

Electronic and Vibrational Spectroscopy of 1-Methylthymine and its Water Clusters: The Dark State Survives Hydration

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Electronic and vibrational gas phase spectra of 1-methylthymine (1MT) and 1-methyluracil (1MU) and their clusters with water are presented. Mass selective IR/UV double resonance spectra confirm the formation of pyrimidine-water clusters and are compared to calculated vibrational spectra obtained from ab initio calculations. In contrast to Y. He, C. Wu, W. Kong; J. Phys. Chem. A, 2004, 108, 94 we are able to detect 1MT/1MU and their water clusters via resonant two-photon delayed ionization under careful control of the applied water-vapor pressure. The long-living dark electronic state of 1MT and 1MU detected by delayed ionization,

survives hydration and the photostability of 1MT/1MU cannot be attributed solely to hydration. Oxygen coexpansions and crossed-beam experiments indicate that the triplet state population is probably small compared to the $^1n\pi^$ and/or hot electronic ground state population. Ab initio theory shows that solvation of 1MT by water does not lead to a substantial modification of the electronic relaxation and quenching of the $^1n\pi^*$ state. Relaxation pathways via $^1\pi\pi^* \rightarrow n\pi^*$ and $^1\pi\pi^* \rightarrow S_0$ conical intersections and barriers have been identified, but are not significantly altered by hydration.*

1. Introduction

Absorption of UV radiation by DNA bases can generate mutations which mainly occur at bipyrimidine sites. The major photoproducts are cyclobutane dimers and pyrimidine-(6-4)-pyrimidone adducts. The detailed mechanism of this photoreaction is still unclear—direct reaction in the photoexcited $^1\pi\pi^*$ state,^[1] a long-living $^1n\pi^*$ or triplet state^[2,3] populated by relaxation of the $^1\pi\pi^*$ state and non-concerted product formation in the hot S_0 ground state come into question.

The pyrimidine bases uracil (U) and thymine (T) feature broad absorptions in the gas phase and in solution^[4–10] with an onset at around 275 nm in the vapor spectrum. This first absorption band has been assigned to a $^1\pi\pi^*$ ($S_2 \rightarrow S_0$) excitation.^[6,10–13] The computed oscillator strength for the $^1n\pi^*$ ($S_1 \rightarrow S_0$) transition is smaller by a factor of about 10^3 , that is, the state is “dark”.^[12] The dominating decay channel of UV-excited thymine is subpicosecond internal conversion from the $^1\pi\pi^*$ state to S_0 ^[14] creating $\approx 90\%$ vibrationally hot T which cools on the ps time scale.^[15] Passage to the dark $^1n\pi^*$ state accounts for approximately 10% of T in solution.^[15] Condensed phase lifetimes of the $^1n\pi^*$ state are typically 30 ps for thymine and $4\times$ longer for thymidine monophosphates (TMP). A small fraction of the $^1n\pi^*$ population is proposed to undergo inter-system crossing to the lowest triplet state T_1 in competition with vibrational cooling.^[15]

The triplet states of uracil and thymine have been characterized in considerable detail. Weak phosphorescence of U at 77 K was observed with a maximum at 450 nm (2.76 eV)^[4] which compares favorably with the computed vertical $T_1 \rightarrow S_0$ deexcitation energy of ≈ 2.73 eV.^[12] The adiabatic transition energy is about 3.2 eV. Phosphorescence is weak because the T_1-S_2 and T_1-S_0 spin-orbit coupling matrix elements are small

whereas the significant T_1-S_1 coupling does not lead to enhanced emission because the S_1 state is dark.^[12] A triplet lifetime of 75 ms was observed for T at 77 K via phosphorescence^[4] in contrast to a unimolecular decay time of 32 μ s in deaerated aqueous solutions as measured by nanosecond transient absorption spectroscopy (ns-TAS).^[16] Triplet state formation has a low quantum yield < 0.02 in water.^[17,18,18–20] However, the triplet state is still of interest due to its long lifetime when considering excited state DNA chemistry. TMP has the lowest triplet energy of all nucleotides^[21] hence interbase triplet energy transfer would lead to accumulated triplet TMP.

Ns-TAS experiments show that UV excitation of the single-stranded oligonucleotide (dT)₂₀ leads to cyclobutane pyrimidine dimers (CPD) in less than 200 ns, whereas the (6-4) adduct is formed within 4 ms probably via intermediate oxetane.^[22] Quantum yields of 0.03 and 0.004 are determined for CPD and (6-4) formation, respectively. Femtosecond time-resolved infrared spectroscopy is used to study the formation of CPD in (dT)₁₈ upon 272 nm excitation.^[1] The ultrafast appearance of CPD marker bands points to dimer formation in less than 1 ps. It is concluded that T dimerization in DNA is an ul-

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trafast photoreaction and occurs directly in the photoexcited singlet $^1\pi\pi^*$ state.^[1]

Spectroscopic experiments in supersonic jets are able to study isolated nucleobases in detail and may shine more light on the puzzle of thymine's excited states. IR and microwave experiments demonstrate that the diketo tautomer of U and T is the most abundant species in the gas phase—probably the only one.^[10,23,24] Resonance enhanced two-photon ionization (R2PI) experiments show broad and diffuse electronic spectra of U and T which are attributed either to mixing of electronic states or to a large geometry change between the ground and excited electronic states.^[10] Kong and co-workers^[25] showed that methyl-substituted uracils and thymine can be ionized with tens to hundreds of nanoseconds delay after electronic excitation pointing to the existence of a long-living dark electronically excited state of T in the gas phase which they assigned to a $^1n\pi^*$ state. The lifetime of this dark state depends on the internal energy and the degree of methyl substitution with the general trend that the lifetime increases the more substituted the ring and the longer the pump wavelength is. They observed a decrease of lifetime of the dark state of T and a gradual loss of the ion signal with increasing water content in the jet and concluded that in water solutions the dark state is not populated significantly anymore because fast internal conversion to the electronic ground states dominates. Hence Kong and co-workers infer that the photostability is not an intrinsic property of the pyrimidine DNA bases but results from their hydration.^[25] The work of Kohler et al.^[15] on the corresponding pyrimidine nucleotides and short DNA strands however showed that this state is clearly not quenched in water. We therefore decided to reinvestigate the photophysical behavior of methyl substituted thymine in the gas phase.

Herein we present the first IR/UV double resonance spectra of hydrated 1-methylthymine (1MT) from which we infer the structures of 1MT-water clusters. In particular we demonstrate that in 1MT (methylation where the sugar sits in the DNA) the dark state survives hydration and delayed ionization is still possible. We observe broad electronic spectra both of 1MT and its water clusters and assign their diffuse appearance to fast internal conversion of the pumped $^1\pi\pi^*$ state. Hence in the isolated and in the hydrated molecule both fast and slow channels are still observable. Extensive high level ab initio calculations are performed to understand this photophysical behavior in detail.

2. Results

2.1. Spectra

Figure 1 shows the time-of-flight mass spectra of 1MT and 1MU and their hydrated clusters at water vapor pressures of 8.2 and 12.3 mbar. Two-photon ionization is performed by using 273.2 nm as excitation wavelength and 193 nm for ionization with 40 ns delay between the two laser pulses. At low water vapor pressure (8 mbar) only the masses of the monomers and the 1:1 water clusters are discernible. We assume that the contribution from fragmenting larger water clusters

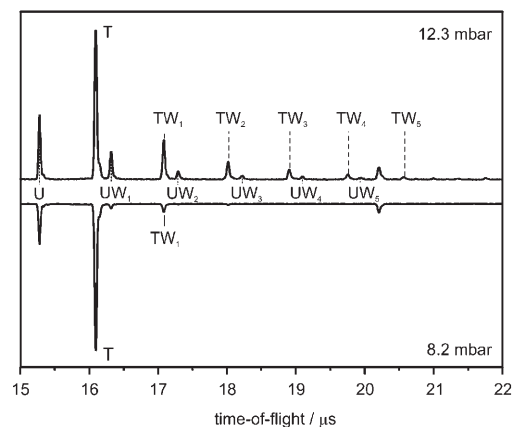


Figure 1. Mass spectra of 1MT and 1MU (abbreviated here as T and U) and their water clusters at water-vapor pressures of 12.3 mbar (top trace) and 8.2 mbar (lower trace). The ion signal of the lower spectrum has been inverted for easier comparison. Excitation at 273.2 nm, ionization at 193 nm; 40 ns delay between the excitation and ionization laser pulses.

plays only a minor role at 8 mbar H₂O pressure. The intensity of the monomer ion signal is about 18-times larger than the water cluster ion signal under these conditions. At a higher water pressure of 12.3 mbar, we observe clusters with up to five water molecules and only four times more monomer than 1:1 cluster signal pointing to extensive monomer-water aggregation and considerable fragmentation contributions to the mass spectrum. At still higher water pressures we monitored increasingly larger water clusters and a continuing decrease of monomer signal due to cluster formation down to zero monomer signal. In contrast to ref. [25], clusters with more than four water molecules were easily detected. With increasing water-vapor pressure, all ion intensities (monomer and cluster masses) decrease and finally vanish, which is probably correlated to a lower ion-detection efficiency under these conditions and the formation of “ice-balls” containing 1MT or 1MU (any traces of 1MT and 1MU are incorporated into very large clusters). As a consequence the overall ion-signal intensities on all small-cluster masses as well as the monomer mass are reduced. This may have been a problem in earlier measurements,^[25] in which a water-vapor pressure of 23 mbar was used to generate thymine-water clusters.

The vibronic spectra of jet-cooled dry 1MT and 1MU detected by R2PI are displayed in Figure 2a. The spectrum of 1MT reveals a distinct peak at 35880 cm^{-1} (278.7 nm) with an onset at $\sim 35700\text{ cm}^{-1}$ followed by a broad, unstructured band which extends up to 41000 cm^{-1} ($\sim 244\text{ nm}$). The electronic spectrum of 1MU is essentially unstructured and blue-shifted relative to the 1MT spectrum, as expected from the lower degree of methylation, with an onset at $\sim 36400\text{ cm}^{-1}$ (274.7 nm). Figure 2b shows the R2PI spectra of 1MT(H₂O) and 1MU(H₂O) clusters detected on the parent masses at low water concentration (8 mbar). As discussed above, we expect preferential formation of small clusters under these experimental conditions. The spectra are again largely unstructured and exhibit only a small shift relative to the monomer spectra. The peak at

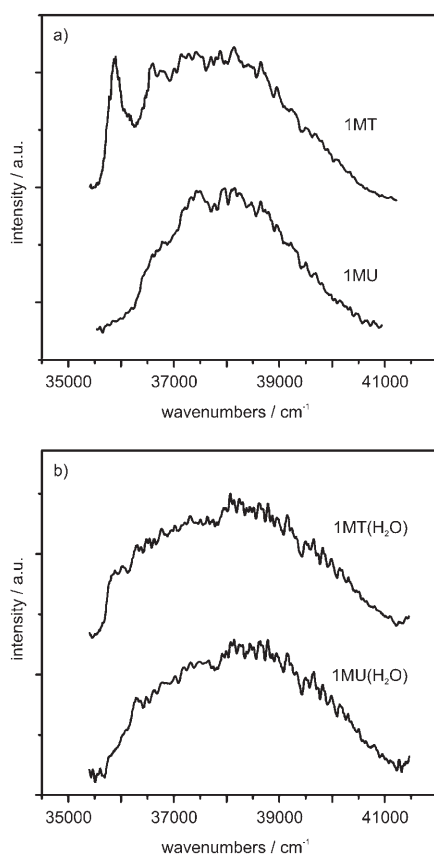


Figure 2. a) Vibronic spectra of 1MT and 1MU monomers (without addition of water) detected by R2PI. b) R2PI spectra of 1MT/1MU-water clusters detected at the 1MT/1MU(H_2O)₁ mass at low water concentration (8 mbar). Ionization at 193 nm; 40 ns delay between the excitation (300 μJ for clusters and 120 μJ for monomers) and ionization (30 μJ) laser pulses.

35880 cm^{-1} (278.8 nm) in the 1MT spectrum is not discernible anymore in the 1MT/water spectrum.

The IR/UV spectrum of dry 1MT is presented in the top trace of Figure 3. The absorption at 3434 cm^{-1} is attributed to the “free” NH stretch vibration. The free NH stretch vibration of 1MU is detected at 3432 cm^{-1} (not shown here). The next trace in Figure 3 shows the IR/UV ion dip spectrum recorded at the 1MT(H_2O)₁ mass at a still lower water pressure of 6 mbar. Cluster structures containing one and two water molecules calculated at the RIMP2 level and their IR stick spectra are displayed for comparison and assignment of the experimental spectrum. The ground-state geometries obtained from CC2/cc-pVDZ calculations of clusters with up to five water molecules are in close agreement with the RIMP2 structures (cf. Figure 7).

Closer analysis shows that the experimental spectrum can be ascribed to the NH and OH stretch vibrations of 1MT(H_2O)₁ with some contributions from 1MT(H_2O)₂ discernible by the shoulder at 3388 cm^{-1} . The IR band of 1MT(H_2O)₂ calculated at $\approx 3000 \text{ cm}^{-1}$ is readily assigned to the OH stretch vibration in the OH...O(H) hydrogen bond and can be expected to be very broad and therefore hard to detect. However, we cannot differentiate between the different isomers of 1MT(H_2O)₁ displayed in Figure 3 because of the close resemblance of the calculated spectra.

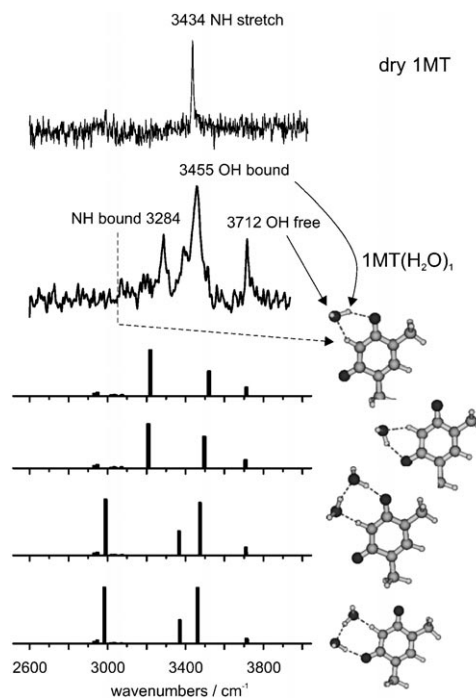


Figure 3. Top trace: IR/UV spectrum of dry 1MT. Lower trace: IR/UV spectrum of 1MT-water detected at the 1MT(H_2O)₁ mass at low water concentration (6 mbar vapor pressure). Excitation at 273.3 nm, ionization at 213 nm. Displayed are the most stable structures of 1MT(H_2O)_{1,2} and their infrared spectra calculated at the RIMP2/ccpVDZ level. The vibrational frequencies were scaled by a factor of 0.9502 to match the calculated free NH-stretch frequency of the 1MT monomer to its experimental value.

2.2. Lifetime Measurements

Figure 4a shows the ion-signal decay of electronically excited dry 1MT and 1MU as a function of the delay time between the excitation (273.2 nm) and ionization (193 nm) laser pulses. Decay times are 107 ns \pm 30 ns for 1MU and 227 ns \pm 30 ns for 1MT. As already pointed out^[25] the lifetime increases with degree of methylation. This finding is supported by ab initio calculations: For bare 1MT we determine the barrier between the $^1\text{n}\pi^*$ minimum and the $^1\pi\pi^*/^1\text{n}\pi^*$ conical intersection to be 1.33 eV (details in the following), whereas this barrier is lower than 1 eV for bare uracil.^[26]

Figure 4b shows the ion-signal decay curves in the presence of water, both, on the monomer masses (1MT and 1MU) and on the 1MT/1MU(H_2O)₁ cluster masses. The ion signals on the cluster masses show a slow, nearly linear decay. In Figure 1, ion signals of clusters with more than four water molecules are readily discernible at a delay time of 40 ns and low water content. At higher water-vapor pressures we measured ion signals for clusters with up to eight water molecules. The decay curves of the larger clusters all show the same, nearly linear decay as the 1MT/1MU(H_2O) clusters. Contrary to earlier measurements^[25] we do not observe a sharp drop of the ion-signal intensities for clusters with more than four water molecules. Instead, the intensities decrease smoothly with increasing cluster size, as in a typical cluster experiment, because more collisions are required to form larger clusters. Figure 4 clearly demonstrates that delayed ionization is possible not only for the

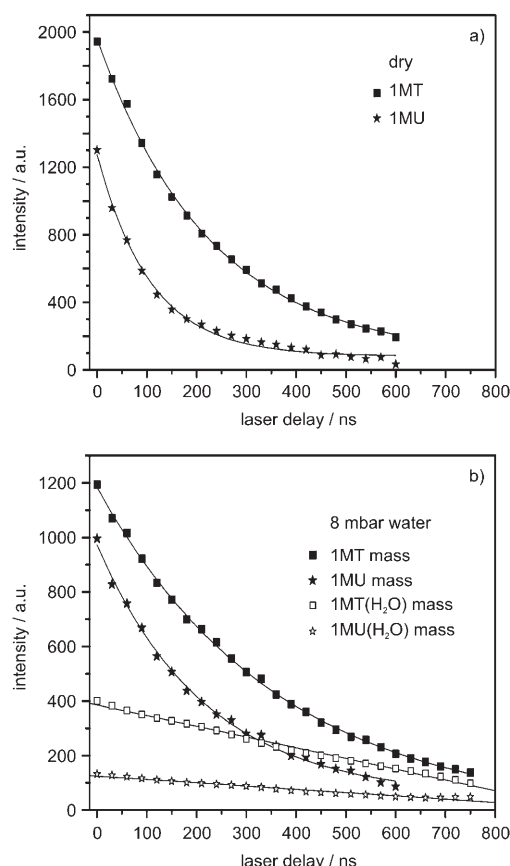


Figure 4. Ion-signal decay of dry 1MT and 1MU (a) and of hydrated 1MT and 1MU (b). Excitation at 273.2 nm (120 μ J) and ionization at 193 nm (30 μ J) with a variable time delay between the excitation and ionization laser pulses. Water-vapor pressure: 8 mbar (4 °C).

monomers, but also for their clusters with water. Identical decay curves are obtained when using 213 nm instead of 193 nm for ionization. Kong and co-workers^[25] used rather high water-vapor pressures in their experiment which leads to a decrease of the ion-detection efficiency up to complete disappearance of the delayed ion signal according to our experience.

The ion-signal decay curves of the clusters in Figure 4b are limited by the time the clusters need to fly out of the ionization volume with the speed of the molecular beam. In our experiment (beam width \approx 2 mm) it takes about 1.5 μ s for all the clusters to fly out of the ionization volume. That time agrees with the extrapolated intersection between the nearly linear decay curves and the time axis in Figure 4b. We confirm this by varying the size of the ionization laser beam. Thus, the long-living dark state of the water clusters decays on a slower timescale ($> 1 \mu$ s) than the size of our observation window.

The lifetime measured on the monomer mass in the presence of water (8 mbar vapor pressure) increases to $218 \text{ ns} \pm 30 \text{ ns}$ for 1MU and $363 \text{ ns} \pm 30 \text{ ns}$ for 1MT which is attributed to a contribution of water clusters fragmenting to the monomer mass, either during the lifetime of the dark state or after ionization.^[27,28]

2.3. Oxygen Coexpansion

The identification of the dark state still remains an open question. Possible candidates are the $^1n\pi^*$ state, a low-lying triplet state or the vibrationally hot electronic ground state populated by efficient internal conversion from the excited-singlet state. Since ground-state triplet oxygen features a low-lying singlet state, efficient 1MT triplet quenching is expected when coexpanding 1MT with oxygen or when crossing a molecular beam of excited 1MT with an oxygen beam. Indeed quenching of T and TMP triplet-triplet absorption with O₂ has been reported to be very effective so that the measurements have to be done in an aqueous solution saturated with N₂ excluding all O₂.^[16] Therefore we decided to use oxygen quenching as a check of the triplet character of the dark state.

In a first step, we coexpanded dry 1MT and 1MU in a mixture of 10% oxygen and 90% helium. The lifetime did not change within the accuracy of our measurements. In a second approach we established an effusive oxygen beam (100% O₂), which propagated perpendicular to the molecular beam carrying 1MT and 1MU and again measured the lifetime of the dark state. We compared the ion-signal intensities under normal expansion and crossed-beam conditions using a delay of 40 ns between the excitation and ionization laser pulses. Crossed-beam experiments with nitrogen and argon were performed as a reference for the attenuation of the molecular beam due to elastic and inelastic collisions in the crossed-beam setup. Comparing the ion intensities gives valuable information, even if the quenching process occurs on a timescale below the detection limit of our apparatus (10 ns). In that case quenching is still observable by a decreased ion-signal intensity.

Indeed we observe a small intensity decrease. However, the effect was similarly large in collisions with nitrogen or argon and can therefore not be attributed to specific triplet quenching. The lifetime of dry 1MT/1MU did not change in the oxygen-beam experiment within the uncertainty of our measurements. Therefore the triplet-state population is probably small compared to the population of the $^1n\pi^*$ or hot vibrational ground state. Kong et al. observed weak fluorescence of 1,3-dimethyluracil with similar lifetimes as the decay times of the delayed ionization signal so that the hot S₀ state seems to us a less likely candidate for the dark state than the $^1n\pi^*$ state. Note, however, that delayed fluorescence in which a low-lying excited-singlet state becomes populated by a thermally activated radiationless transition from the first-excited triplet state is a well-known phenomenon in condensed-phase spectroscopy.^[1,2]

2.4. Calculations

The variance in decay times for bare and hydrated 1MT might be related to the relative energies of the involved electronic states. To gain further insights into the change of the lowest $^1n\pi^*$ and $^1\pi\pi^*$ singlet and triplet states with hydration we performed extensive high-level ab initio calculations. We specifically focus on the following questions: Is a direct decay from the bright $^1\pi\pi^*$ state to the electronic ground state enhanced

by hydration? Does hydration significantly affect the energy barrier for an alternative decay via the dark state?

Our single-point/adiabatic CC2 and LIIC CASSCF/CASPT2 calculations (Supporting Information) enable us to construct an energy level diagram for bare 1MT as shown in Figure 5. In

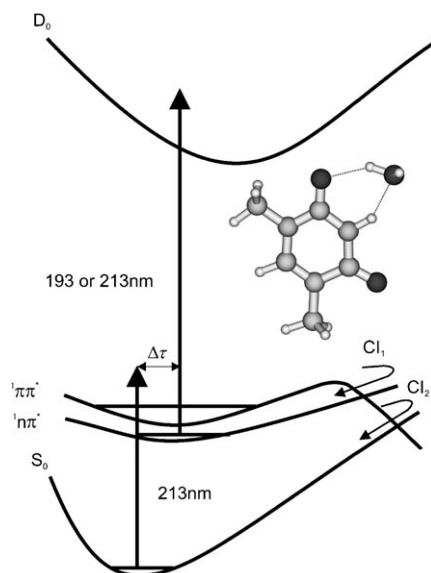


Figure 5. Schematic representation of possible relaxation pathways in 1MT (H_2O)₁ (triplet states excluded).

accord with previous investigations^[29–31] we find conical intersections $^1\pi\pi^*/^1n\pi^*$ (Cl_1) and $^1\pi\pi^*/S_0$ (Cl_2). Furthermore, the two excited states in question exhibit minima on the potential hypersurfaces. The structures at the conical intersections are shown in Figure 6. The structures of the 1MT-water clusters in the electronic ground and $^1n\pi^*$ state optimized at the CC2/cc-pVDZ level are displayed in Figure 7 and in Figure 9 of the Supporting Information.

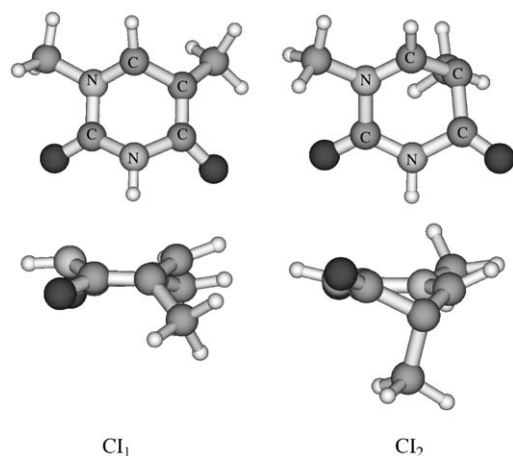


Figure 6. Geometries of the conical intersections in 1MT optimized at the CASSCF(10,8)/6-31G* level: Cl_1 is the intersection between $^1\pi\pi^*$ and $^1n\pi^*$ adiabatic states and Cl_2 is the intersection between $^1\pi\pi^*$ and ground adiabatic states.

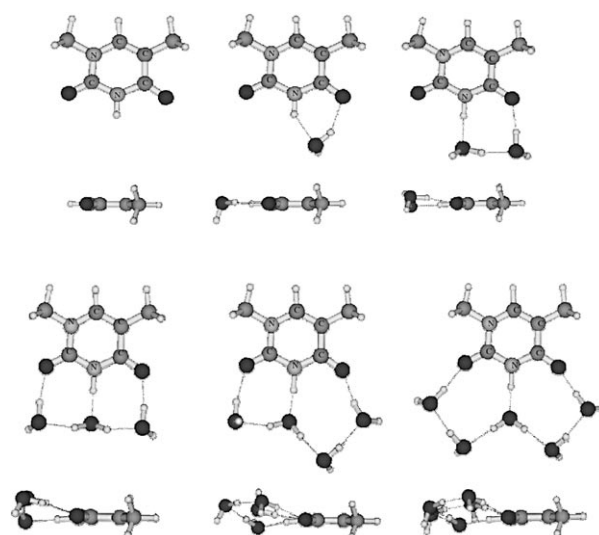


Figure 7. Geometries of ground state 1MT and 1MT-water clusters optimized at the CC2/cc-pVDZ level.

Our calculations show that LIIC paths for bare 1MT and singly-hydrated 1MT are essentially the same. Because of that, we may restrict the discussion to the variance of relative energies due to hydration at crucial points on the hypersurfaces, that is, at the respective minima and conical intersections. From our most accurate calculations (CC2/aug-cc-pVTZ) we determine a $^1n\pi^*$ adiabatic excitation energy of 3.73 eV and a barrier between the minimum of $^1n\pi^*$ and Cl_1 of 1.33 eV for bare 1MT. Upon microhydration with one water molecule, this barrier is lowered to 1.09 eV and the adiabatic energy is increased to 3.88 eV. Due to these findings we expect a higher probability of $^1\pi\pi^*/^1n\pi^*$ internal conversion upon hydration with one water molecule than for bare 1MT.

To address the question of whether increased microhydration might further lower this barrier or lead to a dramatically enhanced direct relaxation from $^1\pi\pi^*$ to S_0 through Cl_2 we have carried out a series of CC2/aug-cc-pVTZ single-point calculations. The results are shown in Figure 8 and Figure 10 (in the Supporting Information) for two points, the equilibrium geometry of the ground state and the equilibrium geometry of the $^1n\pi^*$ excited state. Interestingly, we observe the trend that the $^1n\pi^*$ state is destabilized energetically relative to the $^1\pi\pi^*$ state for structures with one and two water molecules. This points to both an enhanced direct relaxation and a reduced population of the $^1n\pi^*$ state. The trend, however, is broken at the time when the third and subsequent water molecules are attached to the system. In the cluster with five water molecules only a minute deviation of the relative energies from those of bare 1MT remains. We ascribe this energetic reconstitution to a recovery of partial symmetry at the substitution sites (where the hydrogen bonds perturb the electronic valence distribution) as soon as the third water molecule enters the cluster. The preferred formation of water clusters around the oxygen binding sites is shown in Figure 7 where also the symmetry argument becomes obvious. Thus, a substantial modification of the relaxation pathways of 1MT through micro-

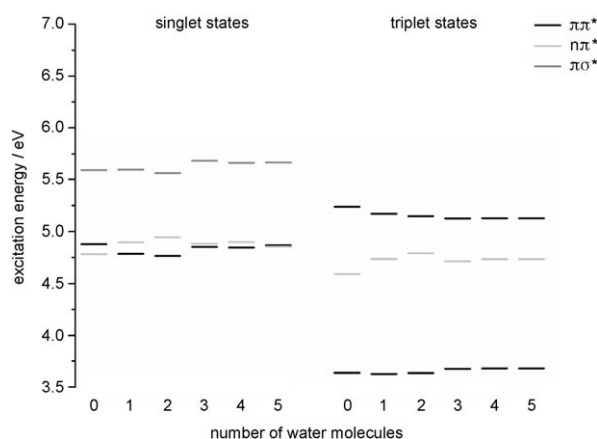


Figure 8. Vertical electronic excitation energies calculated at the ground state geometry at CC2/aug-cc-pVTZ level (the three lowest states). All structures are optimized at the CC2/cc-pVDZ level.

hydration is not observed and a claim that solvation by water would lead to a quenching of the dark state cannot be supported by ab initio theory.

3. Discussion

The decay mechanisms of 1MT/1MU have been investigated and correlated with their water aggregates. We are, to our knowledge, the first to publish R2PI spectra of 1MT/1MU and their water clusters. IR/UV double resonance experiments confirmed the formation of 1MT/1MU(H_2O) clusters, in which the water molecule forms a bridge between the NH bond and a neighboring C=O group. Ab initio calculations reproduce the experimental spectra. The infrared spectra also show that fragmentation of larger water clusters is minimized at low water concentrations in the supersonic expansion. In contrast to the work published by Kong and co-workers,^[25] we were able to detect delayed ionization of 1MT/1MU-water clusters up to 1 μs after excitation under careful control of the applied water-vapor pressure. Even aggregates carrying more than five water molecules were detected. In addition, our ab initio calculations show that the energetics of the excited states and the de-excitation pathways are not significantly altered by hydration.

The decay curves of the bare monomers (1MT and 1MU) in Figure 4a show an exponential decay much faster than our upper detection limit of about 1.5 μs . We can only detect molecules (or clusters) that are ionizable at the wavelength of our ionization laser (193 or 213 nm). This means that on the time-scale of our experiment 1MT and 1MU relax from a higher to a lower state, which is not ionizable anymore. The pyrimidine bases thymine and uracil and their derivatives undergo ultra-fast (sub-picosecond timescale) internal conversion from the initially excited $^1\pi\pi^*$ state to the vibrationally excited ("hot") S_0 ground state and to some extent to the optically dark $^1n\pi^*$ state.^[14,15] For the moment, let us assume that the ionization laser also probes the hot electronic ground state. IVR would spread the energy over the vibrational degrees of freedom on a timescale much faster than our lower detection limit of

10 ns. However, since the gas-phase experiment takes place in the collision-free region of a skimmed molecular beam, the energy will stay in the molecule as long as the radiation lifetime of the vibrational modes ($> 1 \mu\text{s}$). In that case the molecule could be ionized at any time with the same probability, resulting in a linear decay as the molecules fly out of the ionization volume.

This is clearly not the case for the bare monomers in Figure 4a. After 600 ns only a small fraction of the monomers can be ionized anymore, indicating that the hot ground state either cannot be ionized at 193 nm or that the ionization probability is too low for detection due to an unfavorable Franck-Condon overlap. We therefore attribute the exponential decay of the monomer-ion signal to the true relaxation from an electronically excited state (the dark state) to the S_0 ground state.

By contrast, the 1MT- and 1MU-water clusters show a linear decay of the ion signal, meaning that the probed state has a longer lifetime than our upper detection limit. However, we have no reason to believe that the hot ground state of the clusters has a higher ionization probability than the monomer nor do the energetics change significantly between the monomer and clusters, which could have explained a faster decay to the hot ground state. To the contrary, the higher number of vibrational degrees of freedom is likely to facilitate the energy dissipation via IVR. In addition, vibrationally hot clusters tend to fragment and loose energy (evaporative cooling). Therefore we attribute the slow decay of the cluster-ion signal also to the relaxation of an electronically excited state, which is most likely the optically dark $^1n\pi^*$ state. Our upper detection limit is then only a lower limit for the dark state lifetime ($> 1 \mu\text{s}$).

Kohler and co-workers^[32] showed that hydrogen bonds between nucleobases and the solvent strongly enhance vibrational cooling, allowing the nucleobases to dissipate vibrational excess energy to the solvent more effectively. The additional hydrogen bonds between the ribosyl group and the water solvent are responsible for the four-fold larger lifetime of the TMP and UMP nucleotides compared to the nucleobases T and U in solution.^[15] Similar effects might be responsible for the increased dark-state lifetime of the 1MT- and 1MU-water clusters compared to the isolated nucleobases in the gas phase. A more effective energy dissipation of the hydrated monomers as well as cluster fragmentation during the lifetime of the dark state (evaporative cooling) might lead to a slower passage of the $^1n\pi^*/S_0$ barrier and an increase of the dark-state lifetime.

4. Conclusions

Our experimental results demonstrate that hydration does not accelerate internal conversion to the electronic ground state to a degree that the dark state is quenched;^[25] the conclusions of Kong et al.^[25] which state that the photostability is not an intrinsic property of the pyrimidine bases but results from their hydration is not confirmed for 1MT and 1MU. To the contrary, we propose an increase of the dark-state lifetime of 1MT- and 1MU-water clusters in the gas phase. Our results agree with the observation of a long-living dark state upon UV excitation of T and U nucleotides in aqueous solutions.^[15] Ab initio theory

shows that solvation of 1MT by water does not lead to a substantial modification of electronic relaxation and quenching of the $^1n\pi^*$ state. Coexpansions and crossed-beam experiments with oxygen showed no measurable decrease of the dark-state lifetime, so that the triplet-state population is probably small compared to the $^1n\pi^*$ population. Further experimental and theoretical work is directed towards identification of the dark state via vibrational spectroscopy and towards clarification of a possible connection of the dark state to the dimerization of thymine.

Experimental and Theoretical Section

The experimental setup has been described in detail elsewhere.^[27] A mixture of helium and thymine derivatives at 150 to 170°C is expanded through the 300 μm orifice of a pulsed nozzle (General Valve). The skimmed molecular beam (Beam Dynamics Skimmer, 1 mm orifice) crosses the collinear laser beams at a right angle. The ions are extracted in a modified Wiley-McLaren type time-of-flight (TOF) spectrometer perpendicular to the molecular and laser beams.

1-Methylthymine (1MT) and 1-methyluracil (1MU) were purchased from Sigma-Aldrich and used without further purification. Water clusters were formed by bubbling helium through water prior to the expansion. The water content was controlled by the temperature dependence of the water-vapor pressure. Different vapor pressures of 6 mbar (273 K), 8.2 mbar (277 K) and 12.3 mbar (283 K) were investigated.

R2PI (resonance enhanced two-photon ionization) spectra were obtained by exciting the molecules to the first optically accessible electronic state by the frequency doubled output of a Nd:YAG (Spectra Physics, GCR 170) pumped dye laser (LAS, LDL 205) with fluorescein 27, coumarine 307 and coumarine 153. The molecules were ionized by delayed ionization using the 193 nm output of an ArF excimer laser (Neweks, PSX-501-2) or the fifth harmonic of a Nd:YAG laser (213 nm, Innolas, Spitlight 600). Longer ionization wavelengths reduce the excess energy in the ions and thus fragmentation of the 1MT clusters. For the IR/UV double resonance spectra an infrared (IR) laser pulse was fired 36 ns prior to the UV excitation laser. IR light (3000–4000 cm^{-1}) was generated by an IR-OPO/OPA setup (LaserVision), pumped by the fundamental of a Nd:YAG laser (Spectra Physics, Indi 40-10). IR laser frequencies were calibrated by recording an ammonia vapor spectrum.

All calculations were performed with the quantum chemistry program packages TURBOMOLE^[33] and MOLPRO.^[34] We carried out resolution-of-the-identity (RI)-coupled-cluster (CC) 2^[35,36] geometry optimization^[37] of ground- and excited states and RI-CC2 in combination with linear response theory for vertical single-point calculations. We used Dunning's correlation-consistent basis sets cc-pVDZ (C, N, O: 9s4p1d/3s2p1d; H: 4s1p/2s1p) for geometry optimizations and aug-cc-pVTZ (C, N, O: 11s6p3d2f/5s4p3d2f; H: 6s3p2d/4s3p2d) for excitation energies which have provided results of high accuracy in previous CC2 calculations on nucleic acid bases.^[38] Geometries were optimized without symmetry constraints. The optimization of conical intersections and the calculation of linear interpolation in internal coordinates (LIIC) paths have been carried out with state-averaged CASSCF/CASPT2 (complete-active-space self-consistent-field/complete-active-space perturbation theory to 2nd order) methods^[39–41] and Pople's 6-31G* (C, N, O: 10s4p1d/3s2p1d; H: 4s/2s) basis set.^[42] The optimization of conical intersections was carried out with the CASSCF method with an active space of 10 elec-

trons in 8 molecular orbitals (3π , $2n$, $2\pi^*$ and $1\sigma^*$ orbitals) and the 6-31G* basis set. We obtained starting geometries for conical intersection optimization by slightly deforming the ring in order to destabilize the electronic ground state. In all calculations we retained a frozen core comprised by all heavy-atom 1s electrons.

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