

## Ultrafast Internal Conversion of Excited Cytosine via the Lowest $\pi\pi^*$ Electronic Singlet State

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Fluorescence decay times of the DNA/RNA nucleosides and nucleotides have been measured in solution by several groups.<sup>1–5</sup> The excited-state lifetimes of the nucleic acid molecules fall in the subpicosecond time scale, suggesting the presence of an ultrafast internal conversion channel for both nucleosides and nucleotides. Very short lifetimes have also been determined in the gas phase for the isolated purine bases (adenine, guanine) and the pyrimidine bases (thymine, uracil, cytosine).<sup>6</sup> Therefore, the ultrashort lifetime of nucleobases appears to be an intrinsic molecular property. Most likely, life on earth as we know is intimately related to this feature. It is believed that relaxation to the ground state proceeds before photochemical reactions take place. Nucleobases seem to be the optimal biochemical response of evolution to the effects of sunlight radiation. Fascinated by this fact, we are undertaking a research project to characterize the decay mechanisms of the electronically excited DNA/RNA bases, pushing theory up to what current technology can handle at present. Here, we report our conclusions for cytosine. Of course, it would be highly desirable if they could be corroborated experimentally. In this respect, theoretical and experimental findings may complement each other largely, bringing a constructive interplay between experiment and theory.

Recent theoretical work performed at the CASSCF/6-31G\* level led to the proposal that the ultrafast decay of electronically excited singlet cytosine occurs via a  $\pi\pi^*$  to  $n_0\pi^*$  switch prior to the radiationless decay.<sup>7</sup> The CASPT2 results yield a different picture: the conical intersection (CI) between the ground state (gs) and the excited state  $\pi\pi^*$  is predicted as the  $S_1/S_0$  crossing responsible for the ultrafast decay of singlet excited cytosine. The computed spectroscopic properties for cytosine are compiled in Table 1. Unless otherwise stated, the active space comprised 12 electrons distributed in 9 orbitals (7  $\pi$  and 2 lone pairs). The 6-31G\*\* basis set is used throughout. The CI structures have been computed with the Gaussian program,<sup>8</sup> and the remaining calculations were done with the MOLCAS-5 software.<sup>9</sup> In what follows, only CASPT2 results will be discussed.

The vertical transitions are in agreement with the recorded absorption spectra (see Table 1). The  $\pi\pi^*$  and  $n_0\pi^*$  transitions are predicted to have large Stokes shifts, 0.86 and 1.01 eV, respectively, with computed band origins within 0.23 eV. From the calculated CASSCF transition dipole moments and the CASPT2 energies, the radiative lifetimes ( $\tau_{\text{rad}}$ ) have been obtained by using the Strickler–Berg relationship.<sup>11</sup> The theoretically derived  $\tau_{\text{rad}}$  values are consistent with the nature of the states. For the  $n_0\pi^*$  state,  $\tau_{\text{rad}}$  is about 30 times that calculated for the  $\pi\pi^*$  state. Moreover, the theoretical estimate for the  $\pi\pi^*$ , 33 ns, is in reasonable agreement with our estimate based on experimental data, 40 ns.<sup>2,6,12</sup>

Therefore, the relative match between the computed (in vacuo) and observed (in the gas phase) photophysical parameter suggests that the emission measured in solution in transient absorption

**Table 1.** Computed Spectroscopic Properties for the Low-Lying Singlet Excited States of Cytosine

state	vertical transition (eV)		band origin (eV)		$\tau_{\text{rad}}$ (ns) <sup>b</sup>
	CASSCF	CASPT2 <sup>a</sup>	CASSCF	CASPT2	
$\pi\pi^*$	5.43	4.50 (0.065) <sup>c</sup>	3.93	3.64	33
$n_0\pi^*$	5.41	4.88 (0.001)	3.74	3.87	1066
$n_N\pi^*$ <sup>d</sup>	5.73	5.23 (0.003) <sup>e</sup>			

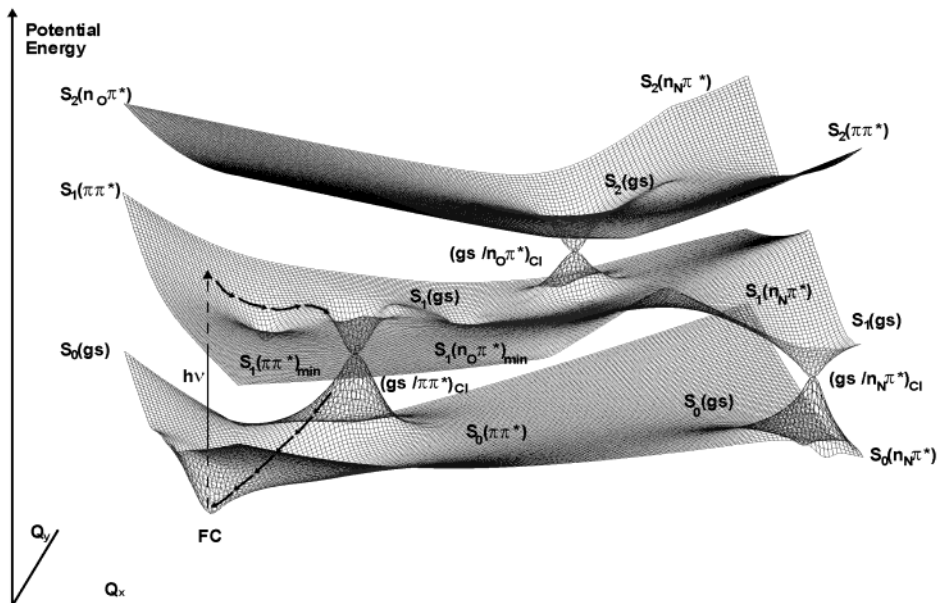
<sup>a</sup> Oscillator strength within parentheses. <sup>b</sup> Estimated radiative lifetime ( $\tau_{\text{rad}}$ ) from experiment: 40 ns. See ref 12. <sup>c</sup> Experimental data: 4.6 eV (0.11). Taken from ref 10. <sup>d</sup> Geometry optimization leads to (gs/ $n_N\pi^*$ )<sub>CI</sub>. <sup>e</sup> Experimental data: 5.3 eV (0.03). See discussion in ref 10.

experiments<sup>3,5</sup> and by femtosecond fluorescence up-conversion techniques<sup>4</sup> originates primarily from the  $\pi\pi^*$  state of cytosine, expected to be the lowest singlet excited state also in solution.<sup>1</sup>

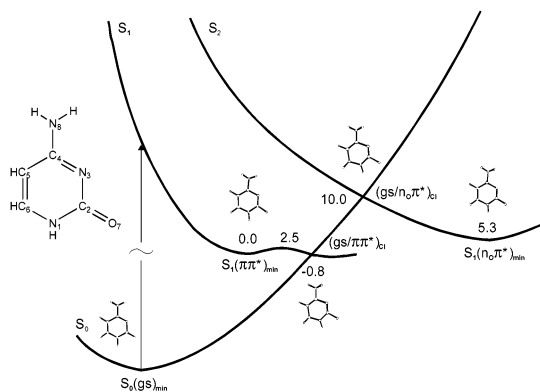
The qualitative picture for the lowest hypersurfaces of cytosine (see Figure 1) is based on the CASPT2 reaction paths built by linearly interpolated internal coordinates (LI-IC) between the CASSCF/6-31G\*\* optimized geometries for the states of interest (see Supporting Information). The relative energies for the main structures considered are included in Figure 2. On the  $S_1$  hypersurface, two minima are located: ( $\pi\pi^*$ )<sub>min</sub> and ( $n_0\pi^*$ )<sub>min</sub>. The CI between the gs and the  $\pi\pi^*$  state, (gs/ $\pi\pi^*$ )<sub>CI</sub>, can be ascribed as the  $S_1/S_0$  crossing responsible for the observed ultrafast radiationless decay. The intersection has an intermediate topology that probably precludes photodegradation. The molecule should accumulate enough vibrational energy during the decay to overcome a small barrier to the (gs/ $\pi\pi^*$ )<sub>CI</sub> funnel (see Figure 2). The initial part of the decay maintains the ring planarity and involves bond inversion.<sup>7</sup> Decay to (gs/ $\pi\pi^*$ )<sub>CI</sub> needs pyramidalization of C<sub>6</sub> and further CO stretching (see atom labeling in Figure 2). At the (gs/ $\pi\pi^*$ )<sub>CI</sub> crossing, the CO bond distance is 1.428 Å. When the CO bond length was increased to 1.462 Å, the (gs/ $n_0\pi^*$ )<sub>CI</sub> crossing is reached. The excited-state  $n_0\pi^*$  is involved in a  $S_2/S_1$  crossing. Therefore, the  $n_0\pi^*$  is not directly implied in the ultrafast repopulation of the ground state after illuminating cytosine with UV light.

The excited-state hydrogen detachment driven by repulsive singlet  $\pi\sigma^*$  states has been proposed as a new paradigm for nonradiative decay in aromatic biomolecules.<sup>13</sup> For cytosine, the dissociation limit of the N<sub>1</sub>–H bond (yielding the C<sub>4</sub>H<sub>4</sub>N<sub>3</sub>O plus H radicals) lies at 4.36 eV with respect to ground-state cytosine. This means that the (gs/ $\pi\sigma^*$ )<sub>CI</sub> is not competitive in cytosine because it is placed at least 0.75 eV higher than the (gs/ $\pi\pi^*$ )<sub>CI</sub>.

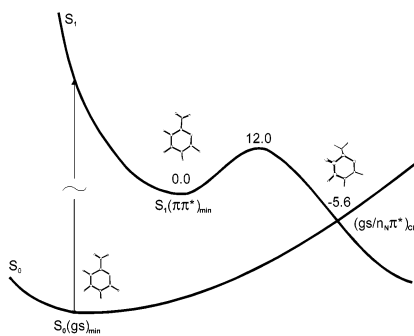
On the other hand, an alternative decay mechanism represents a switch from the  $\pi\pi^*$  to the  $n_N\pi^*$  states and subsequent decay to the ground state (Figure 3). Along the path from the ( $\pi\pi^*$ )<sub>min</sub> to the (gs/ $n_N\pi^*$ )<sub>CI</sub>, an estimated barrier of about 12 kcal/mol is found, considerably higher than the barrier along the  $\pi\pi^*$  path. The minor role of the  $n_N\pi^*$  state in the deactivation of the electronically singlet excited cytosine is supported by recent experimental evidence.<sup>5</sup>



**Figure 1.** Global qualitative picture of the three lowest hypersurfaces in cytosine as suggested from CASPT2 calculations.  $Q_x$  and  $Q_y$  coordinates are related mainly to ring deformation and CO bond stretching. FC: Franck–Condon geometry  $(gs)_{\min}$ .



**Figure 2.** Cut in the cytosine hypersurfaces depicted in Figure 1 and computed relative positions of the  $S_1/S_0$  and  $S_2/S_1$  crossings. Proposed ultrafast decay of singlet-state cytosine through  $(gs/\pi\pi^*)_{Cl}$ . Energies are in kcal/mol. Employed atom labeling is on the left.



**Figure 3.** Path through  $(gs/n_N\pi^*)_{Cl}$  in cytosine. Energies are in kcal/mol.

Protonated cytidine at low pH has a lifetime also in the subpicosecond range despite the loss of the  $n_N\pi^*$  by protonation at  $N_3$ . In light of the present results, the lifetime variations of cytosine species and its derivatives can be most probably rationalized as a consequence of the relative changes in the  $\pi\pi^*$  state with respect to  $(gs/\pi\pi^*)_{Cl}$ . Whether this is a general relaxation mechanism for all excited nucleobases or not is the subject of our current research.

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**Supporting Information Available:** Computational details, Cartesian coordinates for the optimized structures, and LI-IC results (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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