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Characterization of the Relevant Excited States in the Photodissociation of CO-Ligated Hemoglobin and Myoglobin

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Received May 15, 2002

Iron porphyrins play a central role in biology as the active centers or prosthetic groups of hemoproteins,[1] for example, hemoglobin (Hb) or myoglobin (Mb). The first is found in red blood cells and is responsible for the oxygen transport, while the latter is important for the oxygen storage in aerobic muscle tissue. The functionality of Hb and Mb is based on the ability of the iron to reversibly bind diatomic ligands, for example, CO, NO, or O_2.

Upon irridiation of ligated Hb or Mb with light of a wavelength of about 570 nm (2.18 eV), the bond between the iron and the diatomic ligand dissociates. This photodissociation process has been extensively used experimentally to investigate ligand rebinding kinetics and protein relaxation dynamics.[2] Although a vast knowledge about the ground and excited states of Hb and Mb has been accumulated,[3] the nature of the excited states involved in their photodissociation processes is still not identified.[4]

The photodissociation of the CO-ligated species is a complicated process that starts with the excitation of the electronic singlet ground state into the Q_1 state, the energetically lowest $\pi-\pi^*$ transition which is well-known in porphyrins.[5] Recent experimental findings indicate vary rapid decay into a second excited singlet state (E_I) on a time scale of about 50 fs, which is assumed to be the photodissociation step.[4] The time scale of this decay step is too short to allow spin conversion. However, the excited unligated system decays then under spin conversion into its high-spin ground state on a longer time scale (picoseconds).[4] Since we are interested in the initial fast dissociation step, we concentrated on the relevant singlet excited states of Mb and Hb.

During the complete dissociation process, the porphyrin not only changes its spin state but also changes its geometry quite fundamentally. While the iron is strictly in the plane of the porphyrin in the singlet ground state of the CO-ligated Hb or Mb, it is now well established that the iron is about 0.4 Å above the porhyrin plane in the unligated high-spin species.[2] This movement from in-plane to out-of-plane is attributed to the larger size of the iron atom in the high-spin state and the limited space in the rigid porphyrin frame and does not affect the considered states.

In this communication we investigate the initial step of the photodissociation pathway of CO-ligated Hb or Mb by means of time-dependent density functional theory (TDDFT)[6] implemented in the QChem package of programs.[7] For this objective, we use a model complex (Figure 1), which was demonstrated previously to be sufficient to describe the electronic spectra of Hb and Mb.[5] The model complex consists of an iron porphyrin with an imidazole and a CO ligand at the iron. The imidazole ligand mimicks the proximal histidine, which is the only covalent link of the porphyrin to the protein frame in hemoglobin and myoglobin.

As a starting point we optimized the ground-state geometry of the C_s symmetric model complex with DFT using the B3LYP functional and the 6-31G basis set on first-row atoms and the Los Alamos effective core potential[8] on the iron atom. We obtained an equilibrium distance for the iron−carbon bond of 1.80 Å, which is in good agreement with crystallographic data on human CO-ligated hemoglobin (1.77 Å)[9] and myoglobin (1.82 Å).[10] Calculation of the vertical excitation energies of the model complex with TDDFT using the larger 6-311G* basis set and a Stuttgart−Dresden pseudopotential[11] for iron revealed that the lowest-excited singlet state (1 A′) corresponds to a $\pi-\pi^*$ transition strongly localized on the porphyrin ring. This state can be assigned to the Q_1 state of the porphyrin, and the calculated excitation energy of 2.46 eV is in reasonable agreement with the experimental value (2.18 eV).[12] According to our calculations, the 1 A″ state with an excitation energy of 2.48 eV corresponds to the Q_2 state, and the 6 A′ and 7 A″ found at 3.66 and 3.68 eV are the B_1 and B_2 states of the porphyrin representing the B (or Soret) band at 3 eV in the experimental electronic absorption spectrum of Hb.[12] A detailed assignment of the calculated states will be given elsewhere.

To simulate the dissociation process of CO-ligated Hb and Mb, we repeatedly elongated the iron−carbon bond, reoptimized the ground-state geometry at each point, and calculated the vertical excitation energies using the same methods as above. The resulting potential energy curves are displayed in Figure 2. As can be easily seen, the energetically lowest 1 A′ and 1 A″ states at the equilibrium geometry of the ligated complex are not repulsive along the Fe−C bond stretch reaction coordinate. These states rise in energy and cross the repulsive 5 A″ and 3 A′ states at an internuclear Fe−C distance of ca. 2 Å. From this distance on, the repulsive states are the energetically lowest-excited states of the systems, and the Fe−C bond dissociates easily. The transition from the 1 A′ and 1 A″ states into the 5 A″ and 3 A′ states is connected with a small energy barrier of only 0.12 eV.

The 5 A″ and 3 A′ states are strongly repulsive, and to understand this behavior we studied the nature of these states using attachment/detachment electron density plots.[13] The detachment density is that part of the ground-state density which is replaced by the attachment density to define the density of the excited state. The detachment density is shown in the middle, and the attachment density is placed at the right side of Figure 1. The electron density that remains unchanged between the ground and excited state is not shown in these diagrams. The isosurfaces shown are calculated for a 90% density enclosure for the 5 A″ state. A similar picture is found for the repulsive 3 A′ state.

Comparison of the detachment and attachment densities nicely explains the repulsive character of the 5 A″ state. While the detachment density is dominated by a bonding iron−carbon

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10.1021/ja026916i CCC: $22.00

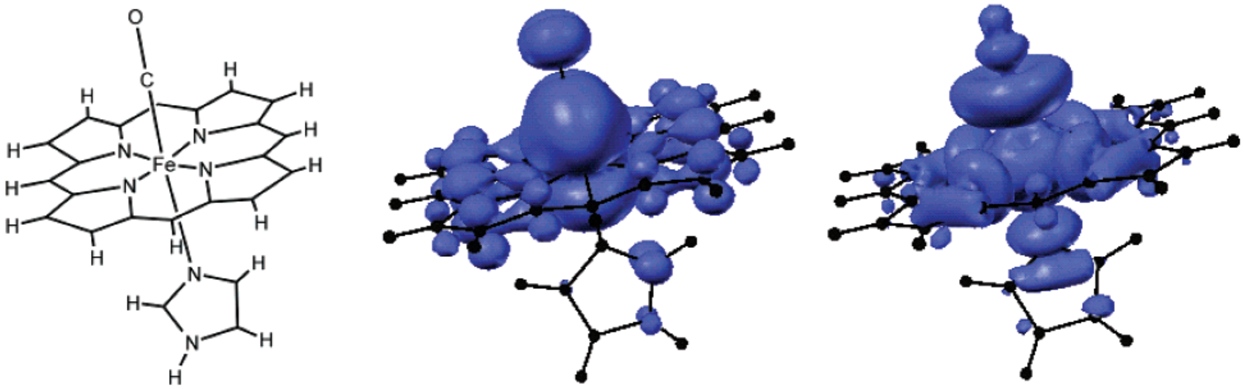

Figure 1. Structure of the myoglobin model complex (left) is shown together with the electron detachment density (middle) and electron attachment density (right) of the repulsive excited singlet state, $5\ A''$.

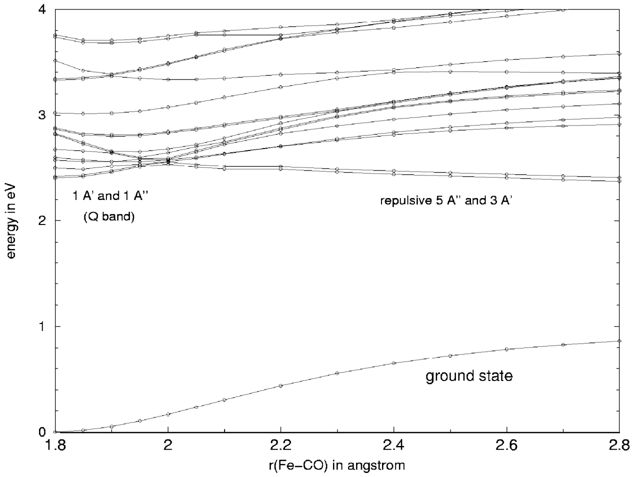

Figure 2. Potential energy curves of the lowest-excited singlet states of the model complex along the Fe−C bond stretch calculated at the level of TDDFT/B3LYP/6-311G*.

interaction, the attachment density has clearly antibonding character, which manifests itself by a node along the Fe−C bond. The bonding interaction between the iron atom and the CO ligand in the displayed detachment density can be understood in chemical terms as back-bonding from the iron d-orbitals into the π^* orbital of CO. The antibonding interaction in the excited $5\ A''$ state is attributed to the antibonding combination of these orbitals.

On the basis of our calculations, the initial step of the photodissociation process can be explained as follows. Upon excitation into the $1\ A'$ or $1\ A''$ state, which is accompanied by vibrational excitation, the excited system crosses the small energy barrier of about 0.12 eV into the dissociative $5\ A''$ and $3\ A'$ states. Hence, these states or one of these states corresponds to the state E_1 seen in the experiment.[4] Being in these states, the complex can decay via spin conversion into the high-spin ground state of the unligated system.

In the present work, we did not explicitly address the motion of the iron atom from the in-plane to the out-of-plane geometric configuration, but we checked the relevance of this coordinate at an Fe−C distance of 2.5 Å. However, it turned out that the iron still prefers to occupy the in-plane position in the singlet states. We expect the iron motion to play a role in the further decay of the excited singlet into the final high-spin state.

In our model calculations we optimized the ground-state structure along the Fe−C bond stretch and calculated vertical electronic excitation energies. In principle, one has to optimize the geometries of the excited states involved, but this is at present not possible for us. Consequently, the shown potential energy curves are not minimum-energy paths, but probably very close to them. In general, we do not expect the underlying physical picture to change when the true minimum-energy path is calculated, although this is a very desirable future refinement.

Acknowledgment. Andreas Dreuw gratefully acknowledges financial support by the Deutsche Forschungsgemeinschaft as an "Emmy Noether" fellow. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. M. Head-Gordon is on appointment as a Miller Research Professor in the Miller Institute for Basic Research in Science.

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JA026916I