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Communication: The dark singlet state as a doorway state in the ultrafast and efficient intersystem crossing dynamics in 2-thiothymine and 2-thiouracil

Marvin Pollum and Carlos E. Crespo-Hernández[a)]
Department of Chemistry and Center for Chemical Dynamics, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106, USA

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Femtosecond broadband transient absorption experiments are reported for 2-thiothymine and 2-thiouracil in aqueous buffer solution and in acetonitrile. It is shown that the $S_1(n\pi^*)$ state acts as a doorway state in the ultrafast and efficient population of the $T_1(\pi\pi^*)$ state upon 316 nm excitation. A sequential kinetic model is presented to explain the excited-state dynamics in 2-thiothymine and 2-thiouracil upon UVA excitation: $S_2(\pi\pi^*) \rightarrow S_1(n\pi^*) \rightarrow T_1(\pi\pi^*)$. The experimental results are also used to scrutinize the excited-state relaxation pathways recently predicted for 2-thiouracil at the CASPT2//CASSCF level of theory [G. Cui and W. Fang, J. Chem. Phys. **138**, 044315 (2013)]. The efficient population of the $T_1(\pi\pi^*)$ state for both 2-thiothymine and 2-thiouracil in a few hundreds of femtoseconds lends further support to the emerging idea that thiobase derivatives exhibit photo-toxic properties that can be effectively harnessed in photo-chemotherapeutic applications. © *2014 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4866447]

Sulfur-substituted DNA/RNA bases, also known as thiobases, are receiving increased attention primarily due to their biological relevance and therapeutic applications.[1–3] While thiobases are present as minor components in natural t-RNAs,[4] they are also members of the family of molecules known as prodrugs, which are widely prescribed for maintenance therapy and immunosuppression.[2,3] Moreover, thiobases are extensively employed as cytotoxic agents of clinical relevance and in photo-chemotherapeutic applications.[3] The skin of patients treated with these drugs is more sensitive to UVA radiation; long-term treatment results in up to a 200-fold increased incidence of sunlight-induced skin cancer.[2] Unravelling the electronic decay mechanisms in thiobases might hold the key to identifying thiobase derivatives with increased potential for medical and photo-chemotherapeutic applications, while simultaneously minimizing the harmful side-effects.

In addition to the medicinal interests, thiobases have recently been used as model compounds to reveal how a single-atom substitution affects the electronic relaxation pathways in the natural DNA/RNA bases.[5–13] The excited-state dynamics of the DNA/RNA chromophores are sensitive to subtle structural modifications, which often restrict access to the ultrafast nonadiabatic relaxation pathways available in the natural bases.[14–19] 4-thiothymidine (4tThd)[6–8] and 6-thioguanosine (6tGuo)[10] have been shown to exhibit femtosecond intersystem crossing dynamics resulting in near unity triplet yields, in contrast to the negligible triplet yields observed in thymidine and guanosine in aqueous solution ($<10^{-3}$ molecules/photon).[20] Clearly, a quantitative understanding of the intersystem crossing dynamics in thiobases is of fundamental and biological relevance because the resulting high triplet yields play a foremost role in determining the efficacy of a sensitizer to cell and DNA damage.

It has become increasingly evident that in order to obtain a quantitative and firm understanding of the relaxation mechanisms of thiobases[6–8,10–12] and DNA/RNA bases,[14–17,21,22] the experimental results should be married with high-level, multiconfigurational calculations that take into account nonadiabatic and spin-orbit coupling interactions between singlet and triplet states. Very recently, and of particular relevance to this work, Cui and Fang reported the excited-state relaxation pathways of 2-thiouracil (2tUra) at the CASPT2/aug-cc-pVDZ//CASSCF(16,11)/6-31+G* level of theory, used as the archetypal chromophore of 2-thiothymine (2tThy).[12] As shown previously for 6tGua,[11] the authors show that multiple relaxation pathways and crossing points could play a major role in the population of the triplet state in 2tUra.[12] Based on the calculations, Cui and Fang proposed three probable nonadiabatic relaxation pathways to rationalize the efficient population of the $T_1(\pi\pi^*)$ state in 2tUra and 2tThy after excitation of the spectroscopic $S_2(\pi\pi^*)$ state: (I) $S_2(\pi\pi^*) \rightarrow S_1(n\pi^*) \rightarrow T_1(\pi\pi^*)$; (II) $S_2(\pi\pi^*) \rightarrow T_2(n\pi^*) \rightarrow T_1(\pi\pi^*)$; and (III) $S_2(\pi\pi^*) \rightarrow T_3(n\pi^*) \rightarrow T_2(n\pi^*) \rightarrow T_1(\pi\pi^*)$.

However, from a computational viewpoint, only dynamical studies can elucidate the pathways that are operative and the regions of the potential energy surfaces that are visited from a set of potential pathways.[22,23] From an experimental perspective, on the other hand, time-resolved experiments can provide a wealth of information to unravel the excited-state relaxation mechanisms and the rate constants for the different nonadiabatic events.[14–17] Kuramochi *et al.*[9] used flash photolysis and time-resolved thermal lensing experiments to show that the triplet state in 2tThy is populated with unity quantum yield. More recently, Taras-Goślińska and co-workers

a)E-mail: cxc302@case.edu

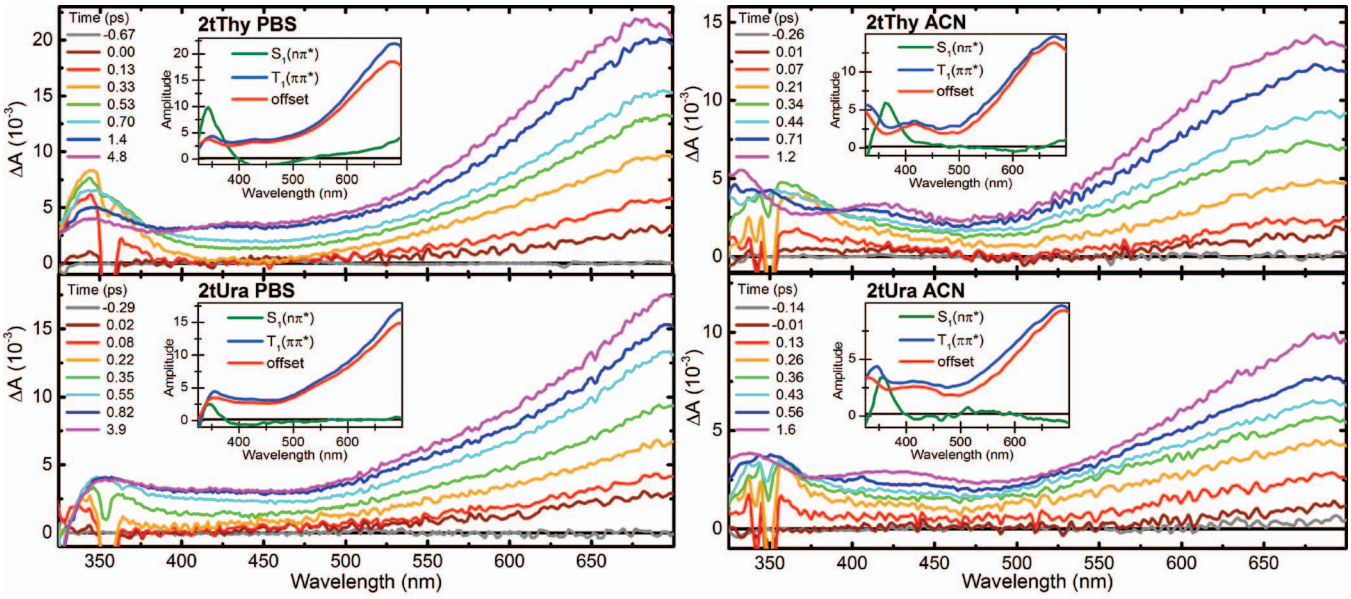


FIG. 1. Transient absorption spectra of 2-thiothymine (2tThy) and 2-thiouracil (2tUra) in pH 7.4 phosphate buffer solution (PBS) and acetonitrile (ACN). Spectra are given from time zero to the peak of the band in the visible. Stimulated Raman emission is observed around 350 nm at short time delays. The decay-associated spectra for each sample are inset in their respective graph.

investigated the relaxation mechanism of the $T_1(\pi\pi^*)$ state of 2tThy and its DNA and RNA nucleosides in acetonitrile.[13] However, due to the limited time resolution, the authors[9,13] were unable to identify the nonadiabatic relaxation pathways leading to the efficient population of the triplet state. Moreover, and to the best of our knowledge, the intersystem crossing rate constants for both 2tThy and 2tUra have not been reported.

Figure 1 shows the first few picoseconds of transient absorption spectra for 2tThy and 2tUra in aqueous buffer solution and in acetonitrile after 316 nm excitation. Figure S1 shows the corresponding contour plots of the transient data in the time window of up to ∼2 ns (see the supplementary material).[24] We have used a global and target analysis method based on a sequential kinetic model (see the supplementary material[24]) to extract the lifetimes and decay-associated spectra (DAS) from the multidimensional transient absorption data.[18,25,26] The DAS for each molecule are inset in Fig. 1 and representative decay traces obtained from this analysis are shown in Fig. 2. Two lifetimes plus a time-independent constant are needed to adequately fit the time-resolved data. The first, sub-picosecond lifetime depends on the thiobase and on the solvent used. For 2tThy, it has a value of 0.62 ± 0.07 ps in aqueous solution, whereas a value of 0.32 ± 0.09 ps was obtained in acetonitrile. For 2tUra, this lifetime has a value of 0.35 ± 0.06 ps and 0.34 ± 0.09 ps in aqueous and acetonitrile solutions, respectively. The second, tens of picoseconds lifetime is concentration and solvent dependent (not shown), as observed previously for other thiobases,[5–7,9,10,27–29] and its detailed investigation will be the subject of a future contribution. Suffice it to say here that this second lifetime corresponds to the bimolecular dynamics of the $T_1(\pi\pi^*)$ state in both 2tThy and 2tUra, as previously observed in 4-thiothymine[6,7] and 4-thiouracil[28] derivatives, and

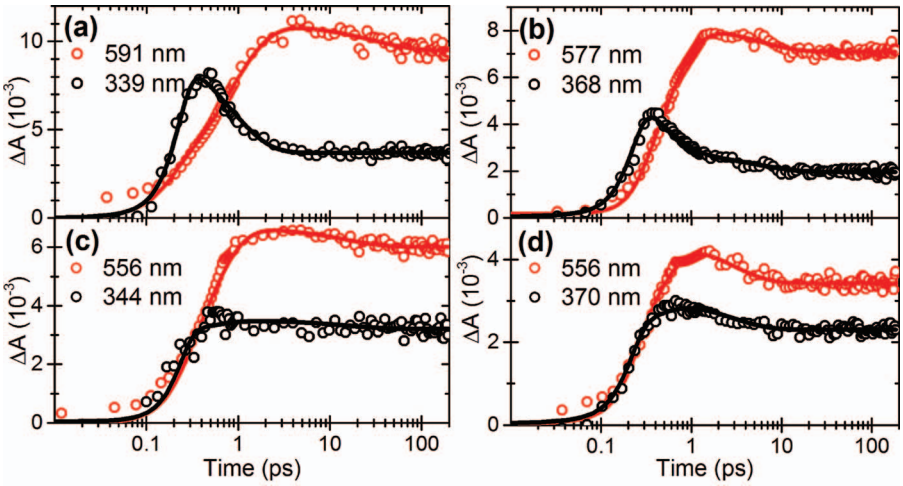


FIG. 2. Kinetic traces of the UV and visible bands for 2tThy (a) and (b) and 2tUra (c) and (d) in aqueous buffer solution and acetonitrile, respectively.

more recently in 2tThy and its nucleosides in acetonitrile.[13] In agreement with previous results for other thiobases,[6–8,10,13] we assign the hundreds of femtoseconds lifetime to intersystem crossing to the $T_1(\pi\pi^*)$ state. We explain the rationale behind this assignment and present a kinetic mechanism explaining the initial excited-state dynamics in 2tThy and 2tUra in the following paragraphs.

Figure 1 shows that a transient absorption species grows in the UV within the cross-correlation of the pump and probe pulses. This transient absorption species has an absorption maximum at ~345 nm in aqueous buffer solution for 2tThy, whereas the absorption maximum shifts to ~360 nm in acetonitrile. As time progresses, the transient species in the UV decays while a band grows and broadens in the visible, leading to the formation of a long-lived transient absorption species with absorption maxima at ~345, 430, and 685 nm in aqueous solution and at ~330, 420, and 680 nm in acetonitrile. This long-lived transient absorption species has been previously assigned to the $T_1(\pi\pi^*)$ state of 2tThy in acetonitrile.[9,13] We assign the corresponding transient absorption species in aqueous buffer solution to the $T_1(\pi\pi^*)$ state as well, which shows room-temperature phosphorescence (not shown), as previously observed in 4tThd,[6] and more recently in 2tThy in acetonitrile solution.[13] We note that this transient species redshifts in going from acetonitrile to aqueous solution, in agreement with its $\pi\pi^*$ character.[9,12,13]

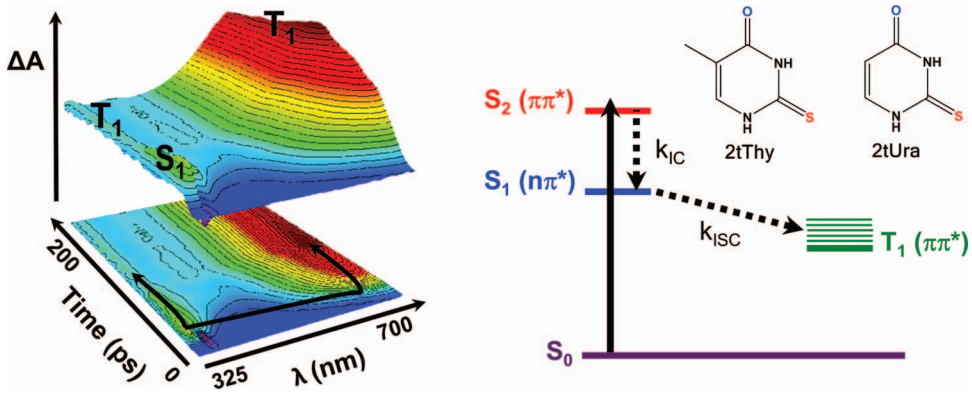
The transient species in the UV, which is populated within the time-resolution of our experiments, is assigned to the excited-state absorption of the $S_1(n\pi^*)$ state based on the following observations: (1) this transient species redshifts by ~15 nm in going from aqueous to acetonitrile solution; (2) an isosbestic point is observed around 383 and 390 nm in aqueous solution and acetonitrile, respectively, suggesting state-to-state dynamics (Fig. 1); (3) intersystem crossing occurs in hundreds of femtoseconds (τ_1 above); and (4) a previous work has reported a triplet yield of unity for this thiobase.[9,13] The assignment of the transient absorption species in the UV to the $S_1(n\pi^*)$ state is also in good agreement with the high-level, multiconfigurational calculations performed recently by Cui and Fang,[12] which shows a spin-orbit coupling interaction of 121.01 cm^{-1} between the $S_1(n\pi^*)$ and $T_1(\pi\pi^*)$ states. We discuss the calculations of Cui and Fang in light of our experimental results in more details below.

As shown in Figs. 1 and 2, the evolution of the transient absorption spectra in 2tUra is very similar to that in 2tThy. Initially, a transient absorption species in the UV grows within the time resolution of our experimental setup. After a few hundred femtoseconds, this transient species decays while a long-lived transient absorption species is populated. This long-lived transient species has absorption maxima at ~352, 423, and 700 nm in aqueous solution, whereas it shows absorption maxima at ~345, 412, and 690 nm in acetonitrile. In line with previous experimental and computational results for 2tUra,[12,29,30] and in analogy to the assignment for 2tThy above, the long-lived transient absorption species is assigned to the $T_1(\pi\pi^*)$ state of 2tUra. In addition, this transient absorption species redshifts in going from acetonitrile to aqueous solution, as expected for a transient species with significant $\pi\pi^*$ character, and does not decay significantly within the ~2 ns timescale of our measurements (see Fig. S1 of the supplementary material).[24] To the best of our knowledge, the triplet yield and transient absorption spectrum of the $T_1(\pi\pi^*)$ state for 2tUra have not been reported until now. Assuming similar absorption cross sections for the triplet-triplet absorption band in both 2tThy and 2tUra in aqueous or acetonitrile solutions, we estimate a triplet yield of (75 ± 20)% for 2tUra exciting at 316 nm from back-to-back transient experiments under identical experimental conditions. We caution, however, that this estimated triplet yield for 2tUra should be regarded as a lower-bound value because the absorption cross sections for the triplet state absorption band are currently unknown.

The solvent dependence of the initial transient species in the UV for 2tUra is, however, more difficult to visualize from the spectra shown in Fig. 1 because of its strong overlap with the absorption spectrum of the long-lived $T_1(\pi\pi^*)$ state. Nevertheless, an isosbestic point is observed in Fig. 1 for 2tUra in acetonitrile near 380 nm, and the DAS (Fig. 1, inset) reveal that the transient species in the UV redshifts in going from aqueous to acetonitrile solution, as observed in 2tThy. Therefore, we assign this transient species to the $S_1(n\pi^*)$ state by invoking similar arguments to those described for 2tThy above. This assignment is further supported by high-level calculations presented by Cui and Fang for 2tUra.[12]

Based on the analysis and discussion presented above, we can now propose a kinetic mechanism that can satisfactorily explain the excited-state dynamics in 2tThy and 2tUra under physiologically relevant (i.e., aqueous solution at pH 7.4) and acetonitrile conditions (Scheme 1). Excitation at 316 nm results in the direct population of the spectroscopic state in both thiobases (see Fig. S2 of the supplementary material).[24] Previous experimental and computational investigations have shown that this spectroscopic state should be assigned to the $S_2(\pi\pi^*)$ state.[9,12,13] The $S_2(\pi\pi^*)$ state internally converts through a conical intersection[12] to populate the $S_1(n\pi^*)$ state with a lifetime shorter than the time resolution of our experimental setup (i.e., $\tau \leq 200$ fs). The population in the $S_1(n\pi^*)$ state intersystem crosses in hundreds of femtoseconds (τ_1 above) to populate the $T_1(\pi\pi^*)$ state with near unity yield. Finally, the $T_1(\pi\pi^*)$ state decays in multiple time scales, which depend on the concentration and the solvent used.

The sequential kinetic model proposed above is in excellent agreement with the primary nonadiabatic relaxation pathway proposed by Cui and Fang based on high-level multiconfigurational calculations for 2tThy and 2tUra in vacuum: (I) $S_2(\pi\pi^*) \rightarrow S_1(n\pi^*) \rightarrow T_1(\pi\pi^*)$.[12] This relaxation pathway involves ultrafast internal conversion from the $S_2(\pi\pi^*)$ state to the $S_1(n\pi^*)$ state, which is mediated by a S_2/S_1 conical intersection.[12] The population reaching the $S_1(n\pi^*)$ state then decays to the $T_1(\pi\pi^*)$ state through a minimum energy crossing point that has a spin-orbit coupling interaction of 121.01 cm^{-1}. Cui and Fang also proposed two other nonadiabatic relaxation pathways that could be competitive with pathway I above: (II) $S_2(\pi\pi^*) \rightarrow T_2(n\pi^*) \rightarrow T_1(\pi\pi^*)$ and (III) $S_2(\pi\pi^*) \rightarrow T_3(n\pi^*) \rightarrow T_2(n\pi^*) \rightarrow T_1(\pi\pi^*)$. Based on the magnitude of the spin-orbit coupling interactions and the relative energy of minimum crossing points calculated between



SCHEME 1. (Left) 3D contour plot obtained for 2-thiothymine in PBS buffer. Each absorption band is labeled with the proposed state it represents. (Right) Sequential kinetic mechanism proposed for the relaxation of 2-thiothymine (2tThy) and 2-thiouracil (2tUra) in PBS buffer and acetonitrile.

these states, the authors proposed that pathways II and III should not be as efficient as pathway I, but should play a role in the population of the $T_1(\pi\pi^*)$ state under physiologically relevant conditions.[12]

We considered the assignment of the transient intermediate species in Fig. 1 to the $T_2(n\pi^*)$ state, but dismissed it based on the following premises. Previous studies have shown that the transient absorption spectrum of the $S_1(n\pi^*)$ state in uracil and thymine monomers has a maximum around 340 nm in solution.[31–33] This absorption maximum is in good agreement with those estimated from the DAS shown in Fig. 1 for the intermediate transient species of 2tUra and 2tThy. In addition, energetic and structural considerations from the CASPT2 calculations suggest that the ultrafast $S_2(\pi\pi^*) \rightarrow S_1(n\pi^*)$ internal conversion is more competitive than the $S_2(\pi\pi^*) \rightarrow T_2(n\pi^*)$ intersystem crossing pathway:[12] (1) the relative adiabatic energy minimum of the $S_2(\pi\pi^*)$ state (89.1kcal/mol) is closer in energy to the $S_1(n\pi^*)$ state minimum (87.9 kcal/mol) than to the $T_2(n\pi^*)$ state minimum (86.6 kcal/mol) at the CASPT2 level of theory; (2) the relative potential energy of the S_2/S_1 conical intersection is lower in energy (90.6 kcal/mol) than the S_2/T_2 crossing point (97.0 kcal/mol); and (3) the S_2/S_1 conical intersection is structurally more similar to the $S_2(\pi\pi^*)$ state minimum than the S_2/T_2 crossing point and there is no transition state barrier between the $S_2(\pi\pi^*)$ state minimum and the S_2/S_1 conical intersection. Furthermore, internal conversion pathways via conical intersections are generally thought to be faster than intersystem crossing pathways via crossing points.

Hence, pathway I plays a preponderant role in the population of the $T_1(\pi\pi^*)$ state in solution according to the results presented in this work, although we cannot exclude the lesser participation of pathway II in the overall relaxation mechanism. Dynamical calculations that include both nonadiabatic and spin orbit coupling interactions are desirable to further scrutinize whether or not the $S_2(\pi\pi^*) \rightarrow T_2(n\pi^*)$ intersystem crossing pathway participates in the population of the T_1 state. It is evident that a close synergy between experimental and computational approaches is essential in order to gather a quantitative and firm understanding of the excited-state dynamics and photochemistry of these biomolecules in solution.

In summary, we present experimental evidence for a sequential kinetic model in which the dark singlet state, $S_1(n\pi^*)$, acts as a doorway state in the ultrafast and efficient population of the $T_1(\pi\pi^*)$ state upon UVA excitation of 2tThy and 2tUra. The solvent dependence and the femtosecond dynamics of this intersystem crossing event are in good agreement with previous observations for other thiobases.[6,7,10] Ultrafast intersystem crossing dynamics seem to be a general property of thiobase derivatives, which results in the efficient population of the $T_1(\pi\pi^*)$ state in yields that approach unity. This unique property leads to the generation of singlet oxygen[5,9,34–36] and other reactive species in high yield, which explains the UVA-photosensitization efficacy of thiobase derivatives and their extensive use in medical and photo-chemotherapeutic applications.[2,3] The results presented in this work lend further support to the observation that thiobase derivatives are effective at damaging DNA and killing cells[2,3,29,30] due to the efficient population of the triplet state, which can ultimately lead to a 200-fold increase in UVA-induced skin cancer in patients treated with these drugs.[2]

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