Effects of water solvation on absorption spectra of firefly oxyluciferin

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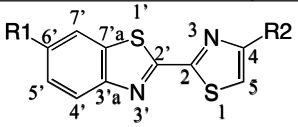
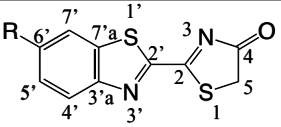
For the understanding of firefly bioluminescence, [1] it is critical to obtain spectroscopic characteristics individually for the emitters, or the oxyluciferin and its conjugate bases, although only a mixture of them is available experimentally. In this context, we theoretically determined relative molar concentration among the emitters in aqueous solutions under various pH conditions and successfully assigned the observed spectra.[2] The next step is to compare our theory with available experimental data[3] regarding the spectral shape of each chemical species to check reliability of our theory and, in addition, to investigate contribution from the molecular vibrations and solvation on the spectra.

Thus, in this study, first, we elucidated the vibronic effect on the absorption and fluorescence spectra of the chemical species in Table 1.[4] While the energies of the excited states were calculated with the time-dependent density functional theory (TD-DFT), the solvent effect was incorporated using the polarized continuum model (PCM). The Franck-Condon factors between the ground and first excited states were evaluated using Barone's method.[5] The calculated absorption energies are slightly lower than the experimental ones. The calculated spectral shapes well reproduce the experimental shapes except for the case of phenolate-keto. The calculations for phenolate-keto gave the very sharp spectral peak, different from the experimental broad shape.

One of the reasons for this difference is the lack of hydrogen bonding effects in our calculations with the PCM model. Then, the effects of hydrogen bonding interactions were clarified through a theoretical study on the stability of the oxyluciferin anions with explicit water molecules using the first-principles molecular dynamics (FPMD) simulations.[6] Fig. 1 shows the snapshots from the FPMD simulations for phenolate-keto, phenolate-enol and

Table 1. Structures and names of oxyluciferin and its conjugate bases

R1	R2		R	
OH	OH	enol	OH	keto
OH	O^-	enolate	O^-	phenolate-keto
O^-	OH	phenolate-enol		
O^-	O^-	$OxyL^{2-}$		

enolate. These simulations showed that phenolate-enol anion is more stable than phenolate-keto anion because of the unique features of the static and dynamical hydration structures, which are difficult to capture using the PCM solvation model.

Next, the absorption spectra of aqueous oxyluciferin anions were derived using the structures obtained from the FPMD simulations at room temperature for each isomeric form, in order to account for the effects of vibrations of oxyluciferin anions and dynamical fluctuations of their hydration structures. For this purpose, we performed QM/MM calculations with the TD-DFT method using an explicit solvent model. Fig. 2 shows the instantaneous absorption spectrum thus obtained for phenolate-keto. The theoretical width of the spectrum peak is in good agreement with the experimental one. This suggests that the experimental absorption spectrum is best reproduced with an explicit solvation model that successfully incorporates the hydrophobic nature of oxyluciferin anions.

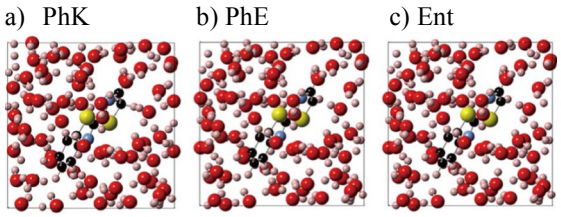


Figure 1. Snapshots from the FPMD simulations for (a) phenolate-keto (PhK), (b) phenolate-enol (PhE) and (c) enolate (Ent).

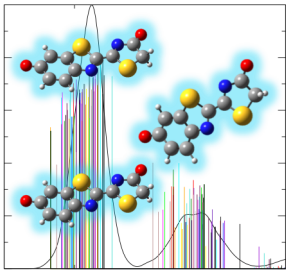


Figure 2. Calculated absorption spectrum for phenolate-keto in aqueous solution.

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