Letter

Decay Pathways of Thymine and Methyl-Substituted Uracil and Thymine in the Gas Phase

Yonggang He, Chengyin Wu, and Wei Kong

J. Phys. Chem. A, **2003**, 107 (26), 5145-5148• DOI: 10.1021/jp034733s • Publication Date (Web): 06 June 2003

THE JOURNAL OF PHYSICAL CHEMISTRY A

 VOLUME 107, NUMBER 26, JULY 3, 2003

LETTERS

Decay Pathways of Thymine and Methyl-Substituted Uracil and Thymine in the Gas Phase

Yonggang He, Chengyin Wu, and Wei Kong*

Department of Chemistry, Oregon State University, Corvallis, Oregon 97331-4003

Received: March 21, 2003; In Final Form: May 15, 2003

We report observations of a dark state in the decay pathways of thymine, 1,3-dimethyl thymine, 1,3-dimethyl uracil, and 1-methyl uracil in the gas phase. After initial excitation by a nanosecond laser, the excited molecules failed to return to the ground state but rather were trapped in a dark state for tens to hundreds of nanoseconds. This result contradicts those reported in water solutions. We therefore propose that the photochemistry of these pyrimidine bases is different in the gas phase from that in the liquid phase and that the dark state is effectively quenched in water solutions. Although we do not have a quantitative measure of the yield of this dark state, the fact that further ionization from this dark state has a high yield in the deep UV makes this pathway important in the chemistry of nucleic acid bases. This result further implies that the photostability of our genetic code may not be an inherent property of the bases themselves.

One of the important risk factors to living organisms is ultraviolet (UV)-induced carcinogenesis from sunlight; the two major cellular targets are DNA and RNA because of their strong absorption in the 200−300 nm range.[1] Fortunately, stratospheric ozone and our own self-defense and enzymatic repair systems offer the necessary protection. However, ozone depletion caused by the buildup of man-made chemicals in the atmosphere has led to increased UV radiation at the earth's surface and consequently a rising rate of skin cancer.[2] In general, two photoinduced processes are believed to trigger lethal mutagenesis and carcinogenesis: one is photoionization of the bases via the absorption of two photons, and the other is formation of cyclobutane dimers between adjacent pyrimidine bases via a long-lived intermediate state.[3,4] Over the past two decades, efforts have been made in the study of the relaxation dynamics of excited-state nucleic acid bases. The lifetimes of the first electric dipole-allowed state (S_1) have been observed to be generally short (around 1 ps), and fast decay to the ground state via internal conversion (IC) has been proposed.[5−13] However, this decay mechanism is far from certain, and other possibilities have also been suggested.[14−16] In this paper, we present evidence of an alternative decay path for isolated species in the gas phase. We observed a dark state in methyl-substituted uracil and thymine bases with lifetimes of 23−209 ns, depending on the excess energy and methyl substitution. Enhanced ion yield in the deep UV region centered at 220 nm from molecules in this dark state manifests the significance of this channel in the photostability of these nucleic acid bases.

Three types of measurements were carried out in this study. In the 1+1′ resonantly enhanced multiphoton ionization (REMPI) experiment, a Nd:YAG pumped dye laser was used to pump the molecules to the S_1 state, while a Nd:YAG pumped OPO laser was used to probe the excited species. Without the pump laser, the probe laser alone generated a 1+1 REMPI signal, and the net 1+1′ REMPI spectrum was obtained by removing the one-laser signal from the two-laser signal. Lifetime measurements were performed by varying the delay of the probe laser relative to the pump laser. In the time-resolved fluorescence measurement, the signal was detected by a photomultiplier tube through two collection lenses. The wavelength region of the

* To whom correspondence should be addressed. E-mail: kongw@chem.orst.edu. Phone: 541-737-6714. Fax: 541-737-2062.

10.1021/jp034733s CCC: $25.00
Published on Web 06/06/2003

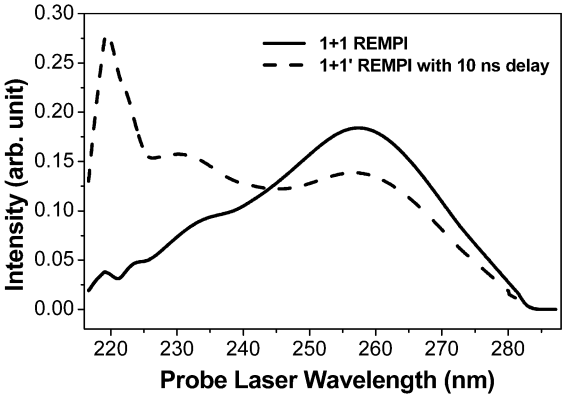

Figure 1. Effect of the pump laser at 250 nm with a time advance of 10 ns on the 1+1 REMPI of the probe laser. The spectra were recorded using the same intensity for the probe laser, and the maximum depletion was 20−25% across the region between 245 and 280 nm.

fluorescence signal was determined using long pass filters. Great care was taken to avoid any saturation effect in the 1+1′ experiment and the fluorescence experiment by recording a linear power dependence of the ion and fluorescence signal on the energy of the pump and probe laser. In the case of the one laser 1+1 REMPI experiment, a second-order power dependence was indeed observed. The samples 1,3-dimethyl uracil (DMU), 1-methyl uracil, and thymine were purchased from Aldrich Co. and used without further purification. 1,3-Dimethyl thymine (DMT) was synthesized from thymine,[17] and its purity was checked by nuclear magnetic resonance (NMR) and infrared absorption (IR) spectra. The powder sample was heated directly inside the pulsed valve to temperatures ranging from 120 (DMU) to 240 °C (thymine) and was supersonically expanded into the interaction region. Limited by the performance of our pulsed valve, we were unsuccessful in conducting the same measurement on uracil, but the resemblance of 1-methyl uracil and uridine and the consistency of our results for all four compounds, including thymine and 1,3-dimethyl thymine, should make our results directly relevant to the photochemistry of DNA.

Figure 1 compares the intensity of the ion signal in the net 1+1′ experiment and the 1+1 experiment. Neither spectrum was normalized by the intensity of the scanning laser; rather, they were smoothed to eliminate structures caused by the fluctuating laser power. The energy of the scanning OPO laser decreased at the blue edge of the spectrum, and this resulted in the missing S_2 state in the 1+1 experiment. The rise in the 1+1′ spectrum in the same energy region, on the other hand, emphasizes the dramatic increase in the total ion signal when the probe laser was in the deep UV. The only difference between the two experiments was an advanced pump beam at 250 nm in the 1+1′ experiment, and the advance time was 10 ns. When the probe laser was at a wavelength between 245 and 280 nm, the early arrival of the pump laser caused a depletion of the ion signal from the probe laser alone, but when the probe laser scanned between 217 and 245 nm, a severalfold increase in the overall ion signal was observed. The maximum depletion observed under the same intensity for the probe laser was 25% of the 1+1 laser signal.

Figure 2 shows the pump−probe transient of 1,3-DMU with the probe wavelength at 220 nm. A single-exponential decay function convoluted with a Gaussian function representing the response of the laser system was used to fit the decay constant. The fitting errors were limited by the time resolution of our laser system to 5 ns. The resulting decay time is dependent on the degree of methyl substitution and the excitation wavelength.

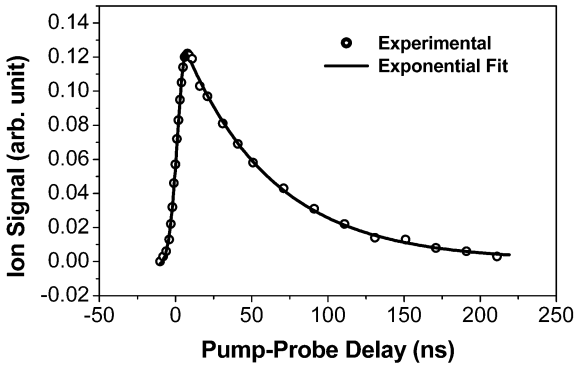

Figure 2. Pump-probe transient ionization signal of 1,3-DMU in the gas phase with the pump and probe wavelengths at 251 and 220 nm, respectively. The exponential decay constant was determined to be 56 ns.

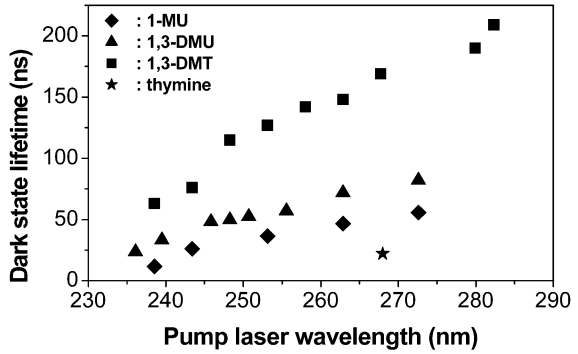

Figure 3. Lifetimes of 1-methyl uracil, 1,3-dimethyl uracil, 1,3-dimethyl thymine, and thymine at different excitation wavelengths.

Figure 3 shows the lifetimes of the four compounds investigated in this study. The general trend is that a lower excitation energy and a higher degree of substitution result in a slower decay process.

To further assess the fate of the molecules in the excited state, we attempted to observe the fluorescence signal, but the signal strength was so low that a quantitative measurement of the fluorescence spectrum was impossible with our existing setup. Qualitatively, the fluorescence lifetime agreed with the decay rate of the REMPI signal, ranging from 18 ns when pumped at 236 nm to 54 ns when pumped at 260 nm for DMU. Using long pass filters, we determined that the peak of the radiation was centered between 370 and 440 nm. In contrast, the fluorescence peak of the S_1 state was measured to be around 300 nm,[18] significantly different from this result.

On the basis of these observations, we propose a new decay mechanism as shown in Figure 4. After initial excitation to the S_1 state, a significant fraction of the gas-phase molecules decay to a dark state (S_d) with lifetimes of tens to hundreds of nanoseconds. This dark state does not absorb in the range of 245−280 nm ($h\nu$) but, instead, in a much higher energy region from 217 to 240 nm ($h\nu'$). In the REMPI experiment of Figure 1, this absence of absorption causes the depletion of the two-laser signal when the probe wavelength is between 245 and 280 nm. When the probe laser scans into the absorption region of this dark state, an enhanced ion yield is observable. The decay process in Figure 2 corresponds to the decay of this dark state, and the fluorescence of this state ($h\nu_f$) is centered between 370 and 440 nm. Methyl substitution seems to stabilize this dark state, while more vibrational energy obtained from the initial excitation step destabilizes this state by opening more decay channels.

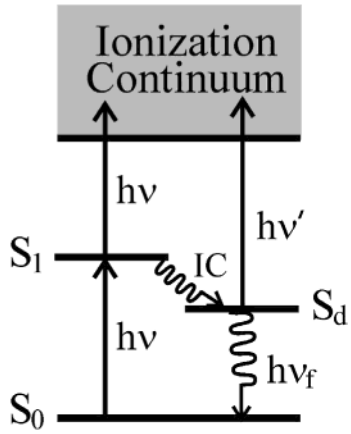

Figure 4. Proposed mechanism of decay for the pyrimidine bases. The dark state, S_d, absorbs in a different energy region ($h\nu'$, $\lambda = 217-245$ nm) from that of the S_1 state ($h\nu$, $\lambda = 245-280$ nm). Absorption or lack of absorption from the dark state populated through internal conversion (or intersystem crossing) results in the enhancement or depletion of the 1+1 REMPI signal in Figure 1.

In Figure 2, the time scale of the decay suggests that the probe beam is not sensing the decay of the highly vibrationally excited states populated from fast internal conversion to the ground state (denoted as IC_0 in the following) and intramolecular vibrational redistribution (IVR) in the dark state.[19] Rather, it should be representative of a different electronic state than S_0 and S_1. It is worth noting that in this gas-phase experiment, molecules in the reaction region can be regarded as collision-free and immobile within the time frame of several hundred nanoseconds. Moreover, with our nanosecond laser systems, we are insensitive to the fast initial steps involving IC_0 and IVR. Therefore, the decay constants in Figure 2 cannot be attributed to the loss of population from S_1, which is known to be several picoseconds.[13] Instead, we should be sensitive to processes after the completion of population redistribution. The decay of the "rediscovered" molecules when the probe beam was between 217 and 240 nm is on a time scale of tens to hundreds of nanoseconds. These constants are much smaller than those from the decay of hot vibrational levels after IVR under collision-free conditions.[19] Thus, Figure 2 should be regarded as characteristic of the dark state rather than of vibrationally excited ground-state species in the molecular beam.

Perhaps the most convincing evidence for the existence of the dark state is the similar decay constants of the ion signal and the fluorescence signal. Although qualitative, the matching of the two decay constants identifies both channels as having the same origin. The possibility for such a fluorescing state to be a manifold of a vibrationally excited ground-state populated from IC_0 and IVR is highly unlikely.

At first glance, our gas-phase results contradict those from the liquid phase. Kohler's group observed red-shifted absorption of the nucleoside in water solutions after excitation to the S_1 state.[10] This spectroscopic shift was regarded as an indication of internal conversion to the ground state, and the absorption was due to the hot bands generated from the IC_0 and IVR. In the gas phase, we failed to observe such an effect, and there was no enhanced ion signal at the red edge of the absorption band. Instead, Figure 1 shows a more or less uniform depletion of the ion signal due to the pump beam across the region between 245 and 280 nm. We attribute this difference to the effect of water solvent on the lifetime of the dark state, and our future report will detail this issue.[20]

A quantitative assessment of the yield of the dark state is impossible without knowledge of the absorption cross section, but an estimate can be obtained from Figure 1. The depletion between 245 and 280 nm should be a result of population loss from the ground state. If we eliminate the possibility of IC_0 and we have neither experimental nor theoretical evidence with regard to dissociation from the S_1 state, the only contributing factor is therefore trapping of the dark state. Assuming an excitation probability of 100% by the pump beam at 250 nm (an overestimate!), a depletion of 20% represents a lower limit for the yield of the dark state. Unfortunately, we were unable to probe the S_1 state and the dark state under saturation conditions, so the enhancement factor at 220 nm cannot be used for a similar estimate.

A few possibilities exist for this dark state, and one such candidate is a $^1n\pi^*$ state. The presence of several heteroatoms with lone pair electrons results in the existence of a number of low-lying $^1n\pi^*$ states close to the $^1\pi\pi^*$ state. These states are readily coupled to the $^1\pi\pi^*$ state by out-of-plane vibrational modes via conical intersections (CI). This type of fast state switch has been invoked to explain the extremely low quantum yield of fluorescence from the $^1\pi\pi^*$ state. On the basis of our assessment of the low fluorescence quantum yield of this $^1n\pi^*$ state, however, we further propose that there exists a weak conical intersection between this $^1n\pi^*$ state and the ground state and an energy barrier on the $^1n\pi^*$ surface that hinders effective population relaxation. The fact that the lifetime of the dark state dropped continuously with increasing pump photon energy offers supporting evidence. A recent calculation by Sobolewski et al. showed that conical intersections between the $^1\pi\pi^*$ state and nearby $^1\sigma\pi^*$ states in aromatic biomolecular systems are ubiquitous.[16] However, the authors stressed that this intersection relies on the polarizability of the NH or OH bond. It is therefore unclear to us whether the same mechanism would be applicable to *N*-methyl-substituted compounds. The multiplicity of the dark state is certainly a point of debate, although we tentatively believe that a triplet state is unlikely because the lifetime of a triplet state in pyrimidine bases has been measured to be on the order of millisecond to second in the condensed phase.[21−23] Moreover, our recent work on water complexes of thymine demonstrates a strong quenching effect on the lifetime of the dark state with the addition of just one or two water molecules.[20] Such a strong effect is unexpected if the involved state is triplet in nature. Although a triplet state for the corresponding nucleosides in solutions is known to be within the same energy region as this dark state,[24] a few theoretical calculations also reported simultaneous existence of a singlet state.[25−27]

The decay mechanism proposed above is consistent with several literature reports.[13,27] The work on cytosine in the gas phase by De Vries' group also reported a long-lived dark state[28] with a lifetime of nearly 300 ns. On the basis of the scale of the lifetime, however, the authors suspected the involvement of a triplet state. Kang et al. measured the lifetime of the S_1 state of all purine and pyrimidine bases in the gas phase using femtosecond pump−probe ionization.[13] The authors observed fast disappearance of the S_1 state within a few picoseconds, but the wavelength of their probe laser at 800 nm was mostly insensitive to the dark state that we observed: a three-photon process corresponds to ionization at 267 nm, while a four-photon process corresponds to 200 nm. In both cases, the ionization cross section was unfavorable for the dark state as seen from Figure 1. Interestingly, the authors reported two decay components in the case of thymine. The longer one with an estimated lifetime of more than 100 ps was suggested to be from a triplet state on the basis of energetic analysis. However, knowing that the $^1n\pi^*$ state in thymine falls in the same energy region as its

triplet state,[25−27] the identity of this component should be considered uncertain. The lifetime measurement of more than 100 ps could simply be a reflection of the time limit in such a femtosecond laser experiment.

Theoretical studies also support our proposal.[29] A recent investigation on cytosine showed the existence of a state switch between $\pi\pi^*$ and $n_O\pi^*$ (the lone pair electron on the oxygen atom) states.[29] The crossing between the two states is weakly avoided, and there exists a small energy barrier between the equilibrium position of the $^1n_O\pi^*$ state and its intersection with the ground state. This barrier might be the very reason that the majority of the molecules are trapped in the dark state after the $^1\pi\pi^*-^1n\pi^*$ internal conversion. More detailed calculations of the decay process from the $^1n_O\pi^*$ state to the ground state will be revealing, as suggested by the authors.

This revelation on the pyrimidine bases parallels that of the purine bases.[10,13,30] In water solutions, purine bases were also determined to exhibit fast internal conversion to the ground state with similar time constants as the pyrimidine bases.[10] In the gas phase, Kang et al. did not observe any evidence of a long-lived dark state.[13] However, Chin et al. attributed the featureless red-shifted fluorescence of four guanine tautomers with lifetimes of tens of nanoseconds to a $n\pi^*$ state.[30] Although the authors cautiously pointed out the lack of direct proof for this claim, previous calculations did reveal such a state for all of the purines.[31]

In summary, we present here evidence for a new decay mechanism of uracil and thymine bases in the gas phase. Our results differ from those of the liquid phase, where fast internal conversion to the ground state dominates the decay path. Instead, the excited-state molecules are funneled into and trapped in a dark state with a lifetime of tens to hundreds of nanoseconds. Ionization from this dark state by deep UV radiation has a substantially high yield, making this channel dangerous in life's early evolution on earth. The very existence of this dark state in isolated molecules further suggests 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. A future report on water complexes of these bases and more extended systems is under preparation.

Acknowledgment. This work was supported by the National Science Foundation, the Petroleum Research Foundation, and the Alfred P. Sloan Foundation. The authors are grateful for discussions with Professor Bern Kohler from The Ohio State University and Professor Charlie Parmenter from Indiana University. The skillful synthesis of 1,3-DMT by T. Suyama is deeply appreciated.

References and Notes

(1) Craighead, J. E., Ed. *Pathology of environmental and occupational disease*; Mosby-Yearbook, Inc.: St. Louis, MO, 1995.
(2) McKenzie, R.; Connor, B.; Bodeker, G. *Science* **1999**, *285*, 1709−1711.
(3) Becker, D.; Sevilla, M. D. In *Advances in Radiation Biology*; Lett, J. T., Adler, H., Eds.; Academic Press: New York, 1993; Vol. 17, pp 121−180.
(4) Brash, D. E. *Photochem. Photobiol.* **1988**, *48*, 59−66.
(5) Daniels, M.; Hauswirth, W. *Science* **1971**, *171*, 675−677.
(6) Callis, P. R. *Annu. Rev. Phys. Chem.* **1983**, *34*, 329−357.
(7) Nikogosyan, D. N.; Angelov, D.; Soep, B.; Lindqvist, L. *Chem. Phys. Lett.* **1996**, *252*, 322−326.
(8) Reuther, A.; Iglev, H.; Laenen, R.; Laubereau, A. *Chem. Phys. Lett.* **2000**, *325*, 360−368.
(9) Pecourt, J.-M. L.; Peon, J.; Kohler, B. *J. Am. Chem. Soc.* **2000**, *122*, 9348−9349.
(10) Pecourt, J.-M. L.; Peon, J.; Kohler, B. *J. Am. Chem. Soc.* **2001**, *123*, 10370−10378.
(11) Peon, J.; Zewail, A. H. *Chem. Phys. Lett.* **2001**, *348*, 255−262.
(12) Gustavsson, T.; Sharonov, A.; Markovitsi, D. *Chem. Phys. Lett.* **2002**, *351*, 195−200.
(13) Kang, H.; Lee, K. T.; Jung, B.; Ko, Y. J.; Kim, S. K. *J. Am. Chem. Soc.* **2002**, *124*, 12958−12959.
(14) Broo, A. *J. Phys. Chem. A* **1998**, *102*, 526−531.
(15) Mennucci, B.; Toniolo, A.; Tomas, J. *J. Phys. Chem. A* **2001**, *105*, 4749−4757.
(16) Sobolewski, A. L.; Domcke, W.; Dedonder-Lardeux, C.; Jouvet, C. *Phys. Chem. Chem. Phys.* **2002**, *4*, 1093−1100.
(17) Hedayatullah, M. *J. Heterocycl. Chem.* **1981**, *18*, 339−342.
(18) Becker, R. S.; Kogan, G. *Photochem. Photobiol.* **1980**, *31*, 5−13.
(19) Hold, U.; Lenzer, T.; Luther, K.; Reihs, K.; Symonds, A. C. *J. Chem. Phys.* **2000**, *112*, 4076−4089.
(20) He, Y.; Wu, C.; Kong, W., manuscript in preparation, 2003.
(21) Salet, C.; Bensasson, R. *Photochem. Photobiol.* **1975**, *22*, 231−235.
(22) Hønnås, P. I.; Steen, H. B. *Photochem. Photobiol.* **1970**, *11*, 67−76.
(23) Görner, H. J. *Photochem. Photobiol., B* **1990**, *5*, 359−377.
(24) Wood, P. D.; Redmond, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 4256−4263.
(25) Lorentzon, J.; Fülscher, M. P.; Roos, B. O. *J. Am. Chem. Soc.* **1995**, *117*, 9265−9273.
(26) Baraldi, I.; Bruni, M. C.; Costi, M. P.; Pecorari, P. *Photochem. Photobiol.* **1990**, *52*, 361−374.
(27) Broo, A.; Pearl, G.; Zerner, M. C. *J. Phys. Chem. A* **1997**, *101*, 2478−2488.
(28) Nir, E.; Müller, M.; Grace, L. I.; de Vries, M. S. *Chem. Phys. Lett.* **2002**, *355*, 59−64.
(29) Ismail, N.; Blancafort, L.; Olivucci, M.; Kohler, B.; Robb, M. A. *J. Am. Chem. Soc.* **2002**, *124*, 6818−6819.
(30) Chin, W.; Mons, M.; Dimicoli, I.; Piuzzi, F.; Tardivel, B.; Elhanine, M. *Eur. Phys. J. D* **2002**, *20*, 347−355.
(31) Lipinski, J. *Spectrochim. Acta. Part A* **1989**, *45*, 557−559.