Ab initio study of a biradical radiationless decay channel of the lowest excited electronic state of cytosine and its derivatives

Marek Z. Zgierski(a) and Serguei Patchkovskii
Steacie Institute for Molecular Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada
Edward C. Lim
Department of Chemistry and The Center for Laser and Optical Spectroscopy, The University of Akron, Akron, Ohio 44325-3601

(Received 20 June 2005; accepted 15 July 2005; published online 30 August 2005)

A theoretical model for the ultrafast \( S_1 \rightarrow S_0 \) internal conversion of cytosine is presented, in which a state switch from the initially prepared \( ^1 \pi \pi^* \) state to the out-of-plane deformed excited state of biradical character controls the rate of the \( S_1 \rightarrow (^1 \pi \pi^*) \) decay. This mechanism successfully accounts for the dramatically longer \( S_1 \) lifetimes of 5-fluorocytosine and \( N\)-acyetylcytosine relative to cytosine. The replacement of the C5 hydrogen atom by a methyl group is predicted to lead to a substantial, but not dramatic, increase in the \( S_1 \) lifetime, also consistent with experiment. It is this ability to correctly predict the substituent effects that distinguishes the present model from the previously proposed mechanisms. © 2005 American Institute of Physics. [DOI: 10.1063/1.2031207]

Because of their biological importance the DNA bases have been the subject of many spectroscopic and photochemical studies. The striking photophysical and dynamical properties of the DNA bases are a very broad absorption spectrum in the UV region and short \( S_1 \) lifetimes, which lead to a low-quantum yield of fluorescence. These features seem to be selected by nature to prevent photochemical damage in the heart of genetic machinery. Transient absorption experiments as well as fluorescence upconversion experiments reveal the \( S_1 \) lifetimes below a few picoseconds in aqueous solutions. Femtosecond pump-probe transient ionization experiments in supersonic free jet yield decay times of ca. 1 ps for purine and pyrimidine bases.

There is a strong substituent dependence of \( S_1 \) lifetime that has an important bearing on the nonradiative decay mechanism of DNA bases. Malone et al. have shown that substitution of the C5 hydrogen atom by fluorine leads to a dramatic increase in the excited-state lifetime. The \( S_1 \) lifetime of 5-fluorocytosine is about 88 ps, which is nearly two orders of magnitude longer than that of cytosine (≈1 ps). We have also observed a very similar effect of fluorination on the \( S_1 \) lifetime of uracil. Even more dramatic lifetime lengthening is observed when a hydrogen atom in the amino group (attached at C4) is replaced by an acetyl group. Thus, the \( S_1 \) lifetime of \( N\)-acetylcytosine is more than two orders of magnitude longer (≈280 ps) than that of cytosine. This is surprising since it is difficult to envision how the acetyl group far removed from the ring could so strongly influence electronic excitation localized in the pyrimidine ring. Clearly, these are the key observations, which a successful model must be able to account for.

To understand the origin of the ultrashort \( S_1 \) lifetimes of DNA bases, and the dependence of their lifetimes on chemical modifications, it is essential to have accurate characterization of the electronic and vibrational structures of the low-lying excited states. Three mechanistic models, based on high-level electronic structure calculations, exist at present. For adenine, the time-dependent density-functional theory (TDDFT) calculation of Sobolewski and Domcke has shown that the potential-energy function of the \( ^1 \pi \pi^* \) state intersects the \( ^1 \pi \sigma^* \) state, which has a conical intersection (CI) with the electronic ground state. The intermediacy of the \( ^1 \pi \sigma^* \) state (which is repulsive along the N9–H coordinate) was proposed to account for the highly efficient \( S_1 \rightarrow (^1 n \pi^*) \rightarrow S_0 \) internal conversion. The applicability of the Sobolewski-Domcke model has, however, been questioned by Kang et al. and Malone et al. as the adenine derivatives that do not contain a N–H hydrogen atom (e.g., N9–CH3 adenine) also exhibit subpicosecond lifetimes in solution and in a supersonic jet. Moreover, there is no observable kinetic isotope (deuterium) effect that might be expected for the decay mechanism involving the intermediacy...
TABLE I. Vertical excitation energies (eV) with CASSCF(8,8)/cc-pVTZ, QDPT2/cc-pVTZ, CIS/cc-pVDZ, and CR-EOM-CCSD(T)/cc-pVDZ approaches calculated at the HF/cc-pVDZ geometries. CASSCF calculations are state-averaged for the four lowest singlet states.

<table>
<thead>
<tr>
<th>Species</th>
<th>State</th>
<th>CASSCF</th>
<th>QDPT2</th>
<th>CIS</th>
<th>CR-EOM-CCSD(T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosine</td>
<td>$1\pi\pi^*$</td>
<td>6.405</td>
<td>4.822</td>
<td>6.294</td>
<td>5.510</td>
</tr>
<tr>
<td></td>
<td>$1\pi\pi$</td>
<td>5.745</td>
<td>5.049</td>
<td>6.942</td>
<td>5.877</td>
</tr>
<tr>
<td>5-methylcytosine</td>
<td>$1\pi\pi^*$</td>
<td>6.334</td>
<td>4.758</td>
<td>6.187</td>
<td>5.478</td>
</tr>
<tr>
<td></td>
<td>$1\pi\pi$</td>
<td>5.862</td>
<td>5.084</td>
<td>6.854</td>
<td>5.873</td>
</tr>
<tr>
<td>5-fluorocytosine</td>
<td>$1\pi\pi^*$</td>
<td>6.218</td>
<td>4.519</td>
<td>6.216</td>
<td>5.433</td>
</tr>
<tr>
<td></td>
<td>$1\pi\pi$</td>
<td>5.687</td>
<td>5.085</td>
<td>6.892</td>
<td>6.450</td>
</tr>
</tbody>
</table>

of the $1\pi\pi^*$ state. For cytosine, complete active space self-consistent field (CASSCF) calculation of Ismail et al.\textsuperscript{15} suggest a three-state decay mechanism involving a state switch from the $1\pi\pi^*$ state to the $1\pi\pi^*$ state (associated with the n orbital of the carbonyl oxygen), which in turn connects to the electronic ground state by $1\pi\pi^*/S_0$ CI. The CASPT2 results of Merchán and Serrano-Andrés,\textsuperscript{15} on the other hand, suggest that the nonradiative decay of cytosine occurs through a $1\pi\pi^*/S_0$ CI. At the CASPT2 level of theory, the $1\pi\pi^*$ state is about 15–20 kcal/mol above the $1\pi\pi^*$ state, which renders the decay mechanism involving the intermediacy of the $1\pi\pi^*$ state much less likely. They also consider the Sobolewski-Domcke mechanism\textsuperscript{15} involving the repulsive $1\pi\pi^*$ state irrelevant for cytosine as the $1\pi\pi^*/S_0$ CI lies at least 0.75 eV above the $1\pi\pi^*/S_0$ CI.

Irrespective of the differing conclusions, none of these existing models is able to provide a reasonable explanation of the dramatic substituent dependence of the $S_1$ lifetime observed in experiment. CASSCF(8,8) (Ref. 16)/cc-pVTZ and the corresponding quasidegenerate second-order perturbation theory, QDPT2 (Refs. 17 and 18)/cc-pVTZ, calculation of $1\pi\pi^*$ and $1\pi\pi^*$ vertical excitation energies at the optimized ground-state geometries for cytosine and two of its derivatives (Table I) give no explanation for the observed variation of the lifetimes of the doorway state. While the state-averaged CASSCF suggests the $1\pi\pi^*$ state as $S_1$ QDPT2 gives correctly the $1\pi\pi^*$ state as the lowest-excited singlet. This agrees with the two other methods used below.

Very recently Sobolewski and Domcke\textsuperscript{19} have indicated that in the guanine-cytosine (GC) base pair it is convenient to transfer the initial excitation from the guanine molecule to cytosine. The latter was suggested to undergo internal conversion involving ethylenic twist with ensuing fast internal conversion. However, the importance of this pathway was not studied in detail, and the reaction coordinate involved in the conversion process was not characterized. Also Shukla and Mishra\textsuperscript{20} reported the relaxation of the $1\pi\pi^*$ state of uracil to a configuration with a twisted C5C6 double bond. Here, we present a detailed theoretical model for the ultrafast $S_1 \rightarrow S_0$ internal conversion in cytosine and its derivatives (and uracil) involving twisting of the HCCH bond with the creation of a biradical state, which accounts for all the observed substituent effects on the $S_1$ lifetime.

The ab initio study leading to the model involves the application of a simple configuration interaction singles (CIS)/cc-pVDZ method to find partially optimized geometries. Single-reference CIS approach tends to overestimate excitation energies and may lead to incorrect results in situations where multiple excitations become important. Indeed, test calculations using multireference QDPT2/cc-pVTZ approach at optimized restricted Hartree–Fock (RHF) $S_0$ geometries indicate significantly lower excitation energies (Table I). Even larger differences might be expected at distorted geometries, where the ground state will acquire significant multiconfigurational character due to a $\pi$-bond breaking. Unfortunately, the QDPT2 approach is not suitable for following the potential-energy surface (PES) for large structural distortions in an orbital-rich molecule like cytosine, both because of the cost of the calculations and because of the difficulties in maintaining consistent active spaces for the entire reaction path. On the other hand, single-reference completely renormalized coupled-cluster methods\textsuperscript{21,22} provide a qualitatively correct description of potential-energy surfaces along bond-breaking directions, including multireference cases studied so far.\textsuperscript{23} At equilibrium $S_0$ geometries, completely renormalized equation of motion coupled-cluster [CR-EOM-CCSD(T)] calculations with a small basis set recover a large fraction of the QDPT2 excitation energy lowering (Table I). At the present time, analytic energy gradients are not available for the CR-EOM methods, so that a complete PES exploration is not practical. Instead, we perform CR-EOM-CCSD(T) calculations at optimized CIS geometries to confirm energetics of the low-lying excited electronic states. Overall agreement in the shape of the PES sections between these two methods provide an assurance that our results are robust with respect to a more sophisticated treatment of electron correlation. The CIS calculations were performed with the use of the GAUSSIAN98 program.\textsuperscript{24} The coupled-cluster doubles calculations with type-III perturbative corrections for connected triples\textsuperscript{21,22} were done with the GAMESS (Ref. 25) program. The essential results of the computations are given in Fig. 1.

The calculations show that the $1\pi\pi^*$ state, created by optical excitation of the ground-state cytosine, is unstable with respect to the out-of-plane deformation and evolves down the energy slope along the C4C5C6N1 out-of-plane deformation until it intersects the biradical-like state in which there is a strong rehybridization of the C5 and C6 orbitals. In this new state, the C5 and C6 hydrogen atoms are almost perpendicular to the average ring plane and displaced in opposite directions. As a result the $p_z$ orbitals of the C5 and C6 carbon atoms are decoupled from the $\pi$-electron sys-
Fig. 2. (Color online) The same as Fig. 1 but for 5-fluorocytosine. The black lines refer to CIS calculations, while the red lines represent CCSD(T) energies shifted upward by 1.454 55 a.u.

FIG. 1. (Color online) The energy of the \( S_1 \) state as a function of the C4C5C6N1 dihedral angle (left) and of the HC5C6H angle (right). EOM-CCSD(T) values are shifted uniformly upward by the amount indicated in the figure. The inset shows the side view of the structure of the minimum of the biradical state. The blue solid line indicates the energy of the CCSD(T) ground state at biradical configuration geometries. The green dashed lines show the energy for pyramidalization of the amino group reversed with respect to the ground state.

not dramatic, increase in the \( S_1 \) lifetime. Consistent with this expectation, the \( S_1 \) lifetime (~7 ps) (Ref. 5) of 5-methylcytosine is only seven times longer than that of cytosine. Significantly, similar calculations predict that the replacement of one of the hydrogen atoms in the amino group (attached at C4) by an acetyl group raises the energy of the biradical state relative to the \( ^1\pi\pi^* \) state through the formation of a weak intramolecular C–H:O hydrogen bond between the acetyl carbonyl group and C5–H group. This makes it more difficult to pull the C5 hydrogen atom away from the ring plane, and hence the switch of the \( ^1\pi\pi^* \) state to the biradical state is strongly hindered. In aqueous solution, a water molecule can form a hydrogen-bond bridge between the acetyl carbonyl and the C5 hydrogen, which further stabilizes the \( ^1\pi\pi^* \) state relative to the biradical state. The energy barrier along the C4C5C6N1 coordinate from the minimum of the \( ^1\pi\pi^* \) state to the biradical state is about 1.2 kcal/mol in free N-acetylcytosine (NAC) and about 1.6 kcal/mol in the 1:1 hydrogen-bonded complex of NAC with water. The barrier along the HC5C6H coordinate at the minimum of the \( ^1\pi\pi^* \) state is 7.6 kcal/mol for NAC (CIS). More water molecules in the solvent shell will most probably make the switch from the \( ^1\pi\pi^* \) to the biradical state more difficult to activate. These results provide a rational account of the dramatically longer \( S_1 \) lifetime (~280 ps) of NAC relative to that (~1 ps) of cytosine.

Interestingly, our model suggests that \(^1n\pi^* \) state plays no important role in the ultrafast internal conversion of cytosine along the route envisaged in the calculations. It also predicts that the substitution of the C6 hydrogen atom by a fluorine atom has a less significant and opposite effect on the internal conversion. Thus the \( S_1(\pi\pi^\ast) \) lifetime of 6-fluorocytosine should be somewhat shorter than that of cytosine. Unfortunately, no \( S_1 \) lifetime has been reported for 6-fluorocytosine.

To summarize, we have characterized the pathway for the ultrafast \( S_1(\pi\pi^\ast) \rightarrow S_0 \) internal conversion in cytosine. The model, which is based on high-level CR-EOM-CCSD(T) calculations of excited-state energies at CIS geometries and employs the same T1 and T2 matrix elements as in the CIS calculations, predicts a dramatic increase in the \( S_1 \) lifetime of 5-fluorocytosine. The replacement of one of the hydrogen atoms in the amino group (attached at C4) by an acetyl group raises the energy of the biradical state relative to the \( ^1\pi\pi^* \) state through the formation of a weak intramolecular C–H:O hydrogen bond between the acetyl carbonyl group and C5–H group. This makes it more difficult to pull the C5 hydrogen atom away from the ring plane, and hence the switch of the \( ^1\pi\pi^* \) state to the biradical state is strongly hindered. In aqueous solution, a water molecule can form a hydrogen-bond bridge between the acetyl carbonyl and the C5 hydrogen, which further stabilizes the \( ^1\pi\pi^* \) state relative to the biradical state. The energy barrier along the C4C5C6N1 coordinate from the minimum of the \( ^1\pi\pi^* \) state to the biradical state is about 1.2 kcal/mol in free N-acetylcytosine (NAC) and about 1.6 kcal/mol in the 1:1 hydrogen-bonded complex of NAC with water. The barrier along the HC5C6H coordinate at the minimum of the \( ^1\pi\pi^* \) state is 7.6 kcal/mol for NAC (CIS). More water molecules in the solvent shell will most probably make the switch from the \( ^1\pi\pi^* \) to the biradical state more difficult to activate. These results provide a rational account of the dramatically longer \( S_1 \) lifetime (~280 ps) of NAC relative to that (~1 ps) of cytosine.
etry, shows that the barrier for the state switch from the optically excited $^3\pi\pi^*$ state to an out-of-plane deformed excited singlet state of biradical character determines the efficiency and rate of the internal conversion. This mechanism explains the dramatic variations of the excited-state lifetimes among cytosine and its derivatives. It is this ability that clearly separates our model from all previously proposed mechanisms. In subsequent papers we will describe the details of the calculations presented herein and discuss the importance of our model in uracil and its derivatives and in other DNA bases. In passing we mention that CIS calculations suggest that this biradical path of decay can play a very significant role for ultrafast internal conversion of the adenine-thymine (AT) and guanine-cytosine (GC) base pairs, where the biradical state at its equilibrium geometry is either quasidegenerate (GC) or lies below (AT) the closed-shell ground state. Since the biradical decay channel involves only the part of the pyrimidine ring not participating in hydrogen bonding, the binding of the two helix strands is not expected to be affected by the photophysical process.

ACKNOWLEDGMENTS

We are grateful to the U.S. Department of Energy for the support of this work and to the Ohio Supercomputer Center for grants of computer time. One of the authors (E.C.L.) is the Holder of the Goodyear Chair in Chemistry at The University of Akron.