

Scientific Report

Title: Study of Molecular Excited States

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1 Propose of the visit

Understanding energy and charge transfers in electronically excited molecules is of fundamental importance to photochemistry and photobiology [1]. Because of their biological importance, the DNA/RNA bases, have been the subjects of many spectroscopic and photophysical studies over the past four decades [2–4]. The most striking feature of the photophysics of the nucleobases is the ultrafast internal conversion that rapidly returns the photoexcited molecule to the ground state before chemical reaction in the excited state can cause significant UV photodamage, as the formation of a cyclobutyl ring between adjacent thymine residues on the same DNA strand to form an intrastrand thymine dimer (UV radiation of 200 to 300 nm). The goal of understanding how excess electronic energy evolves in DNA at the molecular level appears increasingly within reach. This energy relaxes via a multitude of pathways that include photon emission, nonradiative transitions to the ground or intermediate electronic states, and reactive decay to photoproducts. Highly efficient nonradiative decay to the electronic ground state (S_0) significantly lowers the rate of DNA damage, thereby reducing the workload of an organism's repair machinery [2].

On the other hand, the temporal and spatial binding of proteins on DNA has a great importance in the regulation of genome expression and maintenance. However, examining how the protein-DNA complexes assemble in living cells at the molecular level is still a challenging [5]. The use of UV laser as UV light source allows the number of photons required for crosslinking to be delivered in nano- or pico- or femtosecond intervals, extremely shortening the irradiation time and achieving higher crosslinking efficiency than conventional UV lamp, thus being well suitable for kinetic studies. Ultrashort laser pulses in the femtosecond range have proven to be effective tools in driving photochemical reactions: under the influence of light, electrons change their energy quantum states, followed by the breaking of existing chemical bonds or the formation of

new ones [6]. For the theoretical investigation of such complex processes one needs to consider a detailed analysis of vertical electronic excitations, excited state relaxation through the potential energy surfaces, and in some special cases charge- or proton transfers.

The purpose of my visit at the Organic Chemistry Department of University of Vigo was to focus on the study of the molecular excited states and their relaxation processes on the potential energy surface. During these days, we (together with Prof. Ángel R. de Lera) tried to understand the behavior of 5-benzyluracil and 6-benzyluracil's excited states and their relaxation.

2 Benzyluracil

Benzyluracil can be considered as a model system for thymine (DNA) - phenylalanine (protein) cross-linking [7]. The molecular system is built by two fragments: *a*) benzene for mimic the protein (phenylalanine) part and *b*) uracil for mimic the DNA (thymine) component, which are linked together by a CH₂ bridge. Two different conformers were obtained, depending where the CH₂ bridge was linked to the carbon atom sides of the uracil fragment: 5-benzyluracil and 6-benzyluracil (See figure 1).

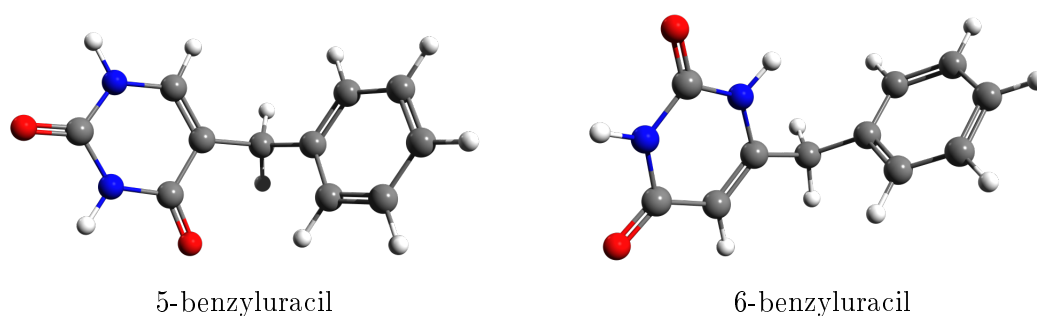


Figure 1. Two molecular conformations of benzyluracil.

According to the Sun [7] findings, the presence of CH₂ bridge is very important in order to have a considerable yield of new covalent bond formation. In the simple case when the CH₂ bridge were missing the yield of binary fragment formation was very low (For an UV irradiation of 144 hours, the yielding of bond formation was only 2%).

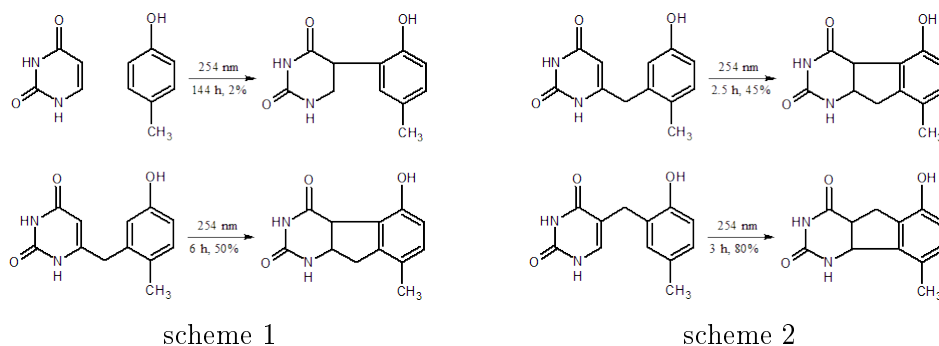


Figure 2. The reaction schemes of UV irradiation of benzene-uracil complex.

Considering an auxiliary CH₂ bridge between uracil and 4-methylphenol the efficiency of a secondary covalent bond formation between a carbon atom of uracil and another carbon atom

of benzene was considerably higher (See scheme 1 on Figure 2). Furthermore, if one consider different carbon atoms of the uracil fragment where the CH₂ bridge is linked, the efficiency of the secondary bond formation is not identical (See scheme 1 on Figure 2). We consider that this different behavior of 5- and 6-benzyluracil is strongly connected with the different excited state relaxation mechanisms.

Analyzing separately, the excited state of the benzene and uracil molecules which were already reported in the scientific literature [8,9] one should observe that the choose of the proper theoretical method is the key of the correct description of these systems. Based on these works, one can see that the multiconfiguration self-consistent method (MCSCF) could be adequate for describing different geometries and potential energy surfaces, while for the transition energies one need to use more performant methods like: multireference configuration interaction (MRCI) or the second order perturbation corrections for the MCSCF method (called CASPT2). In all cases, the crucial point is to define the proper complete active space (CAS) of the molecular orbitals. While in case of the individual subsystems to chose the correct CAS orbital domain is somehow a straightforward task, in case of the binary system it becomes more complicate, because is not obvious how many and which orbitals should be included in the CAS. Since, we are mainly interested in the $\pi \rightarrow \pi^*$ transition one need to include the π orbitals of the benzene aromatic ring as well as the double bonds of the uracil fragment. In this way, for the CAS, we obtain the (18 electrons, 13 orbitals) configuration. We also agreed that as a starting point one need to keep the whole (18,13) orbital configuration even if the excitation will occur only at one of the benzene or uracil fragments, because the orbital constrain could significantly influence the final results.

As a first step we have calculate the vertical excitation energy spectra for both 5- and 6-benzyluracil binary systems (see Figure 3) using the TDDFT/cc-pVTZ method:

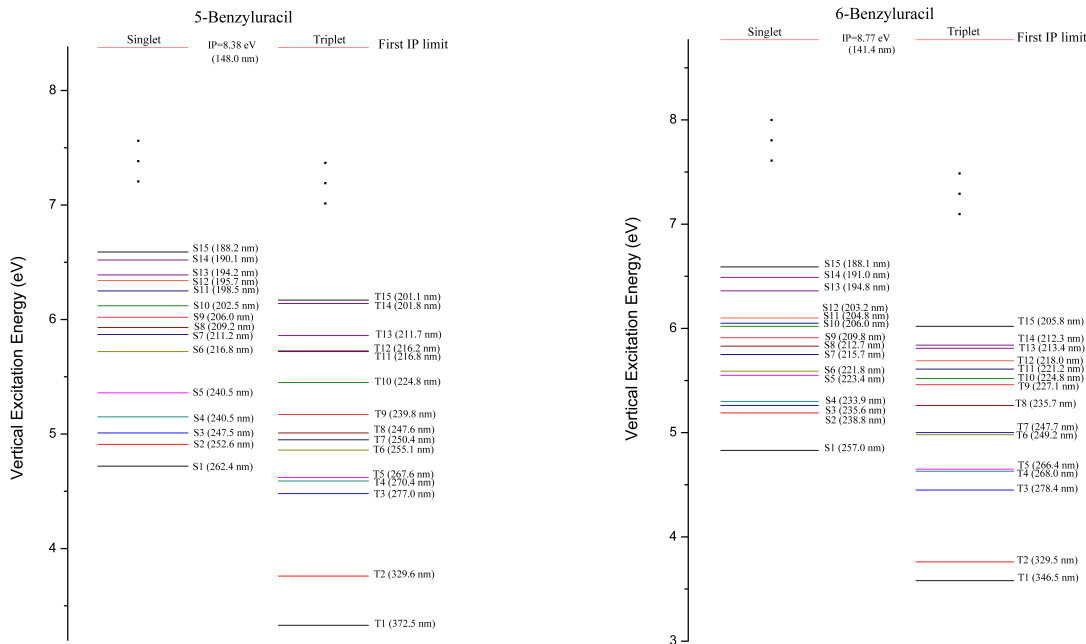


Figure 3. The vertical excitation energy levels of 5- and 6-benzyluracil.

The simulated UV absorbtion spectra is presented in Figure 4.

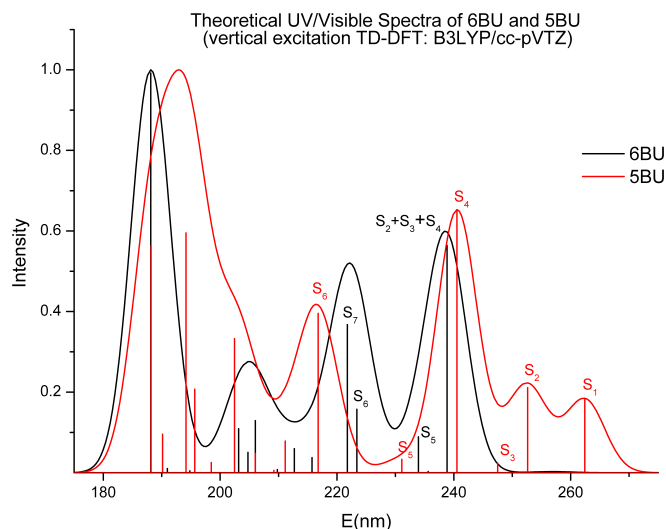


Figure 4. The UV absorption of 5- and 6-benzyluracil.

Analyzing the theoretical UV spectra one can see major differences between 5-benzyluracil (5BU) and 6-benzyluracil (6BU) excited state behaviour. 5BU has active S_1 and S_2 states (red line), while in case of 6BU the S_1 state (black line) has a very small oscillator strength (~ 0.0) and is missing from the absorption spectra as well as the next three 6BU states (S_2 , S_3 and S_4) are very close to each other. Considering the Sun findings [7] (see scheme 2 of the Figure 2) one can see that in the range of 254 nm 6BU has, forsooth, a weaker absorption than 5BU but this yield is not close to the zero value. Accordingly, we agreed that this fact must be confirmed by using other theoretical methods, like MCSCF or equation of motion coupled cluster theory (EOM-CC).

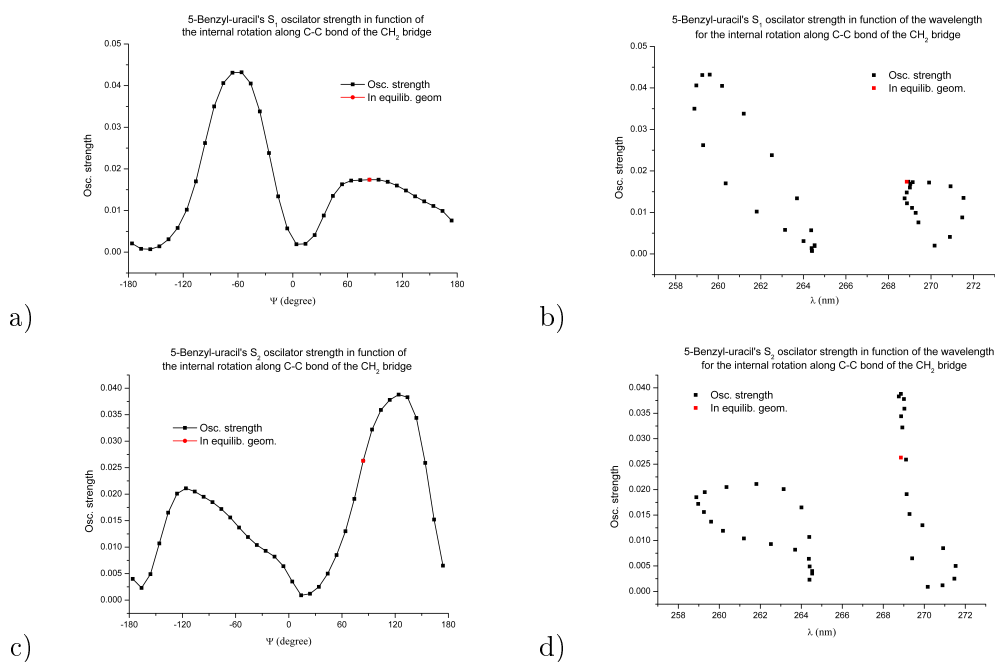


Figure 5. The oscillator strength and absorption wavelength of S_1 and S_2 excited states of 5BU in function of the internal rotation angle.

In the 5BU and 6BU systems the uracil and benzene fragments are linked by the CH₂ bridge and therefore one should have an internal rotation around two different C-C bonds. Analyzing the internal rotation dependence of the vertical excitation energy some important findings were obtained. Here we present the results only for S_1 and S_2 cases of 5BU system (See Figure 5). One can see that in the ground state equilibrium geometry (red point) the oscillator strength does not have the maximum value (see Figure 5a and 5c). On the other hand due to the internal rotation, the absorption band shows a significant band width (see Figure 5b and 5d).

In conclusion, during this visit, we analyzed the vertical excited states of 5-benzyluracil and 6-benzyluracil systems, which were chosen as a model system for DNA-protein crosslinking. Based on these preliminary results we define the proper theoretical methods, in order to describe the potential energy surface relaxation processes and the formation of the secondary covalent bond. We also agreed that the internal rotation plays an important role in the absorption of the radiation and in the vertical excitation of the studied molecular systems.

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