pH effect study for exploring enzyme mechanisms of bioluminescence reaction of bacterial luciferase

Ruchanok Tinikul[1], Chutintorn Suadee[1], Jeerus Sucharitakul[2], Pimchai Chaiyen[3]

[1]Department of Biochemistry and Center for Excellence in Protein and Enzyme Technology, Faculty of Science, Mahidol University, Bangkok, Thailand,

[2]Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University, Henri-Dunant Road, Patumwan,Bangkok 10300, Thailand.

[3]Department of Biomolecular Science and Engineering, School of Biomolecular Science & Engineering, Vidyasirimedhi Institute of Science and Technology (VISTEC), Rayong, Thailand

Bacterial luciferase (Lux) is an enzyme catalyzing the light emitting reaction using reduced FMN, long chain aldehyde and oxygen as substrates. Due to the cheaper price of Lux substrates, it has long been attractive to be used in bioreporter applications. The reaction mechanism of Lux occurs through the generation of a reactive flavin-oxygen adduct called C4a-(hydro)peroxyflavin. The protonation state of C4a-(hydro)peroxyflavin to be C4a-peroxyflavin or C4a-hydroperoxyflavin is very crucial for Lux reaction. It is still unknown about how Lux active site environment controls the protonation status of this flavin adduct. Therefore, in this research, the effects of pH on protonation status of reactive C4a-(hydro)peroxyflavin intermediate was investigated. When the intermediate spectra were monitored in WT enzyme, the intermediate with λ_{max} 385 nm was detected at low pH (pH 6.5) and this was shifted to shorter wavelength (λ_{max} 375 nm) at high pH (pH 10.5). The results suggested that an intermediate with longer λ_{max} (385 nm) may represent the protonated form of C4a-hydroperoxyflavin and shorter λ_{max} (375 nm) may represent deprotonated form of C4a-peroxyflavin. When the same experiment was carried out in His44Ala and His44Asn mutants, only intermediate with λ_{max} 385 nm was observed throughout pH 6.5-10.5, indicating that only protonated form of the intermediate exists in His44Ala and His44Asn mutants. The rate constant for the WT C4a-(hydro)peroxyflavin intermediate decay also decreased upon the pH increment, while no significant change of the decay rate was observed in the mutants. This indicates the crucial function of His44 residue in controlling the intermediate protonation status in Lux reaction. Knowledge gained from this study is useful for further improving light yield of Lux for bioreporter applications.

Keywords: Bioluminescence; Bacterial luciferase; pH effect; C4a-(hydro)peroxyflavin; Protonation status