Communication

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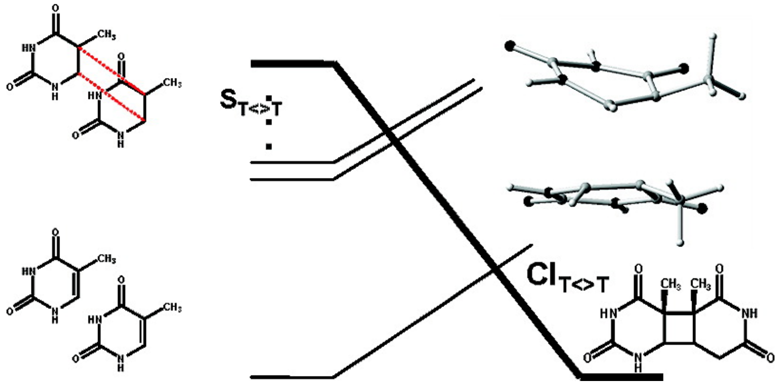
J. Am. Chem. Soc., **2007**, 129 (47), 14540-14541 • DOI: 10.1021/ja074734o



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Modeling Thymine Photodimerizations in DNA: Mechanism and Correlation Diagrams

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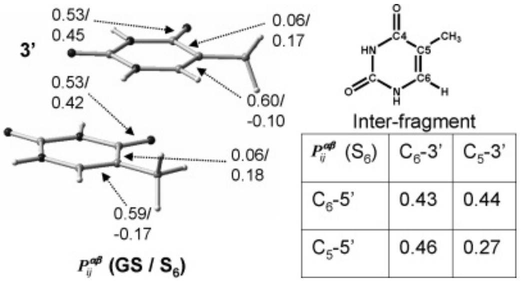
Formation of pyrimidine cycloadducts is one of the most important photochemical reactions in DNA.[1] The quantum yield of formation in isolated DNA (with respect to thymine) and in single-strand, all-thymine oligodeoxynucleotides is 2−3% for the cyclobutane dimer (T<>T), and approximately 1 order of magnitude less for the 6−4 photoproduct, whose precursor is an oxetane adduct.[2,3] Moreover, the formation of T<>T occurs on an ultrafast time scale (1 ps). This suggests the passage through a conical intersection to yield the ground state products during the formal [2 + 2] photocycloaddition.[3] Here the basic mechanistic traits of the T<>T and oxetane formation reactions are established with the help of CASSCF and CASPT2 calculations for a gas-phase model of two stacked thymines (TT pair). Both reactions go through conical intersections between the ground and the excited state connected to the corresponding products. The states responsible for the photoproduct formation correlate with two high-lying states of the pair in its ideal B-DNA conformation, which are different from the delocalized excited states resulting from coupling of the localized ones. Product formation requires population of these states. The results also suggest that the π stacking not only favors the photochemical reactivity by producing suitable conformations but also induces the appearance of an additional state responsible for the T<>T photoproduct.

The calculated excitations and oscillator strengths for the TT pair in its ideal B-DNA conformation (interfragment distance 3.38 Å) are shown in Table 1. These values are only approximate because of the reduced CASSCF active space of six π orbitals per base (see Supporting Information), but they provide a reliable basis for a semiquantitative discussion. The states have been characterized with a valence-bond-based analysis using localized active orbitals, where the spin-transition density matrix elements $P_{ij}^{\alpha\beta}$ can be used as π bond indices between the atom centers that bear the active orbitals.[4] The focus is on the conjugated system formed by the C_4-O_8, C_4-C_5, and C_5-C_6 bonds and the bonds between the C_5 and C_6 atoms of different bases (Figure 1). The ground state bond pattern of both molecules corresponds to the ground state resonance structure. In the four lowest excited states, the excitation is localized on one of the fragments (see Supporting Information). These excitations correspond to the ones found in the monomers (HOMO → LUMO and HOMO-1 → LUMO). The energies of S_1 and S_2 are close to the lowest calculated vertical (π,π*) excitation in isolated thymine (approximately 5 eV).[5] Higher in energy, S_5 and S_7 are charge transfer states between the fragments. More importantly, in S_6, the C−O π bonds are little affected by the excitation, while the bonding of the two intra-annular π bonds is disrupted (see the decrease in the bond indices in Figure 1). Moreover, the electrons on the C_5 and C_6 atoms of one fragment are coupled to the ones on the other fragment, prefiguring the bonding pattern of the cyclobutane adduct (see the table in Figure 1). Thus S_6 (which is S_1 at the CASSCF level) is the precursor state of the T<>T adduct.

Table 1. Calculated Singlet Excitation Energies (E_{exc}, CASPT2/CASSCF(12,12)/6-31G*) and Oscillator Strengths (*f*, Derived from CASSCF(12,12)/6-311+G* Transition Dipole Moments) for Stacked TT in the Ideal B-DNA Conformation[a]

state	E_{exc} (eV)	*f*
S_1 ($LE_{5'}$)	5.12	2×10^{-1}
S_2 ($LE_{3'}$)	5.25	6×10^{-1}
S_3 ($LE_{3'}$)	6.56	8×10^{-4}
S_4 ($LE_{5'}$)	6.61	2×10^{-2}
S_5 ($CT_{3'\rightarrow 5'}$)	6.68	4×10^{-3}
S_6 (T<>T)	6.93	6×10^{-2}
S_7 ($CT_{5'\rightarrow 3'}$)	6.99	9×10^{-3}

[a] $LE_{n'}$: local excitation (n' fragment); $CT_{n'\rightarrow m'}$: charge transfer (n' to m').



$P_{ij}^{\alpha\beta}$ (S_6)	C_6-3'	C_5-3'
C_6-5'	0.43	0.44
C_5-5'	0.46	0.27

Figure 1. Calculated bond indices for the ground state and the T<>T precursor state at the ideal B-DNA conformation.

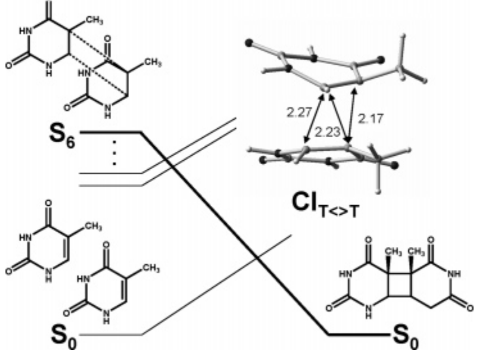


Figure 2. Correlation diagram for T<>T formation and S_1/S_0 conical intersection.

The correlation diagram for the product and reactant states involved in the formation of T<>T is shown in Figure 2. The diagram suggests the presence of an S_1/S_0 conical intersection on the potential energy surface. Density functional theory calculations also suggest this.[6] Here the conical intersection, $\mathbf{CI_{T<>T}}$, has been located at the CASSCF(12,12)/6-31G* level. It is analogous to the one found for the [2 + 2] photocycloaddition of two ethylene molecules.[7] The bonds between the fragments are halfway formed at $\mathbf{CI_{T<>T}}$, and the branching space vectors lie along the reaction

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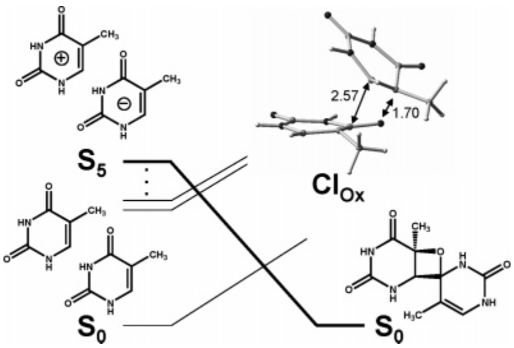

Figure 3. Correlation diagram for oxetane formation and S_1/S_0 conical intersection.

coordinate. Minimum energy path calculations show that the ground state decay from $\mathbf{CI}_{\mathbf{T<>T}}$ can lead to the products or revert to the reactants (see Supporting Information).

A conical intersection between the ground and excited states was also located for the oxetane adduct, precursor of the 6−4 product ($\mathbf{CI}_{\mathbf{Ox}}$, see Figure 3). The cycloaddition is asynchronous, and the new C−O bond is almost completely formed at the conical intersection (1.70 Å). The gradient difference vector corresponds to the C−O stretching coordinate and the excitation to the 3′→5′ charge transfer state (see Supporting Information). Thus, the oxetane can be correlated with S_5 at the B-DNA geometry (see Figure 3). The mechanism for oxetane formation, which seems to be different from the model Paterno-Büchi cycloaddition,[8] is a nucleophilic attack of the negatively charged fragment on the positively charged one. Hydrogen bonding due to Watson−Crick pairing should reduce the reactivity of the charge transfer state, which might partly explain the decrease of the 6−4 adduct yield in double-stranded DNA compared to single-stranded DNA.[2]

One alternative to the photocycloadditions is the unreactive decay of the locally excited state along the methyl out-of-plane bending coordinate described for isolated thymine.[9] A point of degeneracy between the ground and excited states has been located at the CASSCF(12,11)/6-31G* level, $\mathbf{CI}_{\mathbf{LE}}$, where the 5′ fragment has the conformation described for the conical intersection of the isolated nucleobase, and the two thymines retain an approximately stacked arrangement with an interfragment distance of 3.49 Å (see Supporting Information). The energy of $\mathbf{CI}_{\mathbf{LE}}$, calculated at the CASPT2/CASSCF(14,13)/6-31G* level, lies approximately 0.3 eV over $\mathbf{CI}_{\mathbf{T<>T}}$ and $\mathbf{CI}_{\mathbf{Ox}}$. Although $\mathbf{CI}_{\mathbf{LE}}$ is not fully optimized (full optimization leads to an unnatural arrangement with the bases lying in the same plane due to intermolecular hydrogen bonding) and the energies do not take the DNA environment into account, these values indicate that the three conical intersections lie roughly in the same energy range, and the proposed mechanisms are energetically reasonable.

The proposed mechanisms for T<>T and oxetane formation involve passage through the corresponding intersections to access the ground state products. The presence of $\mathbf{CI}_{\mathbf{Ox}}$ also suggests that the 6−4 precursor, whose rate of formation was not determined experimentally,[3] may also be formed at an ultrafast rate. However, the initial part of the mechanism remains unclear. It has been suggested that T<>T formation occurs with a quantum yield close to unity when the molecule is excited in a favorable conformation, while in the more common, unfavorable conformations the excited state will be unreactive.[3] The present results introduce a new aspect. Thus, dimerization does not only depend on the conformation but also requires population of the reactive state. Therefore, we propose two possible mechanisms. The first one corresponds basically to the mechanism suggested on the basis of the experimental results.[3] It involves excitation of the reactive states at favorable conformations, leading directly to the products. However, at the B-DNA conformation, the reactive states lie well above the typical irradiation energies below 5 eV, and the estimated oscillator strengths are lower than for the local excitations. Thus, this mechanism requires a substantial energy lowering of the reactive states by conformational and environmental effects (e.g., approach of the bases), together with an increase in the oscillator strengths. The second mechanism starts with population of a locally excited state. A stacked complex, where the excitation is localized on the 5′ fragment, has been located as a quasi-minimum (see Supporting Information), more than 1 eV above the conical intersections. Decay from this transient, through the unreactive $\mathbf{CI}_{\mathbf{LE}}$, should be the favored process. However, access to the reactive conical intersections through an avoided crossing with the reactive states, involving a small barrier, should also be possible if the transient is long-lived enough in the single- or double-strand environment. Attempts to optimize these paths in the gas phase failed because of the floppy nature of the locally excited minimum. Calculation of the excitation spectra for different conformations and optimization of the excited state reaction paths in the DNA environment are required to further study these mechanisms and the competition with other paths such as excited state hydrogen transfer.[10]

Acknowledgment. Financial support was provided by the Ramón y Cajal program (L.B.) and Project No. CTQ2005-04563 of the Spanish Ministerio de Educación y Ciencia, and the European COST Action P9 "Radiation Damage in Biomolecular Systems". This research has been carried out in part using the resources of the Center for Supercomputation of Catalonia (CESCA).

Supporting Information Available: Computational details, further figures, and Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) After submission of our manuscript, a paper on the same topic appeared [Boggio-Pasqua, M.; Groenhoef, G.; Schäfer, L.; Grubmüller, H.; Robb, M. A. *J. Am. Chem. Soc.* **2007**, *129*, 10096−10097], where the $\mathbf{CI}_{\mathbf{T<>T}}$ intersection is also described.

JA074734O