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Glutathione sensing mechanism of a fluorescent probe: Excited state intramolecular proton transfer and photoinduced electron transfer

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ABSTRACT

In this work, the fluorescence turn-on mechanism of glutathione probe azido-substituted 2-(2′-hydroxyphenyl) benzoxazole derivative (**AHBO**) has been thoroughly studied based on the density functional theory and time-dependent density functional theory methods. The constructed potential energy curves demonstrate that the proton transfer (PT) processes of the probe **AHBO** and the final product **AHBOG** after the glutathione-azide reaction are more likely to occur in the first excited state than in the ground state. Results of frontier molecular orbital analyses show that the S_1 state of **AHBO** is a complete charge-separation state, and the non-radiative acceptor-excited photoinduced electron transfer (a-PET, fluorophore as the electron acceptor) from the excited azide group to the 2-(2′-hydroxyphenyl)benzoxazole (HBO) would take place upon photoexcitation, which is responsible for the fluorescence quenching of the probe **AHBO**. Whereas, without the electron-rich azide group, the product **AHBOG** undergoes the excited state intramolecular proton transfer (ESIPT) in conjunction with the weak intramolecular charge transfer (ICT) process in the S_1 state. The absence of the a-PET and the two processes mentioned above provide explanations for the fluorescent enhancement observed with the product **AHBOG**.

1. Introduction

Biogenic thiols, such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy) are the key points for cellular redox homeostasis in the antioxidant defense systems and mitigate damage from free radicals and toxins [1,2]. However, abnormal levels of cellular thiols could induce a variety of serious diseases. For instance, GSH is the most abundant intracellular non-protein thiol and its abnormal level is directly associated with diabetes, neurodegenerative diseases, cancer, cystic fibrosis, HIV and aging [3–5]. Cys is an essential amino acid for protein synthesis and deficiency of Cys gives rise to slow growth, hair depigmentation, edema, lethargy, liver damage, neuronal degeneration and fat loss [6,7]. Meanwhile, elevated Hcy levels may result in various diseases such as brain atrophy, cardiovascular disease, abdominal aortic aneurysm, and Alzheimer's disease [8–10].

Consequently, sensitive and selective detections of these biothiols are of vital importance for the early diagnosis of diseases. In the recent past, fluorescence-based probe method, owing to its high sensitivity, simplicity of operation and non-invasiveness, has become a popular approach for thiols detection [11–13]. As an example, Yu et al. designed a near-infrared fluorescent probe for monitoring the changes of $ONOO^-$/GSH levels in cells and in vivo [12]. Although many fluorescent probes have been developed for biothiols, only several probes allow to distinguish among GSH, Cys and Hcy because of the similar structures and reaction activities of these biothiols, especially in living cells [14–17]. Among them, Xu and his co-workers developed an azido-substituted 2-(2′-hydroxyphenyl)benzoxazole (HBO) derivative fluorescent probe **AHBO** showing a selective turn-on response to GSH and Cys over Hcy, sulphide and other amino acids (see Scheme 1) [17]. There is one thing to emphasize that the probe **AHBO** is non-fluorescent; only with the addition of GSH and Cys to **AHBO** in CH_3CN/HEPES buffer the fluorescence intensity evidently increases at 488 nm, and other amino acids cause no detectable spectral change. Presently, the detailed mechanism of the fluorescence quenching of **AHBO** is still at a stage of exploration and requires to be further studied. In addition, some interesting questions are still waiting to be addressed more thoroughly, such as, since the probe **AHBO** is an azido-substituted HBO derivative and since HBO is a well-known type of proton transfer (PT)

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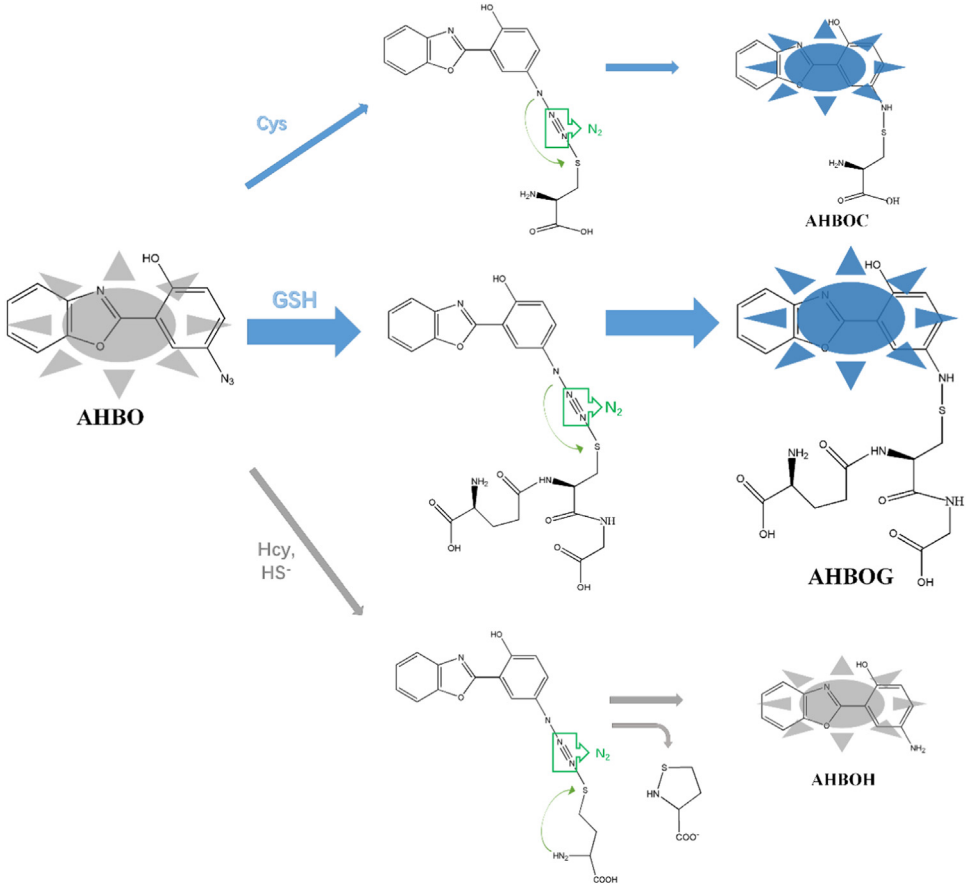

Scheme 1. The proposed reaction mechanism of the probe **AHBO** with GSH and Cys.

dye [18–20], so, whether the PT process of **AHBO** occurs in the ground state or in the excited state requires further research. Meanwhile, how does the PT process take place for the product **AHBOG**, **AHBOC** and **AHBOH** after the thiol-azide reactions (see Scheme 1) still leaves a question open.

For further understanding the sensing mechanism of the probe **AHBO** and these questions mentioned above, a theoretical calculation based on density functional theory (DFT) and time-dependent density functional theory (TDDFT) methods has been carried out to explore the properties of both the S_0 and S_1 states of the relevant molecules. That is, we optimized the structures of the S_0 and S_1 states and calculated the homologous potential energy curves (PECs) of the probe **AHBO** and the product **AHBOG**, **AHBOC** and **AHBOH** to explore the PT and the excited state intramolecular proton transfer (ESIPT) processes. Then the absorption/emission spectra and frontier molecular orbitals have been discussed to elucidate the fluorescent sensing mechanism.

2. Theoretical methods

All the theoretical calculations in the present contribution were performed with DFT and TDDFT methods using the Gaussian 16 program [21]. Geometry optimizations of the S_0 and S_1 states were completed with CAM-B3LYP [22] functional and the 6-31 + G(d,p) [23,24] basis set, which can describe the properties of the charge transfer excited state accurately [25]. Considering the experiments were conducted in CH_3CN/HEPES buffer, the integral equation formalism (IEF) [26,27] version of polarizable continuum model (PCM) [28] with the dielectric constant of acetonitrile (ε = 35.69) was employed in all calculations. In addition, vibrational frequency analysis has been performed to confirm that the optimized structures correspond to the local minima (no imaginary frequency). The S_0 and S_1 PECs have been scanned by constrained optimizations with the variable parameter of O-H bond length alone from 0.95 Å to 2.05 Å in steps of 0.05 Å.

3. Results and discussion

3.1. The optimization of structures and excited-state intramolecular proton transfer

It is well known that the molecular geometry of a state determines its photophysical properties. Thus, geometric structures of the S_0 and S_1 states of the probe **AHBO** and the product **AHBOG** have been optimized by the DFT/TDDFT methods at the CAM-B3LYP/6-31 + G(d,p) level and are shown in Fig. 1. As seen here, the O-H bond in the phenolic hydroxyl group is lengthened from 0.99 Å in the S_0 state of enol form **AHBO/AHBOG** to 1.02 Å in the S_1 state of enol form **AHBO/AHBOG**. The intramolecular hydrogen bond H···N is shortened from 1.77 Å/1.70 Å in the S_0 state of enol form **AHBO/AHBOG** to 1.63 Å in the S_1 state of enol form **AHBO/AHBOG**. These changes can implicitly demonstrate that there should have ESIPT process in the enol form **AHBO/AHBOG** [29–31]. As previously mentioned, the 2-(2′-hydroxyphenyl)benzoxazole (HBO) is a typical intramolecular PT molecule and then the PT or the ESIPT process could occur in **AHBO** and **AHBOG**. To make sure whether the probe **AHBO** and the product **AHBOG** involve the PT and ESIPT process via intramolecular hydrogen bond from O to N, the PECs on S_0 and S_1 states of **AHBO** and **AHBOG** are scanned by varying the O-H bond length in steps of fixed value, 0.05 Å (see Fig. 2). It is then found that the PT processes of **AHBO** and **AHBOG** in the ground state should overcome the barrier of 7.91 and 9.26 kcal/mol respectively and are endothermal. In the S_1 state, however, the rather low energy barriers of 1.66 and 2.14 kcal/mol indicate that the ESIPT processes of **AHBO** and **AHBOG** are thermodynamically