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COMMUNICATION

TD-DFT Study on the pH Related and Site-Specific Quenching Mechanism of 6-Formylpterin

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Abstract: In this communication, the pH related fluorescence quenching mechanism of 6-formylpterin (FPT) has been investigated by using time-dependent density functional theory (TD-DFT). The origin of fluorescence quenching of FPT in acid condition in the presence of acetate ion is originated from the site specific excited state proton transfer (ESPT) on the N1 site whereas the absence of ESPT in basic condition sustains FPT's fluorescence. This ESPT process is found to be modulated by hydrogen bonding patterns which not only controls the molecule's fluorescence but also coordinates its proton transfer site. Excitation process and relaxation process are further studied which give deeper insights into the origination of the proton transfer specificity.

AMS subject classifications: 65D18, 74E40, 78M50
KEYWORDS: Site-specific excited state proton transfer, pH related fluorescence quenching, time-dependent density functional theory.

Pterins exist extensively in living creatures and absorb strongly in UV-A (320-420 nm) region which are generally believed to serve as photo-oxidants [1]. Abundant researches have been made to investigate their photophysical and photochemical properties, most of which focus on the electron transfer processes between triplet pterins and guest molecules [2]. Theoretical studies into the optic properties of singlet excited state pterins, especially the origin of fluorescence changes when they interact with guest molecules are still quite rare.

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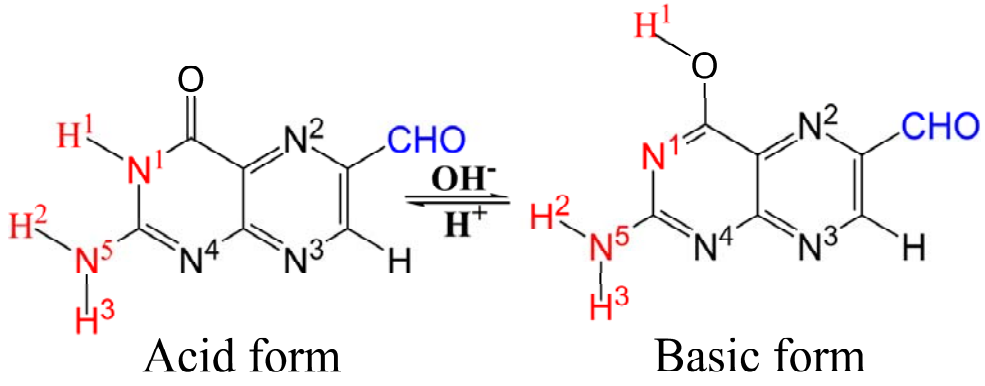
Pterins have different structures in different pH regions which give strong fluorescence emissions [3]. As revealed by Thomas and co-workers, pterin as well as its non-conjugated pterin derivatives namely 6-formylpterin (FPT), neopterin (NPT) and bio-pterin (BPT) exhibit interesting pH-related fluorescence quenching towards hydrogen accepting anions [4]. In acid condition (pH region 4.9–5.5), pterins' fluorescence is significantly quenched by acetate and phosphate whereas in basic condition (pH region 10.0–10.5), pterins' fluorescence remains unchanged. As is known, fluorescence quenching is always a phenomenon generated by ESPT which plays important roles in pterins induced photo-damages. Thus, having a clear understanding of this pH related quenching mechanism would be of biological significances.

Hydrogen bonding interaction is a fundamental and widespread weak force which, as put forward by Han et al, varies in different electronic states and modulates fluorescence properties of chromophores [5]. With the aid of hydrogen bonds, electronic excitation frequently triggers proton transfer from the hydrogen donors to hydrogen acceptors which always lead to strong fluorescence quenching [6]. In the case of pterins, the fluorescence quenching in acid condition is likely to stem from proton transfer process as pterins have several hydrogen donating sites (**Scheme 1**) meanwhile acetate and phosphate can serve as good hydrogen acceptors. The absence of fluorescence quenching in basic condition may also closely related to hydrogen bonds which still remains unknown.

Herein, we perform a full investigation on the pH related fluorescence quenching mechanism of FPT in the presence of acetate by using quantum chemical calculations. In this communication, we first clarified the origin of the pH related fluorescence quenching. Then, excitation and relaxation processes of FPT are analyzed to further look into this pH related quenching mechanism. Density functional theory (DFT) and time-dependent functional theory (TD-DFT) approaches are applied to study the ground and excited state geometries as well as proton transfer potential energy curves. The ground and excited state geometry

Scheme 1: Molecule diagrams of 6-formylpterin and the acid-base equilibrium in aqueous solution.



optimizations for all structures are performed at B3LYP/6-31+G(d,p) level of theory. A series of relaxed scan are performed along the possible proton transfer coordinates to generate potential energy curves. All the single point calculations are calculated at the B3LYP/6-311++G(d,p) theory level. Individual nitrogen atom's contributions to frontier molecular orbits are obtained using Ros-Schuit (SCPA) partition [7]. Solvent effects are considered (except for basis set superposition error corrections) for all the calculations in this article by means of polarizable continuum model [8] (PCM) with water as solvent. All the calculations are performed using Gaussian 09 program suite [9].

Hydrogen bonding patterns for FPT in basic condition is discussed first. For basic form FPT interacting with acetate, a basic model has been built by adding one acetate ion to FPT (BP-Ac). As shown in **Figure 1**, a hydrogen bond forms between the two components with a bond length of 1.709 Å. This hydrogen bond is not very strong with a bonding energy of -28.7 kcal/mol (**Table 1**). This indicates a moderate interaction between BP and acetate in the ground state. Potential energy curve (PEC) for this proton transfer along the transfer coordinates is built to check if this hydrogen bond will induce proton transfer. As plotted in **Figure 2a**, the energy rises as the hydrogen transfers from FPT to acetate. This process shows no saddle points which means the reverse transfer of hydrogen is barrier-less and spontaneous. Energy differential for this proton transfer is as large as 6.69 kcal/mol, which indicates that this process is unfavorable. For the case in the first excited state, the geometry structure is firstly optimized and plotted in **Figure 1b**. Also, the detailed hydrogen bonding energies are reported (**Table 1**). For the S_1 state, the hydrogen bond is stretched significantly from 1.709 Å to 3.115 Å. The hydrogen bonding energy drops sharply from -28.7 kcal/mol to -14.7 kcal/mol. Changes of the stretching vibrational frequencies of the bonds involved in the hydrogen bonding interactions can be used as measurements to probe the change of hydrogen bonding strength [10]. Herein, the stretching vibrational frequency for bond N5-H2 in S1 state is obtained which is significantly blue-shifted by about 637.40 cm^{-1}

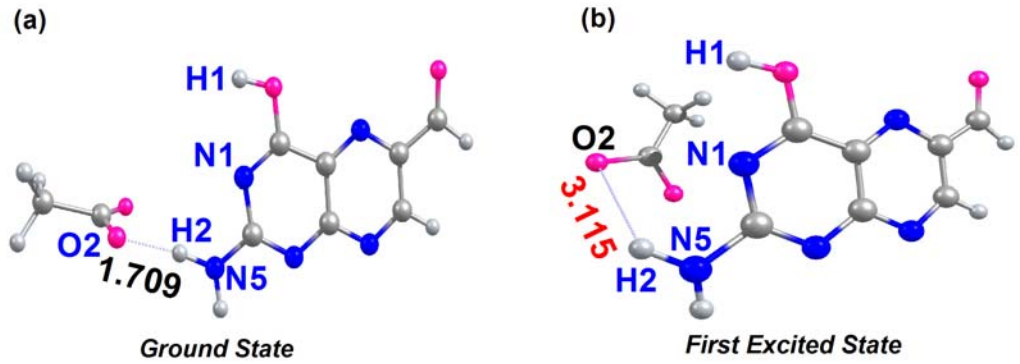


Figure 1: Hydrogen bonding patterns in S_0 (a) and S_1 (b) states for the basic model (BP-Ac).

compared to that of the ground state. These results all demonstrate that the hydrogen bonding interaction between BP and acetate in S_1 state is further weakened which will make the proton transfer even harder. We then perform a series of relaxed scan along the proton transfer coordinates to test the occurrence of ESPT process. Like the case of GSPT, the energy profile for the ESPT rises as the hydrogen transfers (**Figure 2b**). The energy differential is as large as 19.07 kcal/mol which indicates the absence of proton transfer in the first excited state.

From the above information, we find that both ground state proton transfer (GSPT) and ESPT are not likely to occur from basic form FPT to acetate. The hydrogen bonding interaction in basic solution is quite weak and the bonding pattern does not facilitate proton transfer. The absence of fluorescence quenching for FPT in basic environment stems from the lack of proton transfer.

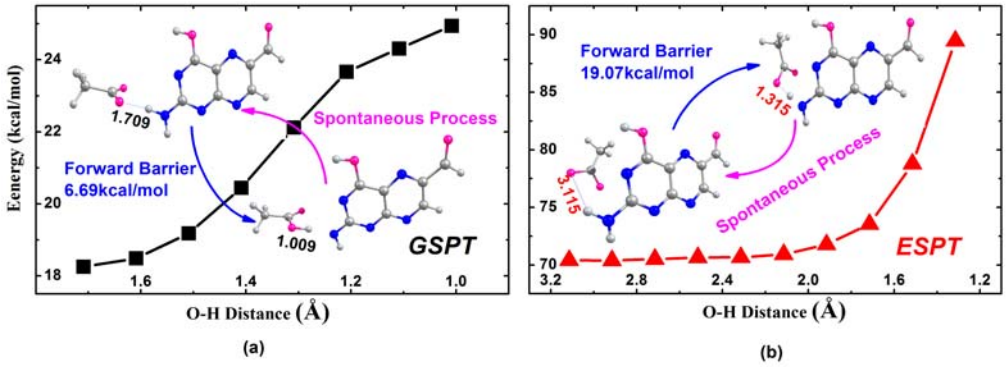


Figure 2: Potential energy curves for ground state proton transfer (GSPT) (c) and excited state proton transfer (ESPT) (d) processes.

Table 1. Calculated vibration and energy information for the two models[a].

Models	States	(N1-H1)*vib*	(N5-H2)*vib*	HB Energy
Basic Model	S_0	— —	2951.12	-28.7
	S_1	— —	3588.52	-14.7
Acid Model	S_0	2716.44	2969.32	-53.3
	S_1	2605.54	3027.69	-54.7

[a](N-H)*vib* stands for the vibrational frequency of the N-H bond (cm^{-1}). BSSE corrected hydrogen bonding energies (HB energy) are given in kcal/mol.

For the case of acid model, two strong hydrogen bonds are observed between FPT and acetate with a nearly planar geometry (**Figure 3**). The total hydrogen bonding energy in S_0

state and S_1 state is -53.3 kcal/mol and -54.7 kcal/mol (**Table 1**), respectively, which are much larger than those of the basic model. This indicates that in both S_0 and S_1 the interactions between acid form FPT and acetate are very strong. Thus, we can preliminarily expect that, the proton transfer processes may occur much easier in acid environment which induce the fluorescence quenching of pterin. As reported in **Table 1**, HB1 is shorter than HB2 in S_0 state. Besides, the stretching vibrational frequency of N5-H2 is blue-shifted by 252.9 cm^{-1} compared to that of N1-H1. These mean that HB1 is stronger than HB2 in the ground state and N1 may be the preferable proton transfer site in the ground state. Then, two PECs are plotted in **Figure 4a** and **4b**. As is shown in both cases, the energies rise as protons approach acetates. The energy differentials for proton transfer from N1 and N5 sites are 3.57 kcal/mol and 6.92 kcal/mol respectively. The transfer of H2 requires larger energy which stems from the weaker hydrogen bonding interaction between H2 and O2. However, both energy profiles show much easier reverse proton transfer feature, making the two GSPT pathways thermodynamically unfavorable.

As shown in **Figure** 3, in the first electronic excited state HB1 is slightly shortened to 1.620 Å whereas HB2 is slightly lengthened to 1.732 Å. The shifts of the stretching vibrational frequencies further support these changes. This enhancement of HB1 in S1 state may facilitate the ESPT process. The proton transfer processes in the first electronic excited state are then studied. By scanning along the transfer coordinates, potential energy curves for ESPT from N1 and N5 sites are plotted. As shown in **Figure 4c**, ESPT from N1 site has to overcome a barrier (2.31 kcal/mol) to reach a relatively stable product. This barrier is quite small which means that ESPT could happen on N1 site smoothly. For the case of N5 site (**Figure 4d**), the forward proton transfer barrier is 5.32 kcal/mol while that of the backward proton transfer barrier is relatively small (3.06 kcal/mol). This suggests that ESPT from N5 site is quite difficult which leads to an unstable product. Thus, N5 site is the unfavorable proton transfer site.

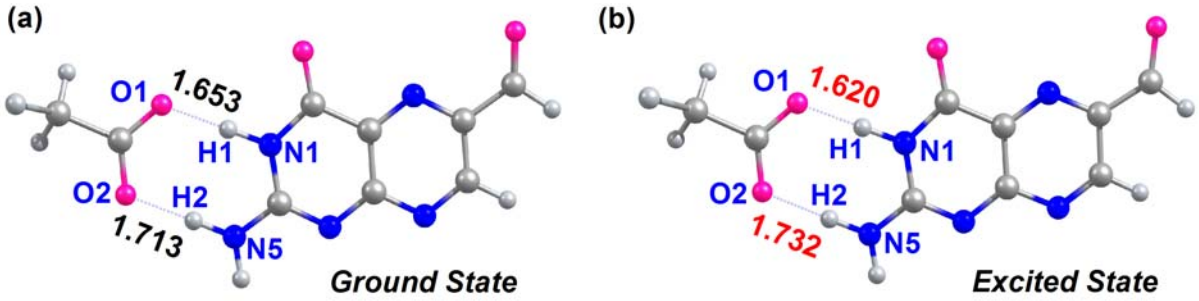


Figure 3: Hydrogen bonding patterns of acid form FPT in S_0 (a) and S_1 (b) states.

The ESPT process in the acid model shows interesting site specificity. N1 site is proved to be the favorable site for ESPT which probably stems from the enhancement of HB1 in the excited state. Hydrogen bonding patterns not only controls the fluorescent properties of pterin but also governs the priority of the proton transfer site. To give a more intuitive study on the origination of this site specificity, the excitation and excited state relaxation processes are investigated.

It is well known that photo-excitation can, sometimes, significantly change the charge distribution all over the molecule which affects its acid-base property [11]. This would change the hydrogen donating abilities of the acidic sites which consequently influences the molecule's hydrogen bonding patterns when interacting with hydrogen acceptors. Thus, the excitation process of AP-Ac is analyzed. As is shown, excitation at the first main absorption peak populates AP-Ac to the S_2 state which corresponds to the HOMO→LUMO transition. **Figure 5** plots the frontier molecular orbits which indicate an ICT process occurs from N5 site to N1 site. This excitation from the ground state (GS) to the S_2 Franck-Condon state (FC) may primarily change the molecule's acidic center. N1 and N5 atoms' contributions for the

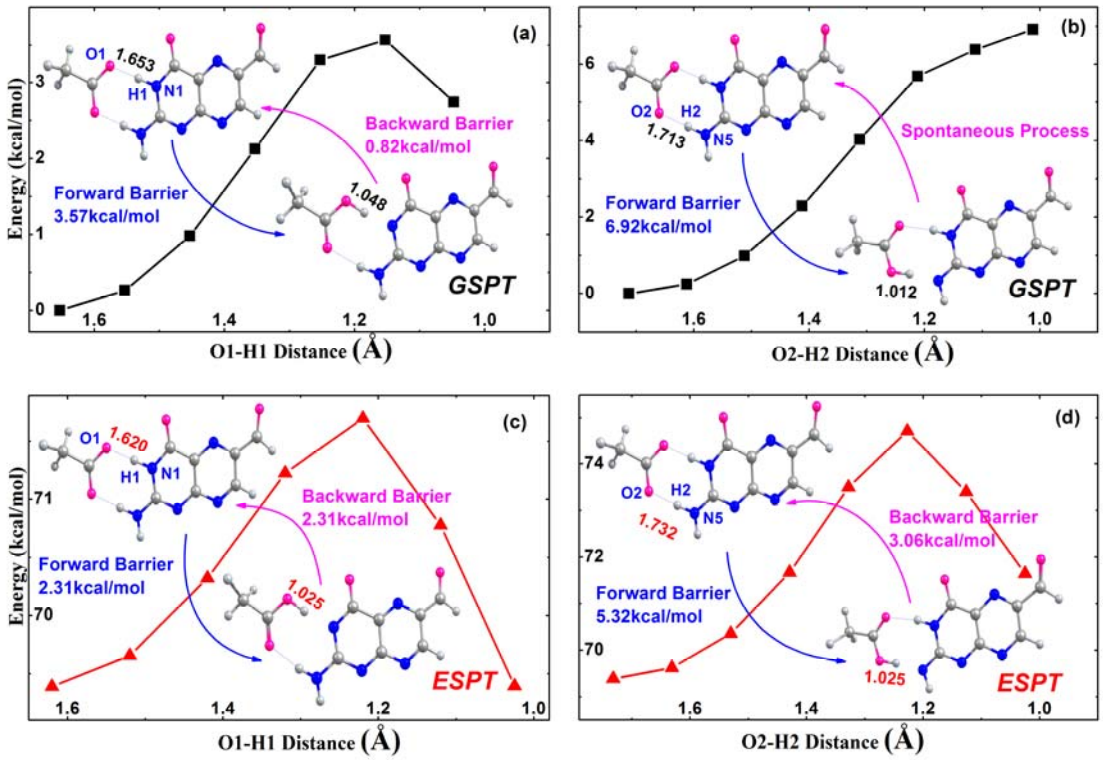


Figure 4: Potential energy curves for GSPT processes from N1 site (a) as well as from N5 site (b). Potential energy curves for ESPT processes from N1 site (c) as well as from N5 site (d). Energy barriers for proton transfer processes are given in blue fonts, reverse transfer energy barriers are given in magenta fonts.

molecular orbits are obtained to give a quantitative description of the ICT processes. As is shown, the contribution of N5 to the molecular orbit drops sharply (from 18.9% to 3.3%) soon after the photo-excitation whereas that of N1 increases to some extent (from 0.3% to 0.5%). This indicates N5 site becomes more electron-deficient. To give further quantitative information, the NBO charges on the two ESPT sites are investigated. It is clearly shown that, upon photo-excitation, the NBO charge on N5 become less negative whereas that on N1 site does not change much. All these mean that N5 site becomes more acidic immediately after excitation. However, this seems to conflict with the PEC results as N1 site is the favorable proton transfer site which probably due to the relaxation of the molecule structure. The relaxation process, during which the hydrogen bonding patterns adjust to the new formation, is thus investigated. From **Figure 5**, NBO charges on N1 sites do not change much during the relaxation process while that on N5 site drops back to -0.774. This means that the acidity of N5 site drops during the relaxation of the molecular bone and N1 site resume its acidity and leads to the enhancement of HB1 in S_1 state. This enhanced hydrogen bond further triggers the site-specific proton transfer which ultimately quenches FPT's fluorescence.

In this work, the pH related fluorescence quenching mechanism of 6-formylpterin in the presence of acetate anions has been fully investigated. We give a clear explanation that the experimentally observed fluorescence quenching in acid environment is originated from the site-specific excited state proton transfer from N1 atom of FPT to O1 atom of acetate anion. While in basic environment, this ESPT process is strongly prohibited by the weakening of

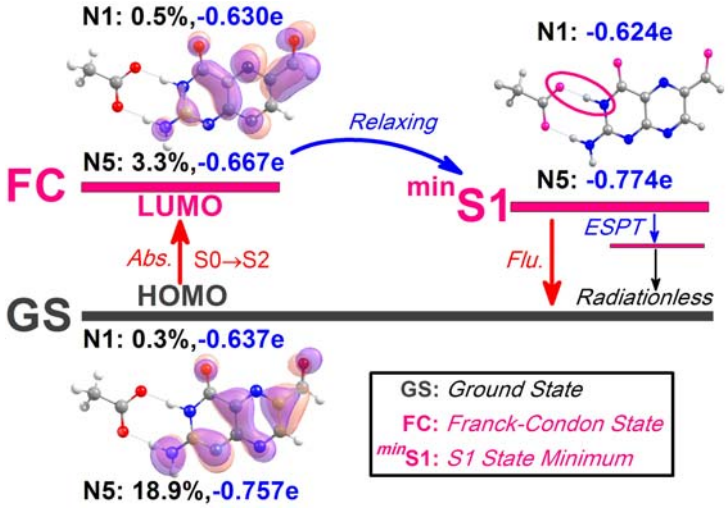


Figure : Excitation and relaxation processes for AP-Ac. Individual nitrogen atom's contributions to the HOMO and LUMO are reported in the form of percentage. NBO charge values are also given beside (blue fonts)

the intermolecular hydrogen bond in S_1 state which sustains FPT's fluorescence. Hydrogen bonds are found to play dominant roles which govern the occurrence of ESPT and consequently modulate the fluorescence of FPT. As revealed in this communication, the proton transfer process shows interesting site-specific due to the shift of hydrogen bonding patterns induced by photo-excitation. This hydrogen bonding pattern shifting is found to intuitively stem from the change of acidic center after photo-excitation and relaxation.

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