origins of the $\pi\pi^*$ and nπ^* states are only 600 cm^{-1} apart,[23] the electronic excitation at our wavelength produces a wave packet widely spread out on the composite $\pi\pi^*$/nπ^* potential energy surface. The nπ^* part of the surface has a conical intersection with the ground-state surface, through which the initially prepared wave packet is readily funneled into the ground state in the nπ^* region of the excited-state surface. A conical intersection almost always plays a vital role in the crossing region, as it is generally associated with the ultrafast dynamics of internal conversion.[24] A recent computational study has proposed an alternative route for the internal conversion of adenine through its lowest $\pi\sigma^*$ state.[25]

Figure 2 shows the transients for the other purine base guanine and the pyrimidine bases cytosine, uracil, and thymine at the same pump wavelength of 267 nm. Again, the transients were decomposed into a Gaussian and a purely single-exponential component, which yielded lifetimes of 0.8, 3.2, and 2.4 ps for guanine, cytosine, and uracil, respectively. Thymine was exceptional in that we had to use a double exponential curve to fit the transient. The shorter component for thymine was 6.4 ps, while the longer one was longer than 100 ps. We carried out a power dependence study for this longer component and found from energetic considerations that it was due to a triplet state. It appears that thymine happens to have energy levels coincident with our ionization scheme that led to the detection of the triplet state.

We note that all the nucleobases decay extremely rapidly in the gas phase on about the same time scale as in solution at the excitation wavelength employed in this study, where all the nucleobases have strong absorption. The ultrashort lifetime of nucleobases appears to be an intrinsic molecular property that is little affected by the medium effect of their chemical environment. The short lifetimes do not necessarily correspond to the lifetimes at the electronic band origin,[26] but we note that in this typical UV absorption region the lifetimes of the pyrimidine bases are significantly longer than those of the purine bases in general. It is interesting to note that the pyrimidine bases, especially thymine, are considerably more prone to photodamage than the purine bases in living cells,[1−3] which may very well be related to their longer intrinsic lifetimes in the excited state.

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