Photostability of thymine and its water complexes

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The photophysical and photochemical properties of electronically excited states of thymine and its water complexes are studied using resonantly enhanced multiphoton ionization spectroscopy. After initial excitation by a nanosecond laser, the excited thymine molecule is trapped in a dark state with a lifetime of tens of nanoseconds. Ionization from this dark state by deep ultraviolet (UV) radiation has a substantially high yield. The lifetime of the dark state is rapidly decreased by adding water molecules. These results indicate that the photostability of our genetic code is not an intrinsic property of the bases themselves. Quenching by solvent water molecules may be the key for the photostability of the DNA bases.

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Solar radiation is known to cause mutagenesis and carcinogenesis in mammalian cells[1]. DNA bases are the dominant chromophres in nucleic acids and have a strong absorption in the 200–300 nm range. They are therefore the central targets for such sunlight induced lethality. The absorption of solar ultraviolet (UV) radiation will photoinitiate some chemical processes in the bases and lead to photodamage. For example, on irradiation of UV light, the adjacent bases of thymine in a DNA strand tend to undergo facile photodimerization, which is believed to be able to trigger the complicated skin cancer process or cause a miscarriage of genetic information during replication of DNA. It is widely accepted that the longer these bases stay in the excited state, the higher the possibility that photodamage might happen. It is quite necessary to measure the lifetimes of the excited state of these DNA bases. In the condensed phase, the lifetimes of the first electric dipole allowed excited state (S_1) of the nucleic acid bases are generally short, on the order of one picosecond. This ultrashort lifetime has been suggested to be the reason for the adoption of these bases as the building blocks of the genetic code during the early stages of life's evolution[2].

Recently, some advanced experimental techniques have been applied to the accurate measurement for the excited-state lifetimes of DNA bases[3]. Kohler's group has measured the lifetimes of these DNA bases in solution using femtosecond transient absorption spectroscopy[4]. The lifetimes have been determined to be on the order of picosecond. They suggest that nonradiative relaxation takes place on an ultrafast time scale to the ground state via internal conversion with the extra energy being transformed into heat. Kim's group has measured the lifetimes of these DNA bases in the gas phase using femtosecond pump-probe technique[5]. The decay of these excited DNA bases has been observed to be extremely fast in the gas phase on about the same time scale as in solution at similar excitation wavelengths. Based on these results, they conclude that the ultrashort lifetime appears to be an intrinsic molecular property of these DNA bases, which is little affected by their chemical environment. We have studied thymine and uracil and their methyl-substituted derivatives in molecular beam experiments using resonantly enhanced multiphoton ionization (REMPI) and laser-induced fluorescence (LIF) spectroscopy[6,7]. The results indicate that, upon excitation to the S_1 state, a significant fraction of the excited molecules will relax to a lower "dark state" (S_d) instead of returning to the ground state. The dark state has a lifetime of tens to hundreds of nanoseconds, depending on the extent of methylation. This dark state is unlikely a triplet state since a triplet state is expected to have an even longer lifetime. We tentatively proposed that this dark state was an $n\pi^*$ singlet state, which was later confirmed by a theoretical calculation by Matsika in Temple University[8]. Although we do not have a qualitative measurement of the yield of this dark state, the high ionization yield from this dark state in the deep UV region makes it very important in the photochemistry of DNA bases. However this dark state has never been observed in the solution phase. A common explanation is that the excited molecules will return directly to the ground state via internal conversion (IC) in the condensed phase. The difference between relaxation process in the gas phase and that in the solution should be attributed to the effect of the water solvent. Based on our own observations and the above analysis, we proposed that the photostability of our genetic code may not be an inherent property of the bases themselves. Rather, it could be the water solvent that effectively quenches the potentially harmful photochemistry of the dark state[6]. To test if it is really the water that protects living organism from photodamage caused by UV irradiation, we have investigated hydration effect on the properties of the excited states of thymine and compared the lifetimes of the dark state for thymine and its solvated water clusters. In the experiment, the sample was housed and heated to 220 °C in the nozzle to obtain sufficient vapor pressure. Then the vapor was seeded in 2 atm of helium gas, and the gaseous mixture was expanded into a vacuum chamber at a 10-Hz repetition rate through a 1-mm orifice. Water complexes were formed by bubbling the carrier gas through a room temperature water reservoir (vapor pressure: $\sim$ 23 mbar) before being routed to the heated sample.

Figure 1 shows the one-color two-photon mass spectra of thymine and its water clusters irradiated by 229- (dashed line) and 268-nm (solid line) laser pulses at an intensity of 1 MW/cm^2. It is clearly seen that mass spectra strongly depend on the excitation wavelength. From 220 to 240 nm (the absorption region of the S_2 state of the bare molecule), small water clusters with n up to 4

were readily observable, which indicate the existence of corresponding neutral clusters in our source. While in the region of 240 to 290 nm (the absorption region of the S_1 state of the bare molecule), the attachment of one water molecule decreases the ion signal to half of its value compared with that of the bare molecule. Additional attachment of one or two water molecules has a similar effect. When four or more water molecules are attached, the S_1 state is no longer observable from the ionization spectra. Since the ionization energy of thymine water clusters were lower than thymine monomer, the lack of large thymine water clusters in the S_1 region cannot be attributed to the insufficiency of the excitation photon energy. This dramatic loss of large thymine water clusters in the S_1 region should therefore be a result of a loss during excitation or ionization. The most reasonable explanation is: hydration highly enhanced vibronic coupling between the S_1 and S_d states. This coupling enhancement then accelerated the population decay from the S_1 to S_d state, and consequently resulted in a much shorter lifetime of the S_1 state. Since the ionization efficiency depends on the lifetime of the corresponding state, the shorter lifetime caused a dramatic decrease of the ionization signal from the S_1 state.

Our previous gas phase results concluded that, upon excitation to the S_1 state, the excited uracil and thymine bases were trapped in a long-lived dark state (S_d state) instead of decaying to the ground state[6,7]. Some dangerous photoreactions may occur through the dark state due to its long lifetime. This fact also indicates that photostability is not an intrinsic property of these genetic code molecules. Since such a dark state has never been detected in the condensed phase, it might be the water that quenches this dark state in the condensed phase and stabilizes these bases when exposure to the UV light. In the following, we compare the lifetime of the dark state of thymine to that of its water clusters.

Figure 2 shows the pump-probe transient of thymine and $T(H_2O)_1$ with the pump wavelength at 267 nm and the ionization wavelength at 220 nm. On the scale of the figure, the ion signal due to either one laser alone is insignificant, and the overall signal intensity shows linear dependence on the power of each laser. A single-exponential decay function convoluted with a Gaussion function representing the response of the laser system has been used to fit the experimental data. The fitting errors are limited by the time resolution of our laser system, which is estimated to be 5 ns. The decay constant corresponds to the decay of the population from the dark state, i.e. the lifetime of the dark state. They are determined to be 22 and 12 ns for thymine and $T(H_2O)_1$, respectively. The fact that the decay profile of $T(H_2O)_1$ contains only a single exponential decay function is an evidence that this measurement has not been contaminated by dissociative products of larger complexes. For clusters with two or three water molecules attached, the two color signals are too weak to accurately determine the decay constants. However, as we increased the delay time between the pump and the probe laser, we observed that heavier clusters disappeared faster than lighter ones. We therefore conclude that this decrease in lifetime with increasing water content is gradual for complexes with $n < 5$. No two color ion signals are observable for clusters with four or more water molecules attached.

Based on the above measurements, we propose the following mechanism displayed in Fig. 3 to explain our observations. For bare thymine molecule, the high barrier between the bottom of the S_d and the S_d/S_0 crossing point is responsible for the weak conical intersection between S_d and S_0. This explains why bare thymine molecule has a long-lived dark state. For thymine water clusters, the thymine-water interactions not only enhance the overlap between S_1 and S_d, but also strongly

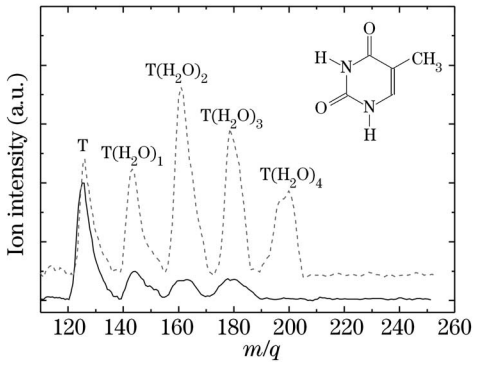

Fig. 1. One-color two-photon mass spectra of thymine (T) and its water clusters obtained at the excitation wavelengths of 229 (dashed line) and 268 nm (solid line), respectively.

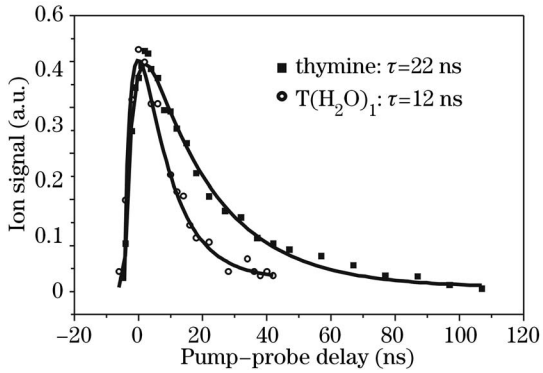

Fig. 2. Pump-probe transient ionization signal of bare thymine and $T(H_2O)_1$ in the gas phase with the pump wavelength at 267 nm and the ionization wavelength at 220 nm. The decay constant corresponds to the lifetime of the dark state. It clearly demonstrates that the lifetime of the dark state is rapidly decreased by adding water molecules.

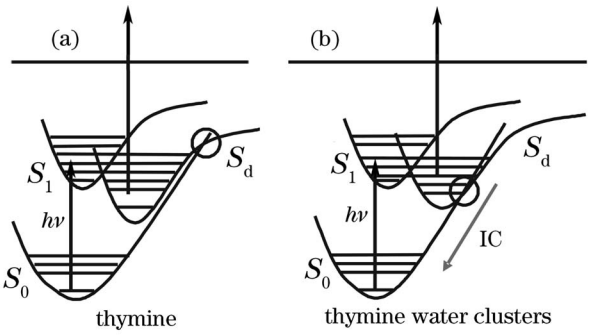

Fig. 3. Proposed potential energy surfaces and processes for thymine (a) and its water clusters (b). Ionization from the dark state S_d produces the two color ion signals in Fig. 2.

influence the crossing point between the S_d and S_0 states. The sum of the hydration effects on the S_d potential surface and the S_0 potential surface leads to a significant decrease of the barrier height. A fast internal conversion thus happens and consequently leads to a much shorter lifetime of the S_d state. It must be noted that the extent of the lowering of the barrier height depends on the number of the water attached to the thymine ring. Thus a gradual decrease of the lifetime of S_d has been observed as thymine became more hydrated.

In summary, we present a decay mechanism of thymine base and its water complexes in the gas phase. After photoexcitation to the S_1 state, the bare molecule is funneled into and trapped in a dark state with a lifetime of tens of nanoseconds. The nature of this dark state is mostly a low lying $^1n\pi^*$ state. Solvent molecules affect the decay pathways by increasing IC from the S_1 to the dark state or to the ground state and from the dark state to the S_0 state. The lifetimes of the S_1 state and the dark state both decrease with the addition of only one or two water molecules. When more than four water molecules are attached, the photophysics of these hydrated clusters rapidly approach that in the condensed phase. Our gas phase results reveal an important function of water in protecting our genetic code from photodamage. The high ionization yield of bare base molecules from the dark state by deep UV radiation and its long lifetime nature indicate that the photostability is not an intrinsic property of the bases themselves. Rather, the interaction of these bases with water molecules provides necessary relaxation mechanism for the excited species. Quenching by solvent molecules may be the key for the photostability of the DNA bases.

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