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First Theoretical Evidence of Blue-Shifted Energy Transfer in Bioluminescence

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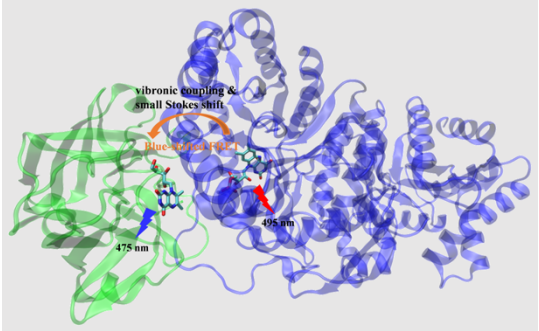
Abstract: Energy transfer (ET) complex is not rare in bioluminescence. Usually, the ET occurs from the donor with higher emission energy to the acceptor with lower absorption energy. However, a blue-shifted ET is observed in the bioluminescence (BL) of *Photobacterium phosphoreum* (*PP*). The luminophore, 4a-hydroxy-5-hydro-flavin mononucleotide at the first singlet excited state (S_1-HFOH), in solitary *PP* luciferase (*PP*Luc) emits light at 495 nm. When a proportional concentration of lumazine protein (LumP) with a substrate of 6,7-dimethyl-8-ribityllumazine (DLZ) is introduced, the emission wavelength changes to 475 nm, accompanied by a 2.1-fold enhancement in intensity. The blueshift is only an observation, whose ET mechanism has not been uncovered over fifty years of research. In the present article, we evidenced that the ET process occurs via a Förster resonance energy transfer (FRET) mechanism by protein-protein docking and molecular dynamics (MD) simulations. Moreover, utilizing the combined quantum mechanics and molecular mechanics (QM/MM) method, we calculated the FRET rate and fluorescence quantum yield. The small Stokes shift of DLZ as well as the strong vibronic couplings of HFOH allow the blue-shifted FRET process. The calculated FRET rate is larger than the radiative and non-radiative decay ones of S_1-HFOH, and the fluorescence quantum yield of S_1-DLZ is higher than the one of S_1-HFOH, which clearly explains the experimentally observed enhancement of the emission intensity. Simultaneously, the blue-shifted FRET mechanism firstly interpreted that the wild-type *PP* emits 475 nm BL rather than 490 nm one as the other species of bioluminescent bacteria do. This first-time deep investigation establishes a theoretical research paradigm for the theoretical study of ET and holds significance in color regulation in the BL field.



Key words: Bacterial bioluminescence, Blue shift, FRET, QM/MM, MD.

1. Introduction

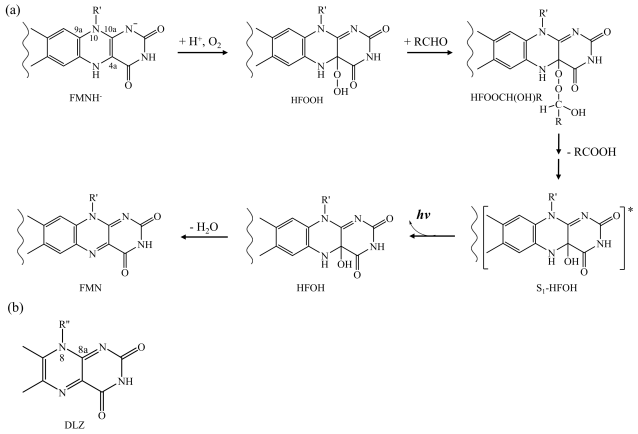
The phenomenon of bioluminescence (BL) is widespread in marine and terrestrial organisms [1], and has been applied in fluorescent probes, medical research, environmental monitoring and so on [2-5]. Among them, the bacterial BL is specially used for real-time in vivo imaging due to the low cost and simple operation [6]. It was widely accepted that all bioluminescent bacteria emit BL through the same mechanism in the bacterial luciferase [7-9]. As described in Scheme 1a, the reaction process includes five stages: the reduced flavin mononucleotide anion ($FMNH^-$) reacts with oxygen to produce 4a-hydroperoxy-5-hydro-FMN (HFOOH); HFOOH reacts with the long-chain aliphatic aldehyde (RCHO) to yield 4a-peroxyhemiacetal-5-hydro-FMN [HFOOCH(OH)R]; HFOOCH(OH)R dissociates to generate the first singlet excited state (S_1-state) 4a-hydroxy-5-hydro-FMN (S_1-HFOH) and carboxylic acid (RCOOH); the bioluminophore S_1-HFOH deexcites to HFOH and emits light [10, 11]; HFOH dehydrates and transforms into flavin mononucleotide (FMN). Most of the known over 30 species of bioluminescent bacteria emit BL of wavelength around 490 nm [12,13]. However, the BL wavelength of *Photobacterium phosphoreum* (*PP*) is 475 nm [9,10]. The BL of 475 nm wavelength is not emitted by the S_1-HFOH in the *PP* luciferase (*PP*Luc), but by the S_1-state 6,7-dimethyl-8-ribityllumazine (DLZ) [14] [see Scheme 1(b)] in an antenna protein, Lumazine Protein (*PP*LumP) [15-17]. The experiments found that S_1-HFOH in solitary *PP*Luc has a maximum BL wavelength at 495 nm [9, 18]. When *PP*LumP is added, the maximum emission wavelength changes to 475 nm [19] accompanied by an enhanced intensity [18]. The extent of intensity enhancement is related to the concentration of the added *PP*LumP. Specifically, when the concentration of the added *PP*LumP is equal to that of *PP*Luc, the BL intensity increases by approximately 2.1-fold (as shown in Table 3 of ref 16) [16]. Lee et al. believed that the 20 nm blueshift is caused by an energy transfer (ET) taking place from HFOH in *PP*Luc to DLZ in *PP*LumP [20]. After more than fifty years of research, the ET mechanism is still unclear. It is difficult to obtain the complex structure of *PP*Luc and *PP*LumP by using the X-ray diffraction or nuclear magnetic resonance (NMR) spectroscopy [10], since the interaction time between energy donor protein (*PP*Luc) and energy acceptor protein (*PP*LumP) is very short.

The purpose of the present study is to investigate the blue-shifted ET mechanism with the aid of computational chemistry. We comprehensively applied the protein-protein docking, molecular dynamics (MD) simulations and combined quantum mechanics and molecular mechanics (QM/MM) calculations to thoroughly understand the ET mechanism. By reason of mechanistic similarity, the current research is helpful to understand the photosynthesis process of plant [21] and apply the technology of bioluminescence resonance ET (BRET) [22].

2. Theoretical Method

2.1 Computational systems

Originally, we intended to study the ET from HFOH in *PP*Luc to DLZ in *PP*LumP. However, both the crystal structures of *PP*Luc and *PP*LumP have not yet been obtained experimentally. The amino acid sequence of *Photobacterium kishitanii* LumP (*PK*Lump, PDB: 3A3G)



Scheme 1. (a) The rough reaction process in bacterial BL. R and R′ represent long-chain aliphatic hydrocarbons and $CH_2(CHOH)_3OPO_3H_2$ respectively. (b) The structure of 6,7-dimethyl-8-ribityllumazine (DLZ). R″ represents $CH_2(CHOH)_3CH_2OH$.

[23] is identical to that of *PP*LumP (Figure S1). The amino acid sequence of α subunit of *Vibrio harveyi* luciferase (*VH*Luc, PDB: 3FGC) [24] has 59.6% identity and 74.1% similarity with that of *PP*Luc [Figure S2 (a)]. For β subunit, the amino acid sequence of *VH*Luc has 47.6% identity and 67.5% similarity with that of *PP*Luc (Figure S2 (b)). Additionally, the predicted structure of *PP*Luc using the SWISS MODEL server [25-27] is found to closely resemble the structure of *VH*Luc [Figure S2 (c)]. As experiments evidenced, *PP*LumP forms 1:1 complex with VHLuc [28]. The addition of *PP*LumP shifts the BL in *VH*Luc at 485 nm to 475 nm [29]. Therefore, it is reasonable to replace *PP*Luc by *VH*Luc (3FGC) and *PP*LumP by *PK*LumP (3A3G). In other words, in this article, to uncover the ET mechanism in *PP* BL, we should study the ET from HFOH in *PP*Luc to DLZ in *PK*LumP, but for computational convenience, we reasonably replace the study on this ET by the study on the ET from HFOH in *VH*Luc to DLZ in *PK*LumP.

2.2 *VH*Luc-*PK*LumP docking and MD simulations

Before MD simulations, we used the ModLoop server to model the missing loop residues 284-289 in *VH*Luc and 87-93 in *PK*LumP [30, 31]. Subsequently, 10 ns MD simulations were performed for HFOH-*VH*Luc and DLZ-*PK*LumP, respectively. Finally, in the equilibrated region of the MD trajectories, the equilibrated HFOH-*VH*Luc and DLZ-*PK*LumP structures were used to perform protein-protein docking by the PatchDock [32]. The FireDock was used to refine the docked models [33]. Among the top ten protein-protein complex, one with the closest distance between DLZ and HFOH (about 18 Å) was selected for subsequent 100 ns MD simulation. For convenience, we refer to the obtained protein-protein complex as the *VH*Luc-*PK*LumP. The RMS derivation analyses were presented in Figures S3, S4 and S5. The MD simulation is in the periodic boundary conditions at constant temperature of 300 K and pressure of 1 atm via the Amber16 package [34]. The time step was 2 fs. For the details of protein-protein docking and MD simulation, see SI.

2.3 QM calculations