Excited-State Dynamics in the RNA Nucleotide Uridine 5'-Monophosphate Investigated using Femtosecond Broadband Transient Absorption Spectroscopy

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Excited-State Dynamics in the RNA Nucleotide Uridine 5'-Monophosphate Investigated using Femtosecond Broadband Transient Absorption Spectroscopy

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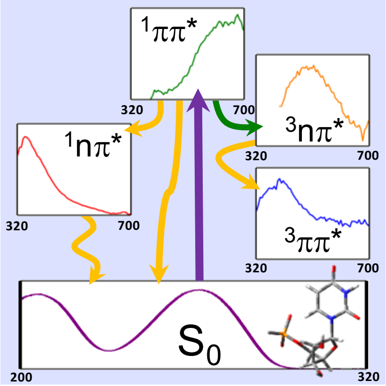
Abstract

Damage to RNA from ultraviolet radiation induce chemical modifications to the nucleobases. Unraveling the excited states involved in these reactions is essential, however, investigations aimed at understanding the electronic-energy relaxation pathways of the RNA nucleotide uridine 5'-monophosphate (UMP) have not received enough attention. In this Letter, the excited-state dynamics of UMP is investigated in aqueous solution. Excitation at 267 nm results in a trifurcation event that leads to the simultaneous population of the vibrationally-excited ground state, a long-lived $^1n\pi^*$ state, and a receiver triplet state within 200 fs. The receiver state internally converts to the long-lived $^3\pi\pi^*$ state in an ultrafast time scale. The results elucidate the electronic relaxation pathways and clarify earlier transient absorption experiments performed for uracil derivatives in solution. This mechanistic information is important because long-lived $n\pi^*$ and $\pi\pi^*$ excited states of both singlet and triplet multiplicities are thought to lead to the formation of harmful photoproducts.

TOC



Excitation of the DNA nucleobases, nucleosides, and nucleotides with UV radiation of ca. 267 nm leads to ultrafast internal conversion to the electronic ground state as the primary relaxation pathway.[1-5] In aqueous solution, most of the excess electronic energy reaching the ground state is transformed into intramolecular vibrational energy, which is transferred to vibrational modes of the solvent molecules in few picoseconds.[6] This electronic-to-vibrational energy transfer event is thought to play a major role in protecting the DNA building blocks from UV photodamage,[1-2] and as an important intrinsic property for their persistence during the prebiotic era.[7-11]

However, there is increasing evidence for the participation of longer-lived excited states in the photochemistry of the DNA and RNA monomers in solution.[2-3, 12] The extent of participation of these long-lived excited states depend sensitively on the solvent and the nucleobase monomer under investigation.[12-19] In particular, the pyrimidine DNA monomers have been at the center of attention because long-lived singlet and triplet states are thought to act as precursors of cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidone photoproducts [(6-4)-PPs], and pyrimidine photohydrates.[2, 14, 18-25] In contrast, focused investigations aimed at unraveling the excited-state dynamics of the RNA nucleotide uridine 5'-monophosphate (UMP) have been largely overlooked. This undertaking is essential because UV radiation can also damage RNA, inducing chemical modifications to nucleobases that include the formation of CPDs, (6-4)-PPs, pyrimidine photohydrates, and oxidation products.[26-29]

Earlier femtosecond transient absorption investigations for 1-cyclohexyluracil[13] and UMP[12] exciting at 267 nm and probing at discrete wavelengths revealed the ultrafast bifurcation of population in the $^1\pi\pi^*$ state to populate the vibrationally-excited ground state and a long-lived $^1n\pi^*$ state. Strikingly, up to 40% of the population in the $^1\pi\pi^*$ state was shown to internally convert to the $^1n\pi^*$ state of UMP in aqueous solution.[12] The population of the triplet state in these uracil derivatives have also been documented,[12-13, 30] but the doorway state leading to intersystem crossing event and the time scale at which this process occur are poorly understood. Recently, Pepino et al.[31-32] performed high-level excited-state calculations including explicit water molecules for the pyrimidine nucleosides. In particular, the authors reported the excited-state absorption (ESA) spectra of the $^1\pi\pi^*$, $^1n\pi^*$, and $^3\pi\pi^*$ minima for uridine (Urd) in water. These quantum-mechanical studies motivated the re-investigation of the excited state dynamics of UMP reported in this Letter using femtosecond broadband transient absorption spectroscopy. Comparison of broadband transient absorption spectra with the simulated ESA spectra of local minima has been established as a very effective method for characterizing the transient species of the nucleobases and their derivatives in solution.[18, 33-35]

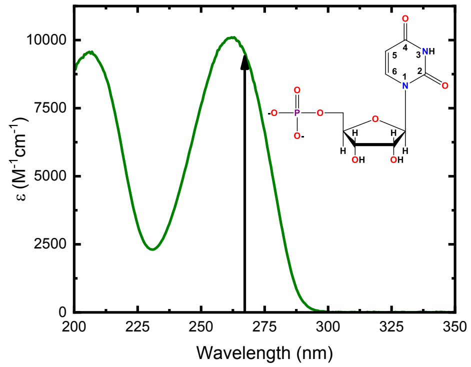


Figure 1. Ground-state absorptivity spectrum of UMP in PBS at a pH of 7.3 using the molar absorptivity coefficients reported by Cavaluzzi et al. for a 0.1 M PBS solution at pH of 7.0.[36] A black arrow depicts the excitation wavelength at 267 nm.

Figure 1 depicts the ground state absorptivity spectrum of UMP in phosphate buffer saline (PBS) solution at a pH of 7.3, whereas the broadband transient absorption spectra are shown in Figure 2. A broad absorption band with maximum at longer wavelengths than 650 nm and a negative amplitude band at shorter wavelengths than 400 nm are observed within the cross-correlation of the pump and probe beams (Figure 2a). The negative amplitude signal decays in lock step with the decay of the absorption band at 650 nm (Figure 2b). This process is followed by the observation of a long absorption tail at ca. 0.8 ps, which encompasses the entire spectral probe window. At a delay time of ca. 2 ps, a transient absorption band with a maximum around 355 nm is observed (Figure 2c). This absorption band decays within a time delay of ca. 1.3 ns (Figure 2c) and another absorption band with a maximum around 400 nm is resolved. The latter absorption band persists for a time delay longer than 3 ns (Figure 2c). Figure 3a depicts kinetic traces at selected probe wavelengths to illustrate the dynamics. The evolution associated difference spectra (EADS) extracted from a global fit analysis of the broadband data reported in Figure 2 are shown in Figure 3b. A three-component sequential kinetic model plus a constant offset was required to fit the broadband data satisfactorily, yielding the following lifetimes: 0.20 ± 0.05, 0.43 ± 0.05, and 365 ± 20 ps.

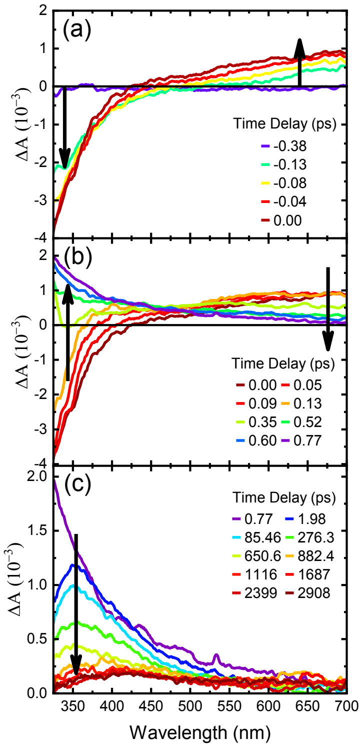


Figure 2. Transient absorption spectra of UMP (a to c) in PBS at pH 7.3. Black arrows are used to guide the eye.

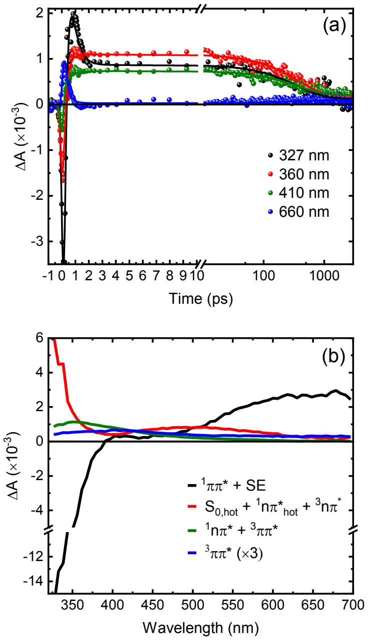


Figure 3. (a) Representative kinetic traces and (b) evolution associated difference spectra for UMP determined from a global fit analysis.

The initial transient absorption band observed at probe wavelengths longer than 500 nm in Figure 2a and Figure 3b is assigned to ESA from the $^1\pi\pi^*$ state, while the negative amplitude band is assigned to stimulated emission from the same state (Figure 3b). The assignment of the negative amplitude band to stimulated emission is supported by the observation of steady-state fluorescence in the same spectral region for uracil[37] at room temperature and for UMP[38] at 77 K. The assignment of the absorption band at wavelengths longer than 500 nm to a $^1\pi\pi^*$ state is also in good agreement with the calculations performed by Pepino et al. using a hybrid quantum mechanics/molecular mechanics method (MS-CASPT2/MM).[31-32] Specifically, the authors computed two bright $^1\pi\pi^*$ excited states for Urd in water, which they labeled as $^1\pi_2\pi^*$ and $^1\pi_H\pi^*$ states. The authors predicted that only the $^1\pi_H\pi^*$ excited state can be populated directly when an excitation wavelength of 267 nm is used.[31] According to these calculations,[31] the vertical population of $^1\pi_H\pi^*$ state leads to a steep path to a shallow planar minimum, which then has a barrierless decay to a lower-lying and slightly twisted (puckered) $^1\pi_H\pi^*$ minimum.

Further evidence for this assignment comes from the ESA spectrum of the $^1\pi_H\pi^*$ state calculated by Pepino et al.[31-32] Figure 4a shows the calculated ESA spectra for both the planar and twisted $^1\pi_H\pi^*$ minima of Urd in water,[31-32] whereas Figure 4b depicts the extracted EADS for UMP in PBS at pH 7.3. Note that both planar and twisted $^1\pi_H\pi^*$ minima absorb at wavelengths longer than 500 nm, with the predicted absorption band of the twisted $^1\pi_H\pi^*$ minimum matching better the EADS shown in Figure 4b in this spectral region. As can be observed in Figure 2a, the transient spectra exhibit an ultrafast blue shift in this spectral probe region, which lends support for a barrierless path from the planar to the twisted $^1\pi_H\pi^*$ minima, as predicted by the calculations for Urd. We remark that the EADS shown in Figure 4b may actually represents a weighted linear combination of the ESA spectra of both planar and twisted $^1\pi_H\pi^*$ minima. Importantly, Figure 4a also shows that both planar and twisted $^1\pi_H\pi^*$ minima absorb at shorter wavelengths than 500 nm, with the planar minimum exhibiting a significantly larger absorption intensity around 400 nm than the twisted minimum. However, the absorption band at 400 nm is not reproduced in the EADS shown in Figure 4b. We propose that the stimulated emission signal (see Figures 2a,b and 3d) dominates the absorption of the $^1\pi\pi^*$ state in this spectral probe region. In support of this idea, we note that the predicted vertical emissions for the twisted and planar $^1\pi_H\pi^*$ minima of Urd are 479 and 330 nm in water, respectively.[32]

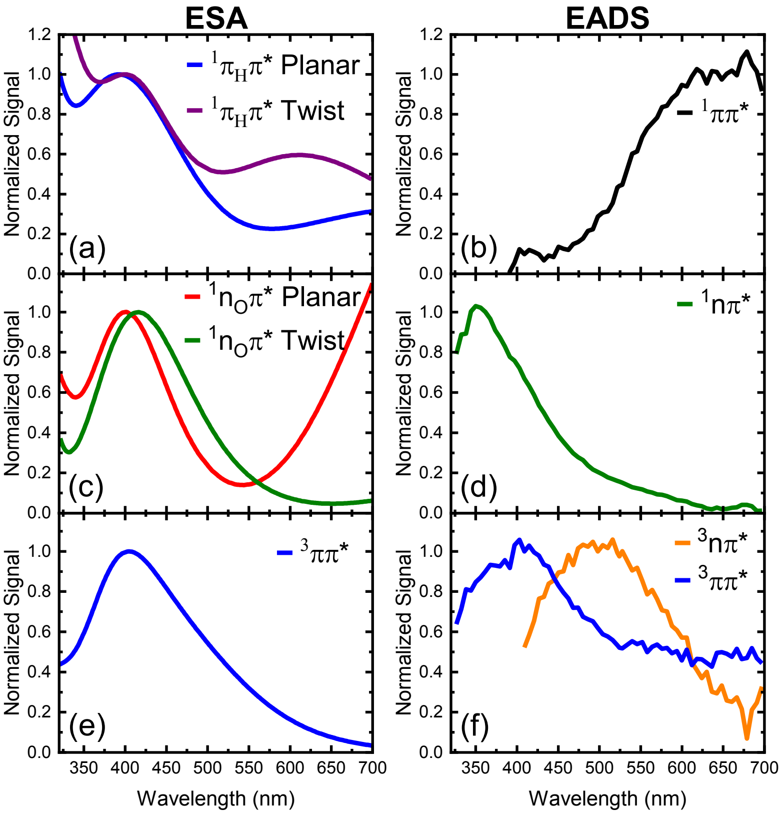


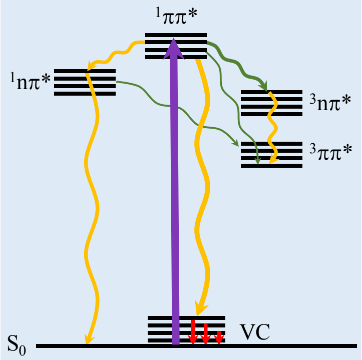
Figure 4. Panels (a), (c), and (e) depict the normalized ESA spectra calculated by Pepino et al.[31-32] for Urd in water. Panels (b), (d), and (f) show the normalized EADS for UMP in PBS at pH 7.3 determined from a global fit analysis of the multidimensional transient data reported in Figure 2. Note that the EADS representing linear combinations of the $^{1}\pi\pi^{*}$ + SE (black line) and the $S_{0,hot}$ + $^{1}n\pi^{*}_{hot}$ + $^{3}n\pi^{*}$ (red line) in Figure 3(b) are shown in panels (b) and (f) only at wavelengths longer than 400 nm in order to highlight the spectral component from these linear combinations that are presumed to be associated with the EAS of the $^{1}\pi\pi^{*}$ and $^{3}n\pi^{*}$ states, respectively.

Figure 2b shows that the population in the $^{1}\pi\pi^{*}$ state decays in an ultrafast time scale to a transient spectrum that covers the entire spectral probe window at a time delay of ca. 0.8 ps. We assign this transient spectrum, and the corresponding EADS in Figure 3b, to a weighted linear combination of the absorption spectra of the vibrationally-excited ground and $^{1}n\pi^{*}$ states, and an intermediate species (discussed below), which are populated from an ultrafast trifurcation process in the $^{1}\pi\pi^{*}$ potential energy surface. According to the global analysis of the broadband data, these transient species are populated within 0.20 ± 0.05 ps, in good agreement with previous transient absorption investigations for the uracil monomers in solution.[12-13, 30, 39] This assignment is also supported by the calculations,[31] which predict a small energy barrier (0.09 eV) to access a conical intersection between the $^{1}\pi_{H}\pi^{*}$ state and the ground state, $(^{1}\pi_{H}\pi^{*}/S_{0})_{CI}$, along the puckering coordinate for Urd in water. For UMP, vibrational cooling dynamics in the ground state occurs within an average (i.e., global) lifetime of 0.43 ± 0.05 ps.

The calculations also predict that there is a small energy barrier (0.31 eV) to access a $(^{1}n_{O}\pi^{*}/^{1}\pi_{H}\pi^{*})_{CI}$ from which the $^{1}n\pi^{*}$ state is populated.[32] High-frequency and out-of-plane vibrations, particularly due to a carbonyl stretching mode, are thought to allow for $^1n_O\pi^*/^1\pi_H\pi^*$ crossing, which facilitates the partial population of the $^1n\pi^*$ state before the out-of-plane C5 puckering motion drives the system toward the $(^1\pi_H\pi^*/S_0)_{CI}$.[32, 40-41] The population reaching the $^1n\pi^*$ state is then predicted to relax towards a planar $^1n_O\pi^*$ minimum, which further decays to a slightly twisted $^1n_O\pi^*$ minimum.[32] The middle panel of Figure 4c compares the ESA for both the planar and twisted $^1n_O\pi^*$ minima with the extracted EADS for UMP (Figure 4d). The agreement of the ESA spectrum for the twisted $^1n_O\pi^*$ minimum with the EADS is remarkable and lends strong support for the population of this minimum in the excited-state dynamics of UMP in aqueous solution. The ESA for the planar $^1n_O\pi^*$ minimum exhibits relatively intense absorption at wavelengths longer than 550 nm, which is not reproduced in the EADS. However, we remark that it is possible that the spectral features of this minimum are obscured in the broadband transient absorption data reported in Figure 2 because of the expected strong spectral overlap between both the stimulated emission and ESA of the $^1\pi\pi^*$ state with the ESA of the planar $^1n_O\pi^*$ minimum. Importantly, the predicted energy barrier to access a $(^1n_O\pi^*/S_0)_{CI}$ from the twisted $^1n_O\pi^*$ minimum is relatively high (0.77 eV) for Urd in water, consistent with the hundreds of picosecond lifetime (365 ± 20) ps obtained from a global analysis of the transient data for UMP.

As the transient spectrum associated with the $^1n\pi^*$ state decays, a residual long-lived transient species is observed (Figure 2c). This transient species does not decay within the 3 ns time window used in this study. The blue curves in Figure 4e and 4f compare the calculated ESA spectrum of the $^3\pi\pi^*$ state of Urd in water with the EADS for UMP, respectively.[32] The agreement between these two spectra is remarkable, lending strong support to the assignment of the transient species to the $^3\pi\pi^*$ state of UMP. This assignment is also supported by early transient absorption experiments,[12, 42-43] which show that the absorption spectrum of the $^3\pi\pi^*$ state of UMP is broad, with a maximum near 400 nm in aqueous solution. Surprisingly, Figure 2c shows that the decay of the $^1n\pi^*$ population, likely from the twisted $^1n_O\pi^*$ minimum, does not populate the $^3\pi\pi^*$ state, but decays independently of triplet state population. A closer inspection of the decay traces at 360 and 410 nm shown in Figure 3a (i.e., near the absorption maxima of the $^1n\pi^*$ and $^3\pi\pi^*$ minima), does not show evidence for a rise at 410 nm as the signal at 360 nm decays (see Figure S1). In addition, if intersystem crossing from the twisted $^1n_O\pi^*$ minimum to the $^3\pi\pi^*$ state was indeed occurring, an isosbestic point would be observed in the transient spectra shown in Figure 2c indicative of a state-to-state transition, but it is not observed. Interestingly, this experimental observation is in conflict with the calculations,[32] which predict an efficient intersystem crossing at the vicinity of the twisted $^1n_O\pi^*$ minimum through a $(^3\pi_H\pi^*/^1n_O\pi^*)_{ISC}$, with a spin orbit coupling term of ~42 cm^{-1}. Note that the transient data shown in Figure 2 do not allow us to support or rule out the hypothesis that intersystem crossing may occur directly from the $^1n\pi^*_{hot}$ state.[12-13, 30] Importantly, however, the same calculations predict that an intersystem crossing event could also occur directly from the $^1\pi_H\pi^*$ state.[32] According to the calculations, there are two efficient intersystem crossing regions from the $^1\pi_H\pi^*$ state. One is near the Franck-Condon region connecting the $^1\pi_H\pi^*$ state with a high-lying $^3n_O\pi^*$ state directly through a $(^3n_O\pi^*/^1\pi_H\pi^*)_{ISC}$. The second is closer to the crossing region with the ground state, which connects the $^1\pi_H\pi^*$ state with the $^3\pi_H\pi^*$ state through a $(^3\pi_H\pi^*/^1\pi_H\pi^*)_{ISC}$, as has been reported in vacuum.[44-47] These two intersystem crossing regions have spin orbit coupling terms of 9 and 5 cm^{-1}, respectively, for Urd in water.[32]

Figure 4f also shows a segment of the EADS at wavelengths longer than 400 nm, which overlaps strongly with the vibrationally-excited ground and $^1n\pi^*$ states ESA spectra at wavelengths shorter than ca. 400 nm (i.e., red EADS in Figure 3b). Unfortunately, Pepino et al.[31-32] did not report the ESA for either the $^3\pi_H\pi^*$ or the $^3n_O\pi^*$ state, so we cannot unequivocally assign this segment of the EADS to absorption from either state. However, it seems reasonable to assign it to ESA from a $^3n_O\pi^*$ state, as expected from the El-Sayed rules,[48] because the $(^3n_O\pi^*/^1\pi_H\pi^*)_{ISC}$ region has a somewhat larger spin orbit term than the $(^3\pi_H\pi^*/^1\pi_H\pi^*)_{ISC}$ region.[32] If this assignment holds true, it would imply that the transient spectrum observed at ca. 0.8 ps in Figure 2 has contribution from the ESA of the $^3n_O\pi^*$ doorway state, particularly in the spectral region above ca. 400 nm. Therefore, a comparison of the excited-state calculations reported for Urd in water with the transient absorption data in Figures 2 through 4 suggests that excitation of UMP at 267 nm leads to a trifurcation event from the $^1\pi_H\pi^*$ state, in which the $^1n_O\pi^*$, $^3n_O\pi^*$ (or a $^3\pi_H\pi^*$), and ground states are simultaneously populated in a competitive fashion (Scheme 1). We note that Pilles et al.[18] recently revisited the excited state dynamics of thymidine monophosphate (TMP) in solution and proposed a somewhat more complex relaxation mechanism, which is however, in general agreement to that proposed herein for UMP.



Scheme 1. Proposed relaxation mechanism for UMP in PBS at pH 7.3. The thickness of the curvy arrows indicates the likelihood of a given the transition: internal conversion (dark yellow), intersystem crossing (dark green), and vibrational cooling (red).

In summary, we investigated the excited-state dynamics of UMP under physiologically-relevant conditions using femtosecond broadband transient absorption spectroscopy. Excitation of UMP at 267 nm results in a trifurcation event that leads to the simultaneous population of the vibrationally-excited $^1n\pi^*$ and ground states and an intermediate $^3n\pi^*$ (or a $^3\pi\,\pi^*$) excited state within a 200 fs lifetime (Scheme 1). The doorway state, likely a $^3n_O\pi^*$ state, internally converts to the lowest-lying $^3\pi\pi^*$ state in an ultrafast time scale. The relative yields of each individual pathway is largely controlled by their intrinsic rate constants, which could not be extracted from the broadband transient experiments. Nonetheless, the combination of broadband transient absorption results for UMP with the high-level calculations reported by Pepino et al.[31-32] for Urd, is demonstrated to be a powerful approach for unravelling the complex excited-state dynamics of the building blocks of life, and for elucidating the intersystem crossing mechanism of the pyrimidine nucleotides. Specifically, it clarifies earlier transient absorption experiments performed for the uracil derivatives in solution.[12-13, 30] The elucidation of the electronic relaxation mechanisms of the pyrimidine nucleotides is important because long-lived $n\pi^*$ and $\pi\pi^*$ excited states of both singlet and triplet multiplicities have been proposed to lead to the formation of delirious photoproducts in both DNA and RNA.[14, 20-24, 26-27, 29] Future work will link these excited states to specific product formation.

ASSOCIATED CONTENT

Supporting Information

Experimental methodology; kinetic decay traces at 360 and 410 nm.

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Notes

The authors declare no competing financial interest.

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