3P248 青色光受容 BLUF タンパク質 TePixD の光反応ダイナミクス
○田中 啓介¹、中曽根 祐介¹、岡島 公司²、徳富 哲²、池内 昌彦³、寺嶋 正秀¹
¹京大院・理、²阪府大院・理、³東大院・総合文化
Photoreaction Dynamics of a Blue Light Receptor BLUF Protein TePixD
Keisuke Tanaka(1), Yusuke Nakasone(1), Koji Okajima(2), Satoru Tokutomi(2), Masahiko Ikeuchi(3) and Masahide Terazima(1). (1: Department of Chemistry, Graduate School of Science, Kyoto University; 2: Research Institute for Advanced Science and Technology, Osaka Prefecture University; 3: Department of Life Sciences, The University of Tokyo)

TePixD is a photosensory protein from a thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1, which has a flavin-binding blue light receptive domain called BLUF (sensors of Blue Light Using FAD). BLUF proteins show the common features in their photo-induced reactions characterized by a reversible ~ 10 nm redshift of the UV-Vis absorption spectra and a downshift of flavin-C4=O band in the FTIR spectra, which are involved in a rearrangement of a hydrogen bonding network between the FAD and surrounding amino acid residues. These results, however, reflect only the structural change in the vicinity of the chromophore, and cannot give the information about the dynamic conformational changes associated with the signaling state formation. In this study, the photoreaction dynamics of TePixD were investigated by using the time-resolved transient grating and transient lens techniques. After the photoexcitation, several spectrally silent reaction dynamics were detected: changes in the partial molar volume and the diffusion coefficient of the protein with time constants of 40 μs and 2 ms, respectively. The dynamics of the diffusion change is an interesting observation in particular, because it indicates a protein global conformational change during the reaction. In addition, the enthalpy and the heat capacity change of the transient intermediates will be estimated quantitatively. On the basis of these results, the photoreaction dynamics of TePixD will be discussed in detail from the conformational and energetic points of view.

3P250 Photoactive yellow protein の光反応における Val122 の作用
○針貝 美樹¹、山崎 洋一²、片岡 幹雄²、今元 泰³
¹京大・産長研、²奈良先端大・物質、³京大院・理
Function of Val122 on the photoreaction of photoactive yellow protein
Miki Harigai(1), Yoichi Yamazaki(2), Mikio Kataoka(2) and Yasushi Imamoto(3)
(1:Primate Research Inst., Kyoto Univ., 2:Graduate School of Materials Science, Nara Inst. of Science and Technology, 3:Graduate School of Science, Kyoto Univ.)

Photoactive yellow protein (PYP) from a purple photosynthetic bacterium is a water-soluble photoreceptor protein. Photocycle of PYP is triggered by the photo-isomerization of the chromophore, and subsequently PYP undergoes the global conformational change. It has been found that two bleached intermediates, acidic and alkaline forms produced in the millisecond time scale, are in the pH-equilibrium. Compared with the alkaline form, structural change of acidic form is smaller. It was suggested that the protonation state of Glu46 is involved in the switching between these two intermediates. Meanwhile, we demonstrated that an interaction between Phe6 and Lys123 is essential for the native conformational change of PYP. In this work, Val122, which is located in a central β-sheet and proximate to Glu46, was replaced, and the mutants were characterized by UV-visible and FTIR spectroscopy to indicate the propagation pathway of structural change from the chromophore to the N-terminal region. UV-visible absorption spectra of bleached intermediates formed from V122N and V122A were close to those of acidic and alkaline form of WT, respectively, and almost pH-independent. Changes in amide I and II modes in the difference FTIR spectrum of V122N were markedly smaller than that of WT, whereas that of V122A were comparable to WT. Correspondingly, vibrational mode of protonated Glu46 for V122N was largely down-shifted relative to WT. These results indicate that the interactions involving Glu46 were drastically affected by mutation of Val122.

3P249 MLSCMO 法による光受容タンパク質の励起状態計算
○川口 一朋¹、倭 剛久^{1,2}
¹名大院・理・物質理学、²CREST, JST
3aD01 The excited electronic state of solvated photoreceptor protein investigated by the improved MLSCMO method.
Kazutomo Kawaguchi (1), Takahisa Yamato (1,2). (1: Graduate School of Science, Nagoya University; 2: CREST, JST)

We performed excited electronic state calculations for the large system consisting of 10,779 atoms by the hierarchical molecular orbital method called MLSCMO method [1,2]. In the previous study, we considered the electronic state of the *p*-coumaric acid chromophore and 20 amino acid residues of photoactive yellow protein (PYP). It is expected that the intrinsic optical properties are explained by this small model. However, it is possible that the other part of the protein molecule and a large number of water solvent molecules affect the optical property of PYP as well. To estimate this effect quantitatively, we improved the previous MLSCMO method. In the present study, the improved MLSCMO method calculated the electronic state of the chromophore pocket model surrounded by the 3rd-layer consisting of the rest of PYP and a large number of water solvent molecules. In order to save the computational time, the 3rd-layer was treated as a distribution of fixed atomic point charges. As a result of the present calculations, we see that the optical property is essentially determined by the amino acid residues in the chromophore pocket. It is possible that the surrounding residues indirectly affect the electronic structure of the chromophore, because the local structure around the chromophore is affected by these residues. Finally we recognized significant solvation effect on the optical property of PYP.

References
[1] K. Kawaguchi et al. Chem. Phys. Lett. 430 (2006) 386-390
[2] T. Yamato et al. Photochem. and Photobiol. 83 (2007) 323-327

3P251 Rhodobacter capsulatus 由来PYP の光反応における pH 依存性
○浴口 真幸¹、山崎 洋一¹、土久保 裕生¹、片岡 幹雄¹
¹奈良先端大・物質
pH dependent kinetic property on the photoreaction of the Rc-PYP
Masayuki Sakoguchi(1), Yoichi Yamazaki(1), Hironari Kamikubo(1) and Mikio Kataoka(1). (1:Graduate School of Materials Science, Nara Institute of Science and Tecnology)

Photoactive yellow protein (PYP) is a blue light sensor that has a *p*-coumaric acid as a chromophore. The Rc-PYP is one of the PYP homologous proteins from a purple photosynthetic bacterium *Rhodobacter capsulatus*. This protein shows two characteristic absorption peaks at 375 and 436 nm in its spectrum. The protein sequence identity between the Rc-PYP and the Hh-PYP (a typical PYP from *Halorhodospira halophila*) is 43.5%. In the Rc-PYP, light absorption also causes isomerization of chromophore from *trans* to *cis* form, but the recovery rate is extremely slow as compared with that of Hh-PYP. We investigated the origins of the different kinetic properties. In this study, we examined the pH dependence on the thermal recovery rate from the *cis* to the *trans* state. The pH dependency of the kinetic recovery rate of the photoreaction was roughly bell-shape between pH 5 to 8. Maximum recovery rate was obtained at pH 6 suggesting that the photo-product in lower pH than about pH6 facilitates the chromophore re-isomerization. In order to understand the protonation state of Glu45 and the structural change of protein upon light absorption, we measured the difference FTIR spectra. In the difference FTIR spectra, pH dependency of protonation state of Glu45 was verified. Changes in protein back bone confirmed at amideI region were larger at acidic condition than alkaline condition. Since these properties are similar to the Hh-PYP, protein local structural changes coupled with chromophore protonation might cause the kinetic recovery difference.