Journal of Luminescence 196 (2018) 406–424

Contents lists available at ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Molecular engineering of the photo switching in the ortho chromophores of the nanostructured green fluorescence protein

Hossein Roohi*, Roghayeh Nokhostin

Department of Chemistry, Faculty of Science, University of Guilan, Rasht, Iran

ARTICLE INFO

Keywords:
GFP chromophore
p-HBDI
o-HBDI derivatives
ESIPT
PBE0
Fluorescence emission
PES

ABSTRACT

Green fluorescent protein (GFP) has attracted wide attention as an efficient fluorescent probe for photophysical properties studies in the biological and biochemical sciences. In this work, the effect of different substituents (OMe, OEt, NO_2, Br, CN, and CF_3) in the para position of the phenyl ring in 4-(2-hydroxybenzylidene)-1,2-dimethyl-1H-imidazol-5(4H)-one (**o-HBDI**), an analogue of the core chromophore of the GFP, on their photophysical properties were explored in both gas and solution phases by using TD-DFT method at PBE0/6-311++G(d,p) and M06-2X/6-311++G(d,p) levels of theory. The potential energy surfaces (PESs) were evaluated along the reaction coordinate (RC = dOH) at the ground S_0 and excited S_1 states of the three **o-HBDI** derivatives composed of electron donating and accepting substitutes in both gas and solution phases. The structural, electronic and photophysical properties, the atomic charges and electron density at critical points were examined. The results show the photophysical properties of **o-HBDI** are dependent on substitution patterns and solvents. In contrast to S_0 state, the excited-state intramolecular proton transfer (ESIPT) process at S_1 state in these compounds is expected to be approximately less barrier height. The ESIPT process in all three solvents is predicted to be an exergonic reaction. Depending on the strength of the electronic accepting or donating of the substituents, a wide tunable range of the fluorescence emissions from 460 nm (visible blue light) to 642 nm (visible red light) was predicted in the solvent media. By comparing wavelengths, the maximum value of the stoke shift is observed for **CN-o-HBDI** and **TF-o-HBDI**. Chromophores composed of the electron donating substituents (OMe and OEt) represent greatest red-shift in non-polar solvent cyclohexane. Our computed results are in good agreement with the experimental observations and have the potential applications in manipulating and tuning photo-physical properties of the technologically and biologically important fluorescent protein.

1. Introduction

Green fluorescence protein (GFP) has been the topic of great interest in a variety of biological experiments, owing to its remarkable fluorescent properties [1–12]. The significance of fluorescent proteins and related technologies was recognized with the 2008 Nobel Prize in Chemistry [13]. The 2014 Nobel Prize that was awarded jointly to Betzig et al. for the development of super-resolved fluorescence microscopy is also directly relevant to fluorescent proteins [13]. GFP can be used to direct observation at intracellular processes, growth and spreading of cell clones, such as pathogenic bacteria and cancers, and mapping gene expressions, etc. [14]. With the discovery of GFP in 1962 [15,16], extensive experimental and theoretical studies have been started in identifying the structural features, the photo absorption properties and other factors which cause to the wonderful spectroscopic features of this protein.

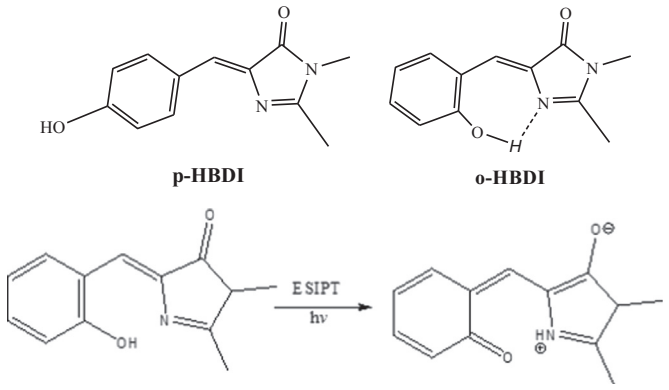
GFP consists of 238 amino acids [17,18], with the size (the molecular weight) about 27 kD and is structurally organized into a barrel containing 11 α-sheets and 4 β-helices [19–21]. Para-hydroxybenzylideneimidazolinone (**p-HBDI**, see Scheme 1) chromophore lies at the heart of GFP and is formed auto-catalytically by the cyclization and oxidation of the serine (Ser65), Tyrosine (Tyr66), and Glycine (Gly67) [22–24]. Wild-type GFP chromophore has two maximum absorption bands of about 395 nm for the neutral chromophore and 475 nm attributed to excitation of the anionic form [25].

Design, synthesis and chemical adjustment of new analogues of GFP chromophore and studying the excited-state proton transfer phenomena, still remains as a challenge. 4-(2-hydroxybenzylidene)-1,2-dimethyl-1H-imidazol-5(4H)-one (**o-HBDI**, see Scheme 1) is an analogue of the native GFP core **(p-HBDI)** that in recent years has been specially investigated. The main reason for this attention is the unique photochemical properties of the **o-HBDI** [26–33].

The **o-HBDI** possesses ESIPT ability [27,34] through the formation of a seven-membered ring intramolecular hydrogen bond. As can be

* Corresponding author.
E-mail address: hroohi@guilan.ac.ir (H. Roohi).

https://doi.org/10.1016/j.jlumin.2017.12.056
Received 21 July 2017; Received in revised form 29 November 2017; Accepted 26 December 2017
Available online 27 December 2017



Scheme 1. Structural isomers of **p-HBDI** and **o-HBDI** and enol to keto tautomerization in GFP chromophore.

seen in Scheme 1, the enol form of **o-HBDI** is converted to keto one upon photothutomerization process. Therefore, this chromophore, curiously emulating the natural excited-state proton transfer reaction, that determines photo-physical properties of wild-type GFP in vivo, produces an ideal sample for further extensive study [35–43]. Recently, **o-HBDI** and its analogues were analyzed and photophysics of **o-HBDI** in the ground and exited states were probed [13]. Based on ultrafast spectroscopic techniques, proton transfer cycle includes: ESIPT→cis-trans transisomerization→deprotonation→ground-state reverse. After excitation, **o-HBDI** is exposed to an ultrafast ESIPT from the hydroxyl to the imidazole group, in less than 25 fs. Only a small part of the ESIPT product (approximately 5%) will decay to trans tautomer at the ground, which in turn, makes an anion type in CH_3CN after being deprotonated [27]. The design, synthesis, luminescent and photo-physical properties of some **o-HBDI** analogues of GFP chromophore have been reported by Chuang et al. [32]. They observed all analogues in solid and nonpolar solvents exist principally as *Z* conformers and demonstrate a seven-membered ring intramolecular hydrogen bonding. The results revealed that photo-physical properties of **o-HBDI-Z** depend on substitution patterns and solvent. Cui et al. computed photo-dynamics of 4-(2-hydroxybenzylidene)-1H-imidazol-5(4H)-one (**o-HBI**) using static ab-initio, density functional calculations and nonadiabatic dynamics simulations [44]. According to this static calculation, the transition from S_0 to S_1 states of **o-HBI** is accompanied by charge transfer. ESIPT, following the induction, occurs very quickly (on the fs scale). The quantum yield of cis-trans isomerization in **o-HBI** is low (less than 5%). Besides, results showed internal conversion in **o-HBI** is ultrafast and therefore the trans conformer is not produced by an excited-state isomerization. Since increasing emission quantum yields are important for advancing of lighting applications, recently, a chemical sample of **o-HBDI** with significant fluorescence emission was synthesized [30]. Hsu et al., knowing the fundamental role of structural rigidity in emission quantum yields, began to experimentally study the locked conformers **p-HBDI** and **o-HBDI** chromophores. The ortho-hydroxyl GFP chromophores, showed considerable enhancements in the quantum yield of fluorescence emission (for example, $\phi = 0.18$ for **o-LHBDI**). Liu et al. investigated the factors of increasing the quantum yield of fluorescence emission of **o-LHBI** and behavioral differences of **o-LHBI** with locked conformers **p-HBDI (p-LHBI)** by Semi empirical OM2/MRCI simulations and CASSCF, MS-CASPT2 and DFT calculations [45]. They as well, examined the influence of locked GFP chromophore on ESIPT reactions. Chemical locking only affected the photo physics of the ortho-hydroxyl GFP chromophores and the excited-state deactivation induced by ultrafast cis-trans isomerization. Eventually fluorescence quantum yields in para substituted GFP chromophores remain low. Recently, an intensive attempt is underway aiming at observing the dual fluorescence in GFP chromophore. Chatterjee et al. observed the dual fluorescence emission in GFP chromophore (p-cyclicamino o-hydroxy benzimidazolidinone, **CHBDI**), for the first time [33]. Moreover, the study showed the direct effect of the functional group modification on the optical behavior difference of GFP chromophores.

The transfer of proton underpins many fundamental life processes, therefore a better understanding of various aspects of this reaction is necessary [46]. GFP is a sample of biological system with the ability to perform proton transfer between proton acceptor and proton donor groups. Actually, the excited-state proton transfer (ESPT) plays a fundamental factor in the exclusive optical behavior of green fluorescent protein. Certainly, a comprehensive understanding the effects of chemical modification on the excited-state dynamics is important to design new GFP analogues with better fluorescence emission [33].

Fine tuning the proton transfer emission can be achieved via chemical derivations of the **o-HBDI**. In continuation of our previous studies on ESIPT process [47–50], In this work, the effect of different functional groups (OCH_3, OC_2H_5, NO_2, Br, CN, and CF_3) (see Fig. 1) in the para position of **o-HBDI** on the mechanism of the ESIPT and the ability of dual fluorescence in both gas and solution phases were investigated. A clear picture of the fluorescence decay channel and the ESIPT process, using the potential energy surfaces (PESs) in the ground and excited states on **o-HBDI** analogues were depicted. Finally, the H-bonding interaction along the proton transfer through Natural Bond Orbital (NBO) [51] and Atoms-In-Molecules theory (AIM) were probed [52–54].

2. Computational details

The ground state (S_0) and the lowest singlet excited-state (S_1) geometry of the **o-HBDI** and six derivatives were optimized using DFT methods (PBE0 and M06-2X for S_0) and (PBE0-TD and M06-2X-TD for S_1) [55–58] in conjunction with the 6-311++G(d,p) basis set. The stationary points on the S_0 and S_1 potential energy surfaces (PESs) of **o-**

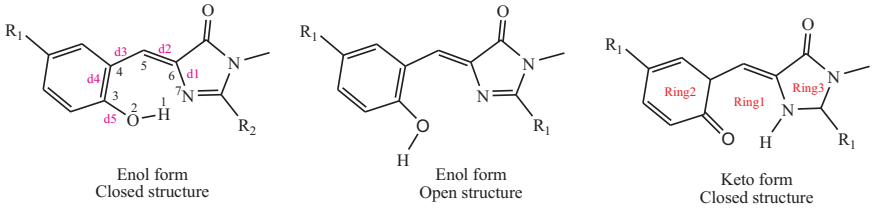


Fig. 1. Closed and open structures of o-HBDI, OMe-o-HBDI, Br-o-HBDI, CN-o-HBDI, NO_2-o-HBDI, TF-o-HBDI and OEt-o-HBDI.

	R1	R2
o-HBDI	H	CH_3
OMe-o-HBDI	OCH_3	CH_3
Br-o-HBDI	Br	CH_3
CN-o-HBDI	CN	CH_3
NO_2-o-HBDI	NO_2	CH_3
TF-o-HBDI	CF_3	CH_3
OEt-o-HBDI	OC_2H_5	CH_3