On the Intrinsic Population of the Lowest Triplet State of Thymine

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The population of the lowest triplet state of thymine after near-UV irradiation has been established, on the basis of CASPT2/CASSCF quantum chemical calculations, to take place via three distinct intersystem crossing mechanisms from the initially populated singlet bright \(^{1}\pi\pi^*\) state. Two singlet—triplet crossings have been found along the minimum-energy path for ultrafast decay of the singlet state at 4.8 and 4.0 eV, involving the lowest \(\tilde{3}\pi\pi^*\) and \(^{3}\pi\pi^*\) states, respectively. Large spin—orbit coupling elements predict efficient intersystem crossing processes in both cases. Another mechanism involving energy transfer from the lowest \(^{3}\pi\pi^*\) state with much larger spin—orbit coupling terms can also be proposed. The wavelength dependence measured for the triplet quantum yield of pyrimidine nucleobases is explained by the location and accessibility of the singlet—triplet crossing regions.

Introduction

Triplet states are frequent intermediates in different types of photoinduced reactions.\(^1\) Both their usual diradical character and their long lifetimes make them reactive species prone to interact with other systems. Among the most important reactions involving triplet states, those related to DNA/RNA purine and pyrimidine nucleobases have undoubtedly attracted more attention, in particular the photodimerization of pyrimidine nucleobases, considered to be the most frequent mutation taking place after UV light irradiation.\(^2-4\) The proposed mechanisms normally suggest photoexcitation of a molecule to a singlet state followed by population of a triplet state by an intersystem crossing mechanism (ISC) and subsequent reaction of the triplet state of the nucleobase monomer with a second molecule in the ground state.\(^2-3\) In particular, the presence of the triplet state of thymine in solution has been observed by using different experimental techniques, such as flash photolysis,\(^5\) whereas specific triplet quenchers and external photosensitizers have been used in its population.\(^2,3,5-8\) It is surprising that, despite extensive work and frequent claims of triplet-state involvement in the fast relaxation processes of nucleobases, in particular for thymine,\(^9\) the actual mechanism for triplet-state formation in these systems is only now starting to be elucidated. We have recently presented a unified model for the rapid internal conversion (IC) of the singlet excited pyrimidine nucleobases uracil, thymine, and cytosine,\(^10\) where the observed ultrafast decay component, both in the gas phase and in solution, can be interpreted in terms of the barrierless character of the minimum-energy path (MEP) associated with the lowest singlet state of \(\pi\pi^*\) type toward a conical intersection (CI) with the ground state, as it has also been reported for adenine.\(^11-13\) Secondary decay paths involving the lowest \(^{1}\pi\pi^*\) state and even a higher \(^{1}\pi\pi^*\) state have also been identified.\(^11,12\) Starting then from the known characteristics of the main deactivation paths—singlet—singlet ICs—it is possible to analyze how the lowest triplet state can be reached efficiently.

The triplet-state population may proceed via endogenous or exogenous photosensitization from other triplet species or by efficient intersystem crossing (ISC) from the initially photoexcited singlet state. There are three couplings enabling the spin flipping mechanism, which enhances the efficiency of the ISC process: solvent-induced spin or spin—lattice relaxation, hyperfine coupling, and spin—orbit coupling (SOC).\(^1\) Whereas the first and second mechanisms are only relevant in the presence of paramagnetic impurities and in polyradicals with distant magnetic centers, respectively, SOC predominates in most photoinduced processes, especially for organic and bioorganic compounds. SOC terms near 0.1—5.0 cm\(^{-1}\) have been considered large enough to yield fast ISC energy transfer.\(^1\) In any case, and considering that the primary step of the photochemical process after light absorption basically involves population of the lowest singlet excited state \((S_1)\), it is necessary to find those channels for energy deactivation and transfer in which the lowest triplet excited state \((T_1)\) can be rapidly and efficiently populated through an ISC mechanism. For simplicity, early studies at the ab initio\(^14,15\) level estimated ISC rates by computing SOC elements between singlet and triplet states at the Franck—Condon (FC) geometry. The well-known qualitative El-Sayed rules\(^16\) to evaluate the coupling between \(\pi\pi^*\) and \(n\pi^*\) states in organic molecules, the largest between alike states and forbidden otherwise, were formulated on those grounds. Efficient ISC requires however simultaneous small singlet—triplet energy gaps and large SOC elements at appropriate geometries. Since product formation in the ground-state hypersurface is very fast and does not allow major conformational changes, it is the geometry of the triplet state yielding more favorable ISC that determines the ratio of the final products.\(^1\) Nowadays, it is possible to locate regions of close degeneracy between the states, singlet—triplet...
crossing regions, and compute there the SOC elements, combining the two required conditions for efficient ISC. As compared to IC, taking place essentially in small regions where the seam of CIs becomes accessible, the sectors of the potential energy hypersurfaces for effective ISC are more extensive. Large efforts have been devoted in previous years to the determination of CIs for IC processes in singlet states.17 The purpose of this Letter is to analyze, on theoretical grounds, the mechanism of population of the lowest triplet state of thymine, as we performed recently for cytosine18 and uracil,19 and rationalize the experimental findings on the basis of the singlet–triplet crossings (STCs) occurring along the major deactivation path responsible for the rapid IC process detected in the molecule. Thus, we address here three main questions: Can the intrinsic population of the lowest triplet state in thymine take place in vacuum? Can the thymine triplet state be expected to have a larger quantum yield of formation ($\phi_{ISC}$) than that of the other nucleobases? And, what is the molecular basis for the wavelength dependence of $\phi_{isc}$? For this purpose, high-level ab initio methods are applied within the framework of the current theoretical paradigm for nonadiabatic photochemistry.17 Since the calculations have been performed in vacuum, without the explicit consideration of solvent effects, the answer provided here can be regarded as a characteristic molecular property of the thymine system, which might be expected to be somewhat disturbed by the specific environment it is exposed to in solution, in solid, or in vivo.

### Computational Details

The present calculations include CASSCF geometry optimizations, minimum-energy paths (MEP), and conical intersection and singlet–triplet crossing searches, followed by multiconfigurational perturbation theory, CASPT2, calculations at the optimized geometries. Spin–orbit coupling terms and transition dipole moments have also been computed. The final results involve an active space of 14 electrons distributed in 10 orbitals, CASPT2(14,10)/CASSCF. Other active spaces were employed in the optimization procedures, following a strategy which was proved successful previously. A one-electron basis set of the ANO-S type contracted to C,N,O[3s2p1d]/H[2s1p] was used throughout. More detailed technical aspects of the calculations can be found in our previous papers10–12,18,19 and in the Supporting Information. All of the calculations reported used the quantum chemical methods implemented in the MOLCAS 6.0 package.20

### Results and Discussion

From thymine ground state and upon near-UV absorption at the FC region, most of the population reaches initially singlet excited states, and in particular, as listed in Table 1, it is the transfer to the $1(\pi\pi^*)$ HOMO (H) → LUMO (L) (hereafter HL) excited state at 4.89 eV, which has the largest oscillator strength up to 6 eV, 0.167. The ultrafast nonradiative decay undergone by thymine in the fs range,21,22 can be rationalized by the barrierless character of the path leading from the FC region toward a CI seam with the ground state, $(gs^*/\pi\pi^*)_{CI}$, as we reported previously,10 and it is shown also here in Figure 1. Unlike simple geometry optimizations, the use of the MEP technique guarantees the absence of energy barriers along the lowest-energy path. The structure of the CI at the end of the MEP can be characterized as ethene-like, involving combined stretching and twisting of the ethylenic bond.10 The presence of an accessible CI also explains the low fluorescence quantum yield ($\sim\phi_F = 10^{-4}$) detected for thymine with a band origin at 4.5 eV in water.23 This weak emission can be related to the presence of a high-lying planar $(\pi\pi^*)$ HL minimum computed adiabatically from the ground state at 4.49 eV, whereas a nonfluorescent $(1(n_0\pi^*)$ minimum is found at 4.05 eV (see Table 1) with a minor contribution to the emissive properties. Measured fluorescence radiative lifetimes, 7–8 ns both for uracil and thymine,23 also agree with our estimation, 9 ns, and confirm the $1(\pi\pi^*)$ nature of the fluorescent state. Similar vertical and adiabatic energy values have been found at other levels of theory.10,24–26

The efficient population of the triplet manifold should take place essentially along the main decay process on $S_1(\pi\pi^*)$ HL. As seen in Figure 1, along the $1(\pi\pi^*)$ HL state MEP, and apart from the occurrence of a CI with the $(1(n_0\pi^*)$ singlet state, it is clear that two singlet–triplet crossings (STC), and therefore two ISC processes, take place, at 4.8 eV with the $1(n_0\pi^*)$ triplet state, $(1(n_0\pi^*)/1(\pi\pi^*)_{STC})$, and at 4.0 eV, further along the relaxation path and near the ethene-like CI with the ground state, directly with the lowest $1(\pi\pi^*)$ T1 triplet state, $(1(\pi\pi^*)/1(\pi\pi^*)_{STC})$, a structure displaying the same type of screw-boat puckered geometry with a stretched and twisted double bond $C_6=C_8$ as that at the $(gs^*/\pi\pi^*)_{CI}$ CI. Figure 2 includes a scheme describing the population of $T_1$ based on our CASPT2 calculations and three possible mechanisms proposed to explain the overall intersystem crossing process. A similar framework was found for uracil,19 although in thymine, the two ISC processes explained so far have similar SOC terms, near 8 cm$^{-1}$, and therefore, a comparable efficiency should be expected. Additionally, the profile of the computed MEP in Figure 1 suggests that, despite the ultrafast decay along the singlet state, the lower-energy ISC mechanism may enhance its efficiency with respect to the other nucleobases, resulting in larger quantum yields because the region for the energy transfer near to the end of the MEP seems to be much more extended.

Regarding the higher-energy ISC process, once the $1(n_0\pi^*)$ triplet state has been populated, a MEP on such a state leads directly to its energy minimum, $(n_0\pi^*)_{min}$, placed at 3.93 eV adiabatically from the ground state (see Supporting Information). Basically degenerated, we have located a conical intersection connecting the triplet states $1(n_0\pi^*)$ and $1(\pi\pi^*)$, favoring the population switch toward the lowest $3(\pi\pi^*)$ state. Another computed MEP on the $T_1$ hypersurfaces leads from $(3(n_0\pi^*)/3(\pi\pi^*)_{CI})$ to the $3(\pi\pi^*)$ state minimum (see Supporting Information). This type of $1(\pi\pi^*)/3(\pi\pi^*)$ ISC mechanism via an intermediate $n_\pi^*$ state, claimed frequently in older literature without the support of hypersurface searches, can be confirmed here as favorable, as it has been recently reported for uracil19 and other biological chromophores such as isoalloxazine27 and psoralen.28 Both mechanisms described here can, in any case, contribute to the overall population of the lowest triplet state. In principle,

### Table 1: Computed Spectroscopic Properties for the Low-Lying Singlet and Triplet Excited States of Thymine at the CASPT2/CASSCF(14,10)/ANO-S C,N,O[3s2p1d]/H[2s1p] Level of Calculation

<table>
<thead>
<tr>
<th>State</th>
<th>Vertical Transition (eV)</th>
<th>Band Origin ($\nu_0$, eV)</th>
<th>$\tau_{rad}$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1(n_0\pi^*)^a$</td>
<td>5.41</td>
<td>4.77 (0.004)</td>
<td>4.23</td>
</tr>
<tr>
<td>$1(\pi\pi^*)^a$</td>
<td>6.52</td>
<td>4.89 (0.167)</td>
<td>6.07</td>
</tr>
<tr>
<td>$1(\pi^*)^a$</td>
<td>7.36</td>
<td>5.94 (0.114)</td>
<td></td>
</tr>
<tr>
<td>$3(n_0\pi^*)^b$</td>
<td>3.95</td>
<td>3.59</td>
<td>2.99</td>
</tr>
<tr>
<td>$3(\pi^*)^b$</td>
<td>5.21</td>
<td>4.75</td>
<td>3.84</td>
</tr>
<tr>
<td>$3(\pi\pi^*)$</td>
<td>5.86</td>
<td>5.14</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Oscillator strengths in parentheses. $^b$ Involving basically $O_{56}$ (in the ortho position with the methyl group).

**Letters**

in different environments, such as in polar solvents, it is expected that the $n^\pi^\ast$-type excited state will destabilize with respect to $\pi\pi^\ast$-type excited states.\textsuperscript{23} Despite those effects, both singlet and triplet $n^\pi^\ast$-type states are estimated to lie in the solvent below the $1(\pi\pi^\ast)^n$ state at the FC geometry,\textsuperscript{23,29} guaranteeing the existence of the STC crossing upon decay along the $1(\pi\pi^\ast)^n$ state. The reported wavelength dependence on the intersystem crossing quantum yield in nucleobases can be understood by the location of the two STC crossing regions and their accessibility upon the initial excitation conditions. In the case of thymine, the value increases from $3.9 \times 10^{-3}$ at 280 nm (4.43 eV) to $5.2 \times 10^{-2}$ at 240 nm (5.17 eV).\textsuperscript{3,30}

Recently, Hare et al.\textsuperscript{29} reported the presence of a second deactivation path in pyrimidine nucleobases in solution via a low-lying dark intermediate state attributed to the singlet $1(n^\pi^\ast)$. In order to explain higher triplet quantum yields in pyrimidine than that in purine nucleobases, a small fraction of the state population is suggested to undergo an ISC process toward the lowest triplet state. In our results, as well as in previous theoretical studies,\textsuperscript{19,24,25} the presence of the low-lying $1(n^\pi^\ast)$ state is confirmed, lying almost isoenergetic with the lowest ethene-like ($tg^\pi^\ast\pi^\pi^\ast$) CI near 4.0 eV.\textsuperscript{2} The $1(n^\pi^\ast)$ state may then be related to the observed dark state. Additionally, we have found that, as both singlet and triplet $1,3(n^\pi^\ast)$ states have almost parallel hypersurfaces because of the typical small singlet—triplet splitting of $n^\pi^\ast$-type states, near degeneracy situations with the lowest $3(\pi\pi^\ast)$ state are equal to both states (see Supporting Information). Therefore, close to the $1(n^\pi^\ast)/3(n^\pi^\ast)$ CI, we have found a singlet—triplet crossing ($1(n^\pi^\ast)/3(n^\pi^\ast)$)STC. The large computed SOC terms, $\sim 61$ cm$^{-1}$, guarantee also an efficient ISC process in the region, confirming this mechanism as relevant for the overall process.

Finally, the lowest triplet state is populated by any of the previous ISC processes and reaches its minimum, as proved by the computed MEPs (see Figure 2). At the state minimum, $3(\pi\pi^\ast)_{\text{min}}$, the molecule displays a distorted structure with a ring deformation, including the dihedral angle C2N1C6C5 as 44° and an increased bond length C5C6 of 1.494 Å. The high reactivity attributed to this triplet state originates from its biradical character on C5 and C6. The minimum is placed at 2.87 eV adiabatically (see Table 1) from the ground-state-optimized minimum, a value somewhat lower than the 3.2 eV estimated for the location of the triplet state for thymine mononucleotide in aqueous solution and room temperature\textsuperscript{7} and consistent with previous theoretical determinations at around 2.8—3.0 eV.\textsuperscript{26} The location of the triplet state in thymine is also lower than that in the other pyrimidine nucleobases, computed adiabatically at 3.15 (uracil)\textsuperscript{18} and 3.04 eV (cytosine),\textsuperscript{19} an effect which can be attributed to methylation.\textsuperscript{31} As a final aspect of the evolution along the triplet manifold in thymine, we have located the singlet—triplet crossing connecting the $3(\pi\pi^\ast)$ and the ground state and mapped the MEP leading from such a STC toward $3(\pi\pi^\ast)_{\text{min}}$ (see Supporting Information). The crossing is placed near 3.0 eV from the ground-state minimum, which means that there is a barrier of 0.13 eV (3.0 kcal/mol) to reach ($tg^\pi^\ast\pi^\pi^\ast$)STC from $3(\pi\pi^\ast)_{\text{min}}$. The molecule recovers the planarity there, and

![Figure 1](image1.png)

**Figure 1.** Evolution of the ground and lowest singlet excited states for thymine from the FC geometry along the $1(\pi\pi^\ast)^n$ MEP computed at the CASPT2/CASCF(14,10)/ANO-S C,N,O[3s2p1d]/H[2s1p] level.

![Figure 2](image2.png)

**Figure 2.** Scheme, based on CASPT2 results, of the photochemistry of thymine focused on the population of the lowest-energy triplet state.
the computed electronic SOC is somewhat low, \( \sim 2 \text{ cm}^{-1} \), predicting a long lifetime and a slow relaxation for the triplet state, becoming therefore prone to react.

In summary, by means of CASPT2/CASSCF calculations and by computing minimum-energy paths, minima, conical intersections, singlet—triplet crossings, and spin—orbit coupling terms, we have identified three different intrinsic mechanisms in which the population can be transferred from the initially populated singlet \( ^1\pi\pi^* \text{HL} \) state to the triplet manifold through respective singlet—triplet crossings (STC), which, in all cases, can be reached in a barrierless manner on the hypersurface. Along the \( ^1(\pi\pi^*) \) decay path, there are two STC at 4.8 eV, \( ^1(\pi\pi^*\pi\pi^*)_{\text{STC}}, \) and at 4.0 eV, \( ^1(\pi\pi^*\pi\pi^*)_{\text{STC}}, \) both with similar SOCs of 8 cm\(^{-1}\). In the former case, the population has to switch from the \( ^3(\pi\pi^*) \) to the \( ^3(\pi\pi^*) \) state through a conical intersection \( ^3(\pi\pi^*\pi\pi^*)_{\text{CI}}. \) The suggested ISC process\(^2^9\) taking place from a less populated singlet \( ^1(\pi\pi^*) \) state toward \( ^3(\pi\pi^*) \) also occurs in the same region of the CI between the lowest triplet states, enhanced by large SOC terms, \( \sim 61 \text{ cm}^{-1}. \) These mechanisms are common to the other pyrimidine nucleobases, at least uracil and cytosine. The observed wavelength dependence of the intersystem crossing quantum yield in nucleobases can be interpreted by the contribution of the three or just the lowest-energy ISC mechanism.

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Supporting Information Available: Details of the calculation of MEPS, conical intersections, singlet—triplet crossings, spin—orbit coupling elements, and radiative lifetimes. Figures describing significant minimum-energy paths on the excited-states hypersurfaces. Table and figure with optimized geometries. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes