THE JOURNAL OF PHYSICAL CHEMISTRY Letters

pubs.acs.org/JPCL

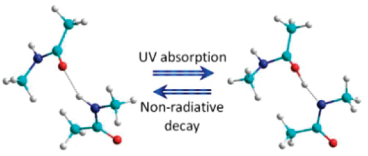
Photoinduced Proton Transfer as a Possible Mechanism for Highly Efficient Excited-State Deactivation in Proteins

Marco Marazzi,[†] Unai Sancho,[†] Obis Castaño,[†] Wolfgang Domcke,[‡] and Luis Manuel Frutos*[,†]

[†]Departamento de Química Física, Universidad de Alcalá, 28871 Alcalá de Henares (Madrid), Spain and [‡]Department of Chemistry, Technische Universität München, D-85747 Garching, Germany

ABSTRACT CASSCF//CASPT2 pathways for a two-glycine minimal model system show that photoinduced electron-driven forward and backward proton transfer could play an important role for the stability of proteins against damage by UV radiation, when a hydrogen bond is located between the two amino acids. The overall photoinduced process involves two electron and proton transfer processes (forward and backward) and results in the reformation of the initial closed-shell electronic structure of the system.

SECTION Molecular Structure, Quantum Chemistry, General Theory



Exposure to UV-radiation represents a serious risk for living matter, since it can cause changes in the structure of proteins and DNA, provoking photodamage in nucleic acids,[1,2] or loss of protein functionality in fundamental enzymatic processes (e.g., substrate recognition,[3−5] or catalysis of chemical reactions[6−9]). It has been proposed experimentally[10−12] and computationally[13−18] that photoinduced proton transfer (PPT) could be a process providing efficient stability against UV radiation for hydrogen-bonded biomolecules, reducing the yield of photodegradation.

In proteins, secondary structure (α-helix, β-sheet, etc.) is crucial in determining their overall structure, with the CO and NH groups of the backbone being primarily important in the formation of hydrogen bonds between different peptides of the same chain.[19,20] These hydrogen bonds have been pointed out to be key structures enabling PPT, where a mechanism involving forward−backward proton transfer along the C═O···H−N hydrogen bond coordinate could be responsible for the photostability of proteins, converting the absorbed energy of the photon into vibrational energy, which is then dissipated by the environment.[17,18]

Multiconfigurational self-consistent-field methods have been demonstrated to be very useful in qualitatively describing the properties of excited-state potential-energy surfaces. Nevertheless, the inclusion of dynamic correlation is crucial in order to quantitatively describe the features of excited states. For this reason, the CASSCF//CASPT2 methodology[21] has been used to study the mechanism of the PPT process in a β-turn model system (see Figure 1). Indeed, the complete optimized reaction pathways have been determined, including the characterization of the electronic crossings involved in the process. The studied model system consists of two amino acids that are properly constrained to mimic two alternate glycines within a β-turn secondary structure. This secondary structure is commonly present in proteins for reversing the direction of the peptide chain, often promoting the formation of antiparallel β-sheets (Figure 1).

By restraining the relative position of the C1 and C2 methyl moieties,[22] the conformation and the corresponding hydrogen bond of the β-turn are correctly described within a minimal model of two hydrogen-bonded amino acids (**A** and **B**) (Figure 1).

Our results show that after excitation to the lowest optically bright state, a charge transfer (CT) state can be efficiently populated via an avoided crossing, giving rise to an intrapeptide electron transfer process. The electron transfer from **B** to **A** promotes the forward proton transfer from the NH group to the CO moiety. Finally, a conical intersection, i.e., a crossing between electronic states of the same spin multiplicity, between CT and ground state (GS) provides the funnel for efficient population of the GS, recovering the initial structure of the system by a second (backward) proton transfer. The overall process provides an efficient photochemical pathway for photostability.

Two locally excited bright states have been identified: one of them localized in the glycine **A**, corresponding to a $^1(\pi_A,\pi_A^*)$ state, and a second one, $^1(\pi_B,\pi_B^*)$, localized on the glycine **B**, the vertical excitation energies of which are 6.85 and 7.16 eV, respectively. We will focus on the evolution of the $^1(\pi_A,\pi_A^*)$ state, i.e., the lowest excited state corresponding to the most feasible excitation. In the following, the $^1(\pi_A,\pi_A^*)$ state will be referred to as the locally excited (LE) state. The $^1(n_A,\pi_A^*)$ and $^1(n_B,\pi_B^*)$ locally excited dark states of both amino acids lie below in energy (5.72 and 6.06 eV for glycine **A** and **B**, respectively). Finally, a $^1(\pi_B \rightarrow \pi_A^*)$ CT state appears at 8.48 eV, involving an electron transfer from glycine **B** to **A**. The vertical excitation to the LE state yields a vibrational excess energy of 14.2 kcal/mol (the 0−0 transition is located at 6.23 eV). These results for the absorption spectrum are in

Received Date: November 12, 2009
Accepted Date: December 10, 2009
Published on Web Date: December 15, 2009

DOI: 10.1021/jz900262w | *J. Phys. Chem. Lett.* **2010**, *1*, 425–428

agreement with previous CASPT2 calculation on other polyglycine systems.[23]

After excitation of the peptide **A** to the LE state, the CT state can be populated via an avoided crossing between the LE and CT energy curves. This avoided crossing acts as a transition state for the electron transfer from glycine **B** to **A**. In order to reach the LE/CT crossing, the O···H distance of the hydrogen bond has to be shortened to ca. 1.3 Å (see Figure 2).

The efficiency of the LE/CT crossing depends mainly on the activation energy and the electronic coupling between the two states. On the one hand, the electronic coupling (ca. 230 cm^{-1}) is large enough for the electron transfer to occur efficiently. It is related mainly to the movement of the proton along the hydrogen-bond coordinate, as can be deduced from the graphical representation of derivative coupling (DC) and gradient difference (GD) vectors[24] (Figure 2). Moreover, the excitation to the $^1(\pi_A,\pi_A^*)$ state provokes the pyramidalization of the carbonyl group of the peptide **A**, giving rise to the shortening of the O···H distance, which in turn, effectively drives the system to the avoided crossing after Franck–Condon excitation.

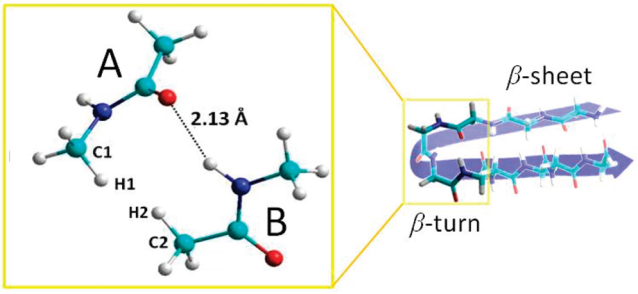


Figure 1. GS optimized structure of the polyglycine model. The relative position of the C1 and C2 methyl groups has been restrained in order to mimic a β-turn structure. The CO hydrogen-bonded glycine is labeled as **A**, while the NH bonded glycine is referred as **B**.

On the other hand, this crossing is estimated to be located only ca. 2 kcal/mol above the minimum of the $^1(\pi,\pi^*)_A$ state. Therefore, the crossing should be accessible from the LE state, permitting the population of the CT state on a time-scale of a few tens of femtoseconds.

The sudden charge separation taking place in the system due to the electron transfer process from glycine **B** to **A** triggers the proton transfer process, in which the proton involved in the hydrogen bond moves to the peptide **A** in order to compensate the charge separation. This proton motion in the CT state does not encounter an energetic barrier, and two more crossings are reached along the minimum energy path. The first, with the $^1(n,\pi^*)_A$ state, may not be efficient in populating this state, since the crossing is avoided and visited only once during the vibrational relaxation process in the CT state. A second crossing (conical intersection) with the GS, on the other hand, provides a funnel for population of the electronic GS (Figure 2).

This CT/GS conical intersection allows the system to decay to S_0. The DC and GD vectors (see Figure 2) of the conical intersection describe the nuclear motion in the relaxation process after the crossing. Therefore, these vectors determine to some extent the formed photoproducts. It should be noted

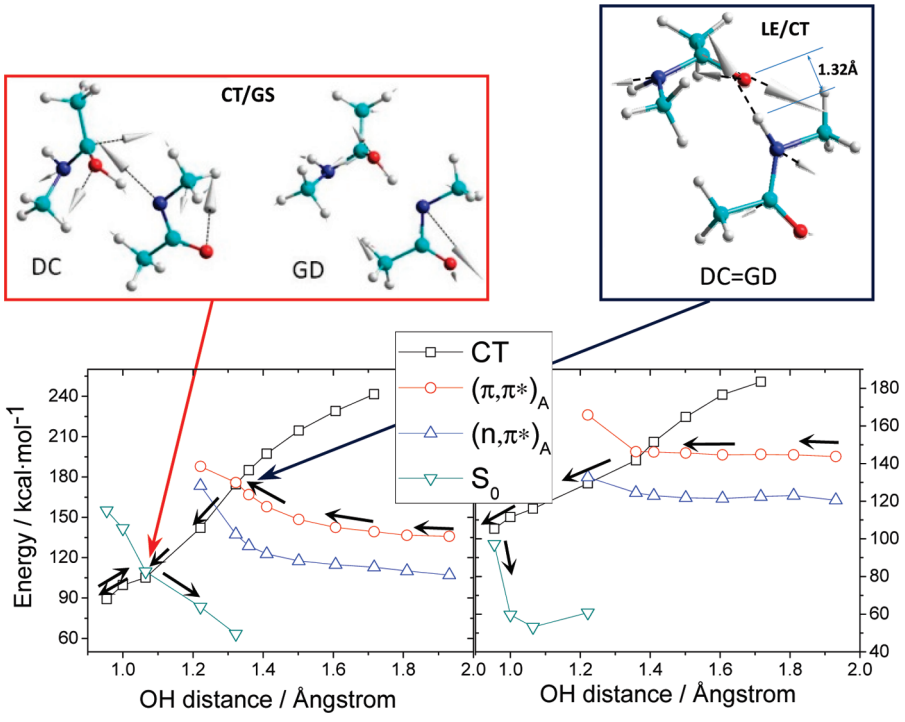


Figure 2. Global view of PPT minimum-energy paths computed at the CASSCF level, as a function of the O···H distance. Both CASSCF (left) and CASPT2 (right) profiles are shown. Note the steepness of the energy profile of the CT state, guiding the proton transfer to **B** after LE/CT crossing leading to the CT/GS conical intersection. The GD and DC vectors are shown for the two most important crossings.

DOI: 10.1021/jz900262w | J. Phys. Chem. Lett. 2010, 1, 425–428

that both vectors for the two crossings have a large component in proton transfer, indicating the efficient proton motion after crossings. Two photoproducts can be formed from this electronic state crossing. For the most stable product, the closed-shell electronic configuration is recovered via a second (backward) electron transfer, in this case from **A** to **B**. This electron transfer drives the proton to its initial position, linked to the nitrogen atom of glycine **B** in the GS, recovering the GS minimum-energy structure. A second high-energy species with CT character can be reached from the CT/GS conical intersection. The corresponding minimum on the potential energy surface lies very close to the conical intersection, with the proton linked to the CO group of peptide **A**. Because of the energetic and structural proximity of this CT intermediate to the CT/GS conical intersection, this CT metastable state is expected to evolve rapidly toward the electronic GS, leading to the formation of the initial minimum-energy structure.

In conclusion, the computed CASSCF//CASPT2 pathways for the photoinduced electron-driven forward and backward proton transfers in a minimal β-turn protein model support the energetic feasibility of this process between hydrogen-bonded amino acids. The overall mechanism provides an efficient way of recovering the initial closed-shell structure after electronic excitation to the lowest bright electronic state. This mechanism could play a significant role in nature, providing to hydrogen-bonded peptides stability against damage by UV radiation. This mechanism may have been especially important for the development of life on the surface of the primitive earth under extreme UV exposure providing to proteins a possible mechanism for photostability.

SUPPORTING INFORMATION AVAILABLE Complete citation of ref 21. Computational details. Cartesian coordinates for optimized structures. Minimum energy paths. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author:

*To whom correspondence should be addressed. Tel: +34-91885-2512. Fax: +34-91885-4763. E-mail: luisma.frutos@uah.es.

ACKNOWLEDGMENT This research has been supported by the projects CTQ2006-07643 and CTQ2009-07120 of the Spanish MEC, and CGO8-UAH/PPQ-3790 of UAH/CAM. M.M. is grateful to the UAH for a doctoral fellowship. U.S. acknowledges receipt of a doctoral fellowship from the CAM and ESF. L.M.F acknowledges a grant from the Alexander von Humboldt Foundation and a "Ramón y Cajal" contract from the MEC.

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(21) Complete Active Space Self Consistent Field (CASSCF) was used to determine minimum energy paths by computing analytical gradients, while Complete Active Space Perturbation Theory to Second Order (CASPT2) was employed to compute single-point correction to energy. CASSCF calculations were performed using Gaussian 03 (see http://www.gaussian.com/), while CASPT2 calculations were performed with MOLCAS, version 6.0 (see http://www.teokem.lu.se/molcas/). See Supporting Information for complete references.

(22) A $(Gly)_3$ structure was previously modelled in a β-turn conformation. After CASSCF geometry optimization, the glycine residue in the middle of the tripeptide was removed, and the remaining two residues were saturated, each one with a hydrogen atom pointing in the direction of the removed glycine backbone (H1 and H2 in Figure 1). The mentioned constraints are set during every geometry optimization. The complete active space was selected in order to include for each peptide unit all π orbitals (2π and $1\pi^*$) and one n orbital on the oxygen, with six corresponding electrons, resulting in a CAS(12,8) (12 electrons in 8 orbitals). See Supporting Information for computational details.

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(24) The DC vector measures the distortion of the system providing the maximum coupling between the electronic states involved in the crossing. The GD vector measures the distortion of the system leading to the largest variation of the energy difference between the two electronic states involved in the crossing.

DOI: 10.1021/jz900262w | *J. Phys. Chem. Lett.* **2010,** *1*, 425–428