J|A|C|S
COMMUNICATIONS

Published on Web 09/15/2004

Triplet (π,π^*) Reactivity of the Guanine−Cytosine DNA Base Pair: Benign Deactivation versus Double Tautomerization via Intermolecular Hydrogen Transfer

Lluís Blancafort,*,† Joan Bertran,‡ and Mariona Sodupe‡

Institut de Química Computacional, Universitat de Girona, E-17071 Girona, Spain, and Departament de Química, Universitat Autònoma de Barcelona, E-08193, Bellaterra, Spain

Received March 28, 2004; E-mail: lluis.blancafort@udg.es

The recent determination of the excited-state lifetimes of the free DNA bases and their nucleosides in the gas phase[1] and in solution[2−5] has provided new insight into the interaction between these DNA components and UV light. The main characteristic is the ultrashort lifetime of the excited states (decay in the subpicosecond or picosecond time scale) due to internal conversion. In cytosine, this occurs via a conical intersection with the ground state.[6,7] However, the presence of "dark states" in the pyrimidine bases has been identified by means of multiphoton ionization techniques.[1,8,9] In the case of cytosine, a triplet has been proposed as the detected species.[8] The intersystem crossing quantum yields of the nucleic acid bases range from 10^{-3} to 10^{-4}, and the formation of pyrimidine dimers in irradiated DNA is a further sign of the relevance of triplet states.[10] In addition, photoinduced hydrogen transfer has been observed in cytosine dimers and clusters and in the guanine−cytosine base pair (**GuCy**),[11] and in this context the well-known proposal of photoinduced tautomerization as a possible mutagenic route[12] merits new attention.

The tautomerization on the *triplet-state surface* of the **GuCy** pair, specifically the $^3(\pi,\pi^*)$ states, was studied with CASSCF/6-31G* and CAS-PT2 calculations, using Gaussian03 and MOLCAS (details in Supporting Information).[13] We focus here on two main points: (1) the competition between unreactive return to the ground state (path A) and hydrogen transfer (path B) and (2) the fate of the base pair after the first hydrogen transfer, i.e., return to the singlet ground state via electron-transfer associated to a conical intersection (B_1) and access to a bifurcation region (B_2) that can lead to regeneration of the canonical pair or H_2 transfer.

Our study, summarized in Figure 1, starts from the minimum of the triplet state for the canonical pair, ^{3}**Can$_{Cy}$**, with cytosine-localized (π,π^*) excitation. This species could be formed by excitation of cytosine and crossing to the triplet surface (in analogy to the route proposed for the dark state of cytosine)[8] or by direct triplet sensitization. ^{3}**Can$_{Cy}$** can evolve along two main pathways. Path A is a benign deactivation route, where the base pair returns to its ground state through an intersystem crossing (**ISC$_1$**) with the triplet state T_1. In ^{3}**Can$_{Cy}$**, the excitation is localized on the C−C bond of the cytosine moiety, and the coordinate that leads to **ISC$_1$** is an out-of-plane bending of the two carbons. The calculated spin−orbit coupling (SOC) at **ISC$_1$** is small (0.6 cm^{-1}). Therefore, the unreactive decay will be only moderately efficient despite the low barrier (approximately 2 kcal mol^{-1}, CAS-PT2//CASSCF), and the phototautomerization path B can compete with it. The minimum where the excitation is localized on guanine, ^{3}**Can$_{Gu}$**, is approximately equal in energy (0.4 kcal mol^{-1}, CAS-PT2//CASSCF). Population of this minimum from ^{3}**Can$_{Cy}$** is unlikely, because the excitation transfer is a nonadiabatic process (almost zero coupling between the two chromophores), and it is associated with a high barrier of 15 kcal mol^{-1}. Moreover, the H_1 transfer path from ^{3}**Can$_{Gu}$** (path B′) leads to the same product as the one from ^{3}**Can$_{Cy}$** (see below).

Path B starts with the transfer of one hydrogen from guanine to cytosine. On the triplet surface, it follows a coupled proton and electron-transfer mechanism such as that described for the singlet excited state of **GuCy**[14,15] and an aminopyridine dimer model.[16] It leads to a biradical species, ^{3}**ST$_{Bir}$**. The CAS-PT2//CASSCF barrier for H_1 transfer from ^{3}**Can$_{Cy}$** is 17 kcal mol^{-1}.[17] The reaction is exothermic (by 11 kcal mol^{-1} at the CAS-PT2//CASSCF level) and leads to a biradical, ^{3}**ST$_{Bir}$**, which is energetically degenerate with the corresponding singlet, ^{1}**ST$_{Bir}$**. The structure corresponds to an intersystem crossing point (**ISC$_2$**), where the reaction path returns to the singlet state (see the right inset of Figure 1). The calculated SOC is <0.1 cm^{-1}, but the change of spin will be efficient because **ISC$_2$** is a minimum on the triplet surface. The alternative processes, transfer of H_2 to form the double tautomer ^{3}**DT** and reverse transfer of H_1, have substantial barriers of approximately 25 kcal mol^{-1} (CAS-PT2//CASSCF), and decay to ^{1}**ST$_{Bir}$** will be the preferred route.

^{1}**ST$_{Bir}$** evolves in two further steps. The first step (B_1) is back electron transfer (ET) from the cytosine to guanine moiety, which leads to a closed-shell, zwitterionic species, ^{1}**ST$_{CS}$**. At the CASSCF-(12,11)/6-31G* level, ^{1}**ST$_{Bir}$** lies on the first excited state, and the gap to the ground state is 8 kcal mol^{-1}. The state order is the same at the CAS-PT2 level. This suggests that the ET has an inverted Marcus topology associated to a sloped[18] conical intersection (**CI$_{Bir/CS}$**) between the biradical and closed-shell states (S_1 and S_0 in the inset of Figure 1). The relevance of ^{1}**ST$_{Bir}$** and the associated **CI$_{Bir/CS}$** for the hydrogen transfer on the *singlet* excited state has been pointed out recently.[15,16] Our assignment of ET in the inverted Marcus region is only tentative because the state order is reversed with a smaller active space (see Computational Details in Supporting Information), and the calculation may not be converged with respect to that. A change in the state order would change the ET topology from inverted to normal Marcus type.

The CASSCF barrier to access **CI$_{Bir/CS}$** from ^{1}**ST$_{Bir}$** is small, approximately 0.2 kcal mol^{-1}, and the reaction coordinate consists of intramolecular rearrangement of the bases at an approximately constant intermolecular distance. The decay from **CI$_{Bir/CS}$** to ^{1}**ST$_{CS}$** has been studied with an IRC calculation and CAS-PT2 single-point calculations along the coordinate (see Computational Details in Supporting Information). It consists of a steep initial decay with intramolecular rearrangements due to the ET, followed by a flatter part where the intermolecular hydrogen bonds adapt to the new charge distribution. The O−H_2 and N−H_1 bonds are shortened by 0.8 and 0.3 Å, respectively, while the third hydrogen bond is stretched by 0.3 Å. The ^{1}**ST$_{CS}$** ground-state tautomer is a shallow

† Universitat de Girona.
‡ Universitat Autònoma de Barcelona.

10.1021/ja048230r CCC: $27.50

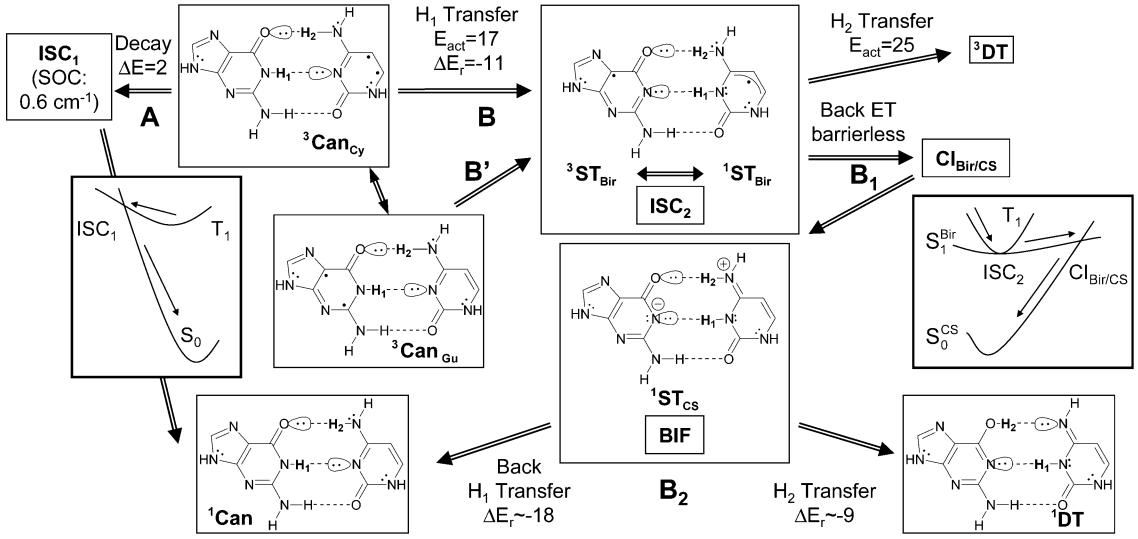

Figure 1. Triplet (π,π^*) reactivity, guanine−cytosine pair (CAS-PT2//CASSCF energies, kcal mol^{-1}). Path A: Benign deactivation. Path B: Tautomerization.

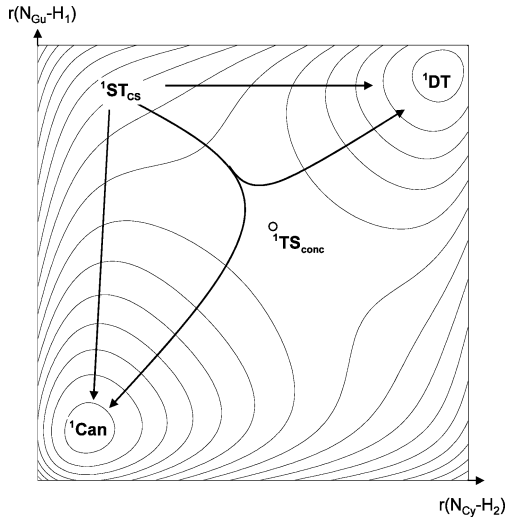

Figure 2. Qualitative S_0 potential energy surface along the H_1 and H_2 transfer coordinates. Arrows mark possible decay paths from **^{1}ST$_{CS}$** (B_2).

minimum at the CASSCF level (Supporting Information), but it is probably a spurious one due to the lack of dynamic correlation, in analogy to previous HF and MP2 results.[19] From additional B3LYP and CAS-PT2 calculations, the ground-state energy surface along the H_1- and H_2-transfer coordinates is the one sketched in Figure 2, where the $N_{Gu}-H_1$ and $N_{Cy}-H_2$ distances stand for the two coordinates. The only two stable minima are the canonical **^{1}Can** pair and the double-transfer tautomer **^{1}DT**, which are connected by a concerted double hydrogen transfer, **^{1}TS$_{conc}$**. The main mechanistic point is that the back ET (path B_1) leads to **^{1}ST$_{CS}$**, which is effectively a bifurcation region between the ^{1}**Can** and ^{1}**DT** tautomers. From there, the decay path (B_2) can continue to any of the tautomers either directly or through the region of **^{1}TS$_{conc}$**. The regeneration of the canonical pair is energetically favored by approximately 9 kcal mol^{-1},[19] but there appear to be no dynamic factors in favor of any of the products. Therefore, the potentially mutagenic double tautomerization will also take place.

In summary, the reactivity of the guanine cytosine base pair in its triplet state, with excitation localized in the cytosine moiety, is characterized by the competition between a benign deactivation route A and a hydrogen transfer (coupled electron and proton transfer) route B with significant probability of triggering the double tautomerization. The calculated barriers favor path A, but the low spin−orbit coupling along this route and the possibility of tunneling in path B (which we have not evaluated) suggest that path B cannot be ruled out completely. Thus paths A and B are probably an alternative to cytidine photodimerization.[10] Many aspects of the reactivity remain to be explored, such as the formation of the initial triplet, the role of (n,π^*) states,[20] and alternative paths from **^{3}Can$_{Gu}$**. Such calculations are in progress.

Acknowledgment. Work was supported by Grant No. BQU2002-04112-C02, Ministerio de Ciencia y Tecnología (MCyT, Spain). L.B. acknowledges a "Ramón y Cajal" fellowship (MCyT).

Supporting Information Available: Computational details and list of geometries (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kang, H.; Lee, K. T.; Jung, B.; Ko, Y. J.; Kim, S. K. *J. Am. Chem. Soc.* **2002**, *124*, 12958−12959.
(2) Peon, J.; Zewail, A. H. *Chem. Phys. Lett.* **2001**, *348*, 255−262.
(3) Pecourt, J. M. L.; Peon, J.; Kohler, B. *J. Am. Chem. Soc.* **2001**, *123*, 10370−10378.
(4) Pecourt, J. M. L.; Peon, J.; Kohler, B. *J. Am. Chem. Soc.* **2000**, *122*, 9348−9349.
(5) Crespo-Hernández, C. E.; Cohen, B.; Hare, P. M.; Kohler, B. *Chem. Rev.* **2004**, *104*, 1977−1202.
(6) Merchán, M.; Serrano-Andrés, L. *J. Am. Chem. Soc.* **2003**, *125*, 8108−8109.
(7) Ismail, N.; Blancafort, L.; Olivucci, M.; Kohler, B.; Robb, M. A. *J. Am. Chem. Soc.* **2002**, *124*, 6818−6819.
(8) Nir, E.; Muller, M.; Grace, L. I.; de Vries, M. S. *Chem. Phys. Lett.* **2002**, *355*, 59−64.
(9) He, Y. G.; Wu, C. Y.; Kong, W. *J. Phys. Chem. A* **2003**, *107*, 5145−5148.
(10) Cadet, J.; Vigny, P. In *Bioorganic Photochemistry*; Morrison, H., Ed.; John Wiley & Sons: New York, 1990; Vol. 1, pp 1−272.
(11) Nir, E.; Kleinermanns, K.; de Vries, M. S. *Nature* **2000**, *408*, 949−951.
(12) Löwdin, P. O. *Adv. Quantum Chem.* **1965**, *2*, 177−179.
(13) Resonance structures of Figure 1 are based on the optimized geometries and the orbitals involved in the excitations.
(14) Guallar, V.; Douhal, A.; Moreno, M.; Lluch, J. M. *J. Phys. Chem. A* **1999**, *103*, 6251−6256.
(15) Sobolewski, A. L.; Domcke, W. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2763−2771.
(16) Sobolewski, A. L.; Domcke, W. *Chem. Phys.* **2003**, *294*, 73−83.
(17) At the DFT level, the size of the barrier depends on the method; see Supporting Information for details.
(18) Atchity, G. J.; Xantheas, S. S.; Ruedenberg, K. *J. Chem. Phys.* **1991**, *95*, 1862−1876.
(19) Florian, J.; Leszczynski, J. *J. Am. Chem. Soc.* **1996**, *118*, 3010−3017.
(20) Triplet-state calculations on isolated cytosine indicate that the lowest $^3(n,\pi^*)$ state lies at least 40 kcal mol^{-1} above the $^3(\pi,\pi^*)$ state.

JA048230R