On the puzzling deactivation mechanism of thymine after light irradiation

Leticia González*, Jesús González-Vázquez*, Elena Samoylova^ and Thomas Schultz^

*Institutfür Physikalische Chemie, Friedrich-Schiller-Universität Jena, Helmholtzweg 4, 07743 Jena, Germany
^Max Born Institute, 12489 Berlin-Adlershof, Germany

Abstract. The possible deactivation mechanisms of thymine after UV light irradiation are reviewed in the light of theoretical calculations. Recent experiments reveal that three transient species with lifetimes in the fs, ps, and ns regime are present in thymine. The possibility of ground or excited state tautomerization is explored and discarded. The role of \( \pi, \pi^* \) states, as well as of the proposed minimum of the \( \pi, \pi^* \) excited state surface are assessed. In view of the obtained calculations and results available from the literature, the measured time scales can be tentatively attributed to a model involving different conical intersections between the \( \pi, \pi^* \) and the electronic ground state, as well as deactivation via the triplet states. Time-resolved photoelectron experiments supported by theoretical calculations are proposed to appraise the validity of this model.

Keywords: thymine, photostability, decay mechanism, conical intersections, CASSCF/CASPT2, RI-CC2, photoelectron spectra


INTRODUCTION

RNA and DNA bases show an extraordinary photostability which has been attributed to the presence of ultrafast nonradiative deactivation processes [1]. After UV irradiation, the main chromophores of RNA or DNA evolve to geometries where two potential energy surfaces are nearly degenerated and touch each other with the form of a double cone. Such points are denominated conical intersections and they are responsible for a very efficient funnel from the upper to the lower surface in few fs [2, 3], thus quenching any photochemical reaction which could otherwise damage our genetic code.

Even if it is widely agreed that low-lying conical intersections are present in all five RNA/DNA bases [4], the detailed deactivation mechanism of these small chromophores is still not completely clarified. Time-resolved pump-probe photoionization experiments on isolated nucleic acid bases show a complex multieponential decay with transients in the femtosecond (fs), picosecond (ps) and nanosecond (ns) time regime. Despite a large number of experimental as well as theoretical studies, both of quantum chemical and dynamical nature, the origin of these times scale remains controverted.

In this contribution, we focus on the deactivation mechanism of thymine (T). The spectra of T, measured by resonance-enhanced multiphoton ionization (REMPI) and laser-induced fluorescence (LIF) experiments, is broad and structureless indicating very short electronic excited state lifetimes [5]. Recent pump-probe ionization spectroscopical investigations [6, 7, 8, 9, 10, 11] agree on the presence of fs, ps and ns lifetimes components for T. In general, a fs relaxation can be only explained by vibrational relaxation or by an early conical intersection close to the Frank-Condon geometry. In most of these DNA bases, such conical intersections occur between the spectroscopically \( S_2 \) active state, the \( \pi, \pi^* \) state, and the lower \( S_1 \), or \( n, \pi^* \) state, which is dark. A ps time scale must be rationalized with a late conical intersection far away from the Frank-Condon region. And, finally the ns relaxation could originate from a spin-forbidden transition or a conical intersection with difficult access. However, as it will be explained below the assignment of these time scales to precise conical intersections and associated reaction paths in T is not straightforward.

Theoretical calculations on thymine have been done at different levels of theory, including complete active space self-consistent-field (CASSCF) corrected with singles and doubles excitations (MR-CISD) [12] or with second-order perturbation theory (CASPT2) [13], configuration interaction including single excitations (CIS) [14], and time-dependent density functional theory (TD-DFT) [15]. These calculations predict that the bright \( \pi, \pi^* \) excited state is higher in energy than the dark \( n, \pi^* \) state. Shukla and Mishra also found a minimum for the \( \pi, \pi^* \) state [14] which could play a role in the decay process. In contrast, two recent calculations by Perun et al. [16] and the group of Merchán [17] predict a barrierless reaction path on the \( \pi, \pi^* \) surface towards a conical intersection with the electronic ground state.
This pathway may account for a rapid (fs) radiationless decay of all \( \pi\pi^* \) vibronic levels. Since no low-lying conical intersection was identified for the \( n\pi^* \) state by Perun and coworkers [16], these authors discussed the idea of a long-lived population in the corresponding dark vibronic levels to account for the ps time scale. On the other end, on-the-fly ab initio dynamics using the multiple spawning method [18] disputes these mechanisms. Although these authors found a \( S_2/S_1 \) conical intersection, at the employed level of calculation, fast internal conversion is prevented by a barrier. Thus, the fs time is attributed to vibrational energy redistribution from the Franck-Condon region to this local minimum, while the conical intersection is responsible for the ps component of the lifetime. Recently, Serrano-Perez et al. predicted an "intrinsic" intersystem crossing (ISC) pathway for populating the lowest triplet state [19]. In agreement with the El-Sayed selection rules [20, 21], a ISC may occur from the singlet \( n\pi^* \) state to the triplet \( \pi\pi^* \), and then three transients for \( \pi\pi^*, n\pi^* \) and \( 2\pi\pi^* \) could be expected.

In conclusion, despite the presence of a conical intersection between the \( \pi\pi^* \) and \( n\pi^* \) states, and between the \( n\pi^* \) and the ground state \( S_0 \), there is no consensus on the origin of the observed three lifetimes. In the following, we discuss possible deactivation mechanisms with the help of ab initio calculations respective to the different hypotheses suggested in the literature.

**COMPUTATIONAL DETAILS**

The electronic ground state geometry of all the stationary points mentioned in this paper have been optimized without any symmetry constraint at the MP2 level of theory with a double polarized basis set [22] (cc-pVDZ) using the Gaussian 03 suit of programs [23]. The nature of all the stationary points has been confirmed by a hessian calculation. Unless otherwise stated, the vertical excitation energies were calculated using coupled cluster of second order in its Resolution of the Identity version, RI-CC2 [24] with a Dunning basis set aug-cc-PVTZ, with triple polarization and augmented with diffuse functions [25] —also as auxiliary basis set [26]. These calculations are performed with the TURBOMOLE software package [27]. The minimum optimization has been done using the CASSCF(14,10) method [28] and an ANO-L basis set contracted to 3s2p in the H atom and 4s3p2d for the heavy atoms. This energy is then corrected with a single point at multi steppe CASPT2 (MS-CASPT2) level of theory [29, 30]. The active space includes all the three \( \pi, \pi^* \) pairs with corresponding six electrons in the double bonds, plus the pairs at the N atoms contributing two electrons and one orbital each to the \( n \) cloud, and two lone pairs at the O atom with another pair of two electrons in one orbital each. These latter calculations are carried out in MOLCAS 6.2 package [31].

**RESULTS AND DISCUSSION**

In first place it is useful to revise the vertical spectrum of T. This has been repeatedly computed in the literature, and here we just emphasize that while the most recent calculation places the bright \( \pi\pi^* \) state above the \( n\pi^* \) one [32], the energy difference between these two states can be very subtle depending on the level of theory employed, or just the choice of the active orbitals considered within CASSCF methods. To illustrate the excitations that take place in T, we show in Figure 1 a scheme of the energies with a molecular orbital representation of the transitions corresponding to the lowest excited states obtained using RI-CC2/aug-pVTZ [11]. The calculated energies are in reasonable agreement with...
other levels of theory, see e.g. [13, 14, 15, 16]. Compared with the experiment, our values are ca. 0.2 eV overestimated, serving as an upper bound for the error in other calculations carried out at this level of theory.

Experimentally, T is excited with 266 nm (4.66 eV). The most recent experiments identify three exponential decays with time scales of 100 fs, 7 ps and 22 ns [11]. In view of the calculated Franck-Condon excitations, it seems most plausible that the $S_2 \pi\pi^*$ electronically excited state is populated. If we accept for the time being that the reaction paths first described by Perun and coworkers [16] are the relevant ones to explain the relaxation mechanism of T, it is possible to propose that the ultrafast time scale (fs) is due to the evolution of the wavepacket on the $\pi\pi^*$ state towards two different conical intersections. The first one is between the $\pi\pi^*$ and the dark $\pi\pi^*$ state. This degeneracy point has been fully optimized by Asturiol and Blancafort [32] and it corresponds to an out-of-plane bending of the methyl group. The second conical intersection is of ethylenic character and connects directly the $\pi\pi^*$ state with the electronic ground state $S_0$ [16]. Following further this model, the ps time scale can be ascribed to the population which has been transferred previously via the first conical intersection ($\pi\pi^*/\pi\pi^*$) and now can relax to the $S_0$ via an additional conical intersection. This stationary point is characterized by an out-of-plane distortion of the carbonyl group next to the CH$_3$ [16, 19, 32]. In this context, the question which can be raised is, which is then the molecular origin of the third, long time scale (ns).

In first place, we have explored whether tautomers other than the canonical tautomer could be involved in the photorelaxation mechanism of T. The six tautomers of T which are connected via different H-migrations are shown in Figure 2. Also shown are their stabilities with respect to the canonical tautomer T1 in kcal/mol, obtained at MP2/cc-pVDZ [11]. Since gas-phase experiments performed on T [11] require temperatures up to 300 °C to evaporate the sample, Boltzmann distributions for temperatures from 0 to 1000 °C have been estimated. The obtained populations are in Table 1. As it can be immediately realized, even at temperatures of 1000 °C, less than 5% of the population can be found in the non-canonical tautomers. In view of this result, it can be safely assumed that in the ground state only the canonical tautomer is present.

TABLE 1. Boltzmann distributions of the thymine tautomers at several temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0°C</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>25°C</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>50°C</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>100°C</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1000°C</td>
<td>95.90</td>
<td>2.02</td>
<td>0.07</td>
<td>1.40</td>
<td>0.60</td>
<td>0.01</td>
</tr>
</tbody>
</table>

The next question we rise is whether tautomerization in the electronic excited states is plausible. For this purpose, the transition states connecting the six tautomers have been optimized and their corresponding vertical spectra have been calculated at the R1-CC2/aug-cc-pVTZ level of theory. The results indicate that in all cases barriers of ca. 1.5 eV need to be overcome to tautomerize, both in the $\pi\pi^*$ and the $\pi\pi^*$ excited states [11]. Since this energy is not available at the experimental laser frequency, tautomerization in the excited states can also be discarded.

In view that tautomerization, both in the ground and excited states, is not relevant in the photostability of T, the nature of the ns transient remains obscure. To shed some light into this problem, three additional hypotheses will be discussed hereafter: i) the role of $\pi\sigma^*$ states, ii) the existence of minima in the $\pi\pi^*$ potential energy surface, and iii) the function of the spin-forbidden transitions.
First we will consider the relevance of \( \pi \sigma^* \) states in T. The existence of an additional relaxation path involving \( \pi \sigma^* \) states has been first suggested by Sobolewski and Domcke [33, 34] in adenine, and later by others, see e.g. [35, 36]. In adenine, there is a conical intersection between the \( \pi \pi^* \) and the \( \pi \sigma^* \) state associated with H atoms bound to the N or O atoms. Moreover, H-atoms have been detected in adenine in the ns time scale by Doppler spectroscopy [37]. This ns transient disappears when adenine is solvated [36] because the \( \pi \sigma^* \) state is very much stabilized in water due to its very large dipole moment. Thus, one way to check the role of \( \pi \sigma^* \) in T is to consider thymine-water complexes.

The RI-CC2/aug-cc-pVTZ vertical absorption energies for T and T(H\(_2\)O)\(_1\) are shown in Figure 3. Also shown is the structure of the most stable thymine-water complex, as predicted by Kabelac et al [38]. As it can be seen, the \( \pi \sigma^* \) state is not stabilized by addition of one water molecule, implying that such states do not play a role in the deactivation of T. This result agrees with the fact that Schneider and coworkers found no evidence for H dissociation in T (using Doppler spectroscopy) [39].

The second hypothesis is related to the existence of a minimum in the \( \pi \pi^* \) excited state. This is a planar minimum which was first found by Merchán and coworkers using CASSCF(14,10)/ANO-S calculations [17]. This minimum is then associated with ps lifetime decays: upon excitation to the \( \pi \pi^* \) excited state the wavepacket is trapped in this minimum and its delayed signal is due to the time employed in climbing the barrier and decaying to the S\(_0\). The presence of a minimum is confirmed by further CASSCF(8,6) calculations and on the fly first-principles quantum molecular dynamics methods [18]. Nevertheless, with these methods an out-of-plane minimum, instead of an in-plane one, is found. Interestingly, in contrast to all previous studies, Hudock et al. suggest that the fs time scale is due to the relaxation into this minimum. Then, the ps transient may correspond to electronic relaxation via the conical intersection between \( \pi \pi^* \) to \( \pi \sigma^* \) states [18]. One should note that this latter ansatz is not directly obtained from their simulations which have not been run to reach the ps time scale. Moreover, although this minimum is confirmed by CASPT2 optimizations on an enlarged CASSCF(8,7) by the same authors, our calculations with a CASPT2/CASSCF(14,10)/ANO-L depict a different situation. In Figure 4 we show that the minimum which is found in the \( \pi \pi^* \) state does not correspond to the lowest \( \pi \pi^* \) excited state but to a higher one! This minimum involves a different starting \( \pi \) orbital centered on the O atom. This conclusion is also reached by Asturiol and Blancafort [32], who also use an enlarged CASPT2/CASSCF(12,9) method.

These calculations point to the fact that the on-the-fly simulations of Ref. [18] are not unambiguous. It is very plausible that these calculations overestimate the probability to go from the \( \pi \pi^* \) state to the \( \pi \sigma^* \) state driving
population to this minimum. In summary, we believe this minimum should not participate in the relaxation mechanism of thymine.

The last hypothesis is the presence of triplet states. A spin-forbidden transition is normally located in the ns regime. Serrano-Perez and coworkers [19] have recently established the population of the lowest triplet state of thymine via different intersystem crossing mechanisms from the $\pi\pi^*$ bright state. This seems a plausible explanation for the longer transient (ns) in the pump-probe spectra of thymine [11].

CONCLUSION AND PERSPECTIVE

Until here we have reviewed the different mechanisms proposed in the literature for the deactivation of thymine. Complicated enough, different levels of theory provide different scenarios. It seems clear that only a close collaboration between theory and experiment can help to find the solution to this puzzle. Here we want to propose a photoelectron experiment which, supported by \textit{ab initio} calculations, should disentangle the rank.

In a photoelectron experiment is possible to measure the velocity of electrons and thereby the energy of the ions. Figure 5 shows a schematic picture of the gedanken experiment. The dynamics of the system can be monitored using a classic pump-probe experiment with two delayed laser pulses. In the Figure 5, the pump pulse initiates the photophysical reaction exciting the molecule to $S_2$ and the probe pulse ionizes the molecule. In our example, there are two competing channels. In the first one, the population can directly be ionized in the $S_2$ state. In the second, part of the population can pass to the $S_1$ state (via a conical intersection or ISC) and then it is ionized in the $S_1$ state. These two channels come from different electronic states so that the velocity of the ejected electron in each case is different. In a pump-probe experiment, the electronic state of the system at each time is observed via the electronic velocity. From a theoretical point of view, the limits of this electronic velocity distribution can be estimated using high level \textit{ab initio} calculations. Ionization far from the adiabatic geometry of the cation leads into high vibronic states and the observation of slow electrons. Ionization closer to the relaxed geometry of the cation leads to faster electrons and the maximum velocity is the energy difference between the minimum of the cation and the laser frequency. This scheme holds true for both, vibrational and electronic relaxation and can be easily extended to thymine deactivation where there are three channels that can proceed from two different states. Then, depending of the deactivation mechanism the photoelectron spectra will show different fingerprints.

In the first hypothesis, which proposes a minimum [17, 18] in the $\pi\pi^*$ state, the first and second transients must come from the same state ($\pi\pi^*$). Then, one could expect that the velocity of the ejected electron corresponds to the cation with one electron in the $\pi$ orbital, i.e. a $^2\pi$ state. The third transient is originated in a $n\pi^*$ state so the cation
would be in a $^2n$ state.

In the second hypothesis, which proposes a spin-forbidden transition as responsible for the third transient [19], the first transient comes from the $\pi\pi^*$ state leading then to a $^2\pi$ cation. The second transient is then produced from a $n\pi^*$ state, so that a $^2n$ cation is expected. Following the El-Sayed rules [20, 21], ISC from the $n\pi^*$ to the $^3\pi\pi^*$ produces a $^2\pi$ cation.

If we compare the two hypotheses, we should expect a $^2\pi$ photoelectron spectra in both cases for the first transient (fs). However, for the second transient (ps), two signals are possible. If the first hypothesis is true, one must see a $^2\pi$ cation coming from the minimum, while if the transient comes from a $\pi\pi^*/n\pi^*$ conical intersection a $^2n$ cation should be observed. In the longer transient (ns), a $^2n$ or a $^2\pi$ cation has to be observed for the first and second hypotheses, respectively.

Such experiments and theoretical calculations are currently in progress in our laboratories.

**ACKNOWLEDGMENTS**

Fruitful discussions with L. Blancafort and access to his submitted paper are gratefully acknowledged. Furthermore, we thank the Deutsche Forschungsgemeinschaft for financial support through the SFB 450 "Analysis and Control of Ultrafast Photoinduced Reactions" and the project GO 1059/6-1. The COST Action RADAM is also acknowledged for its cooperative atmosphere and mobility support.

**REFERENCES**