- 3P252 紅色光合成細菌 *Rhodospirillum centenum* 由来 PYP 様ドメインの光反応サイクルの特性
○小山 知則¹、上久保 裕生¹、山崎 洋一¹、片岡 幹雄¹
¹奈良先端大・物質
Characterization of the photocycle of photoactive yellow protein like domain from *Rhodospirillum centenum*
Tomonori Koyama, Hironari Kamikubo, Yoichi Yamazaki, and Mikio Kataoka.
(Graduate school of Materials Science, Nara Institute of Science and Technology)

The sensor histidine kinase Ppr in *Rhodospirillum centenum* has a homologous domain to photoactive yellow protein (Ppr-PYP hereafter), which acts as a light sensor domain. In order to examine the photoreaction of the Ppr-PYP domain, we overexpressed and purified the Ppr-PYP domain. The pH dependence of the photosteady state was studied by continuous illumination under various pH. The accumulation of M intermediate (Ppr-PYPM) was observed at pH between 4.0 and 9.5. At alkaline condition, the L intermediate (Ppr-PYPL) was also observed which was characterized as the increase of absorbance around 480nm. After the subtraction of the contribution of Ppr-PYPL, the difference absorption spectrum of Ppr-PYPM was obtained at various pH. The λ_{max} of Ppr-PYPM under acidic and alkaline conditions appeared to be at 368nm and 340nm respectively, suggesting two types of M intermediates, which is comparable to PYP of *Halorhodospira halophila* (Hh-PYP). Although two M intermediates of Hh-PYP show substantial conformational change confirmed by CD, both Ppr-PYPM's do not show any changes in CD spectrum, indicating no structural changes. Based on these results, we will discuss the molecular mechanism of photoreaction of Ppr-PYPM.

- 3P254 Photoactive Yellow Protein を用いた中性子結晶構造解析
○山口 繁生¹、上久保 裕生¹、栗原 和男²、山崎 洋一¹、大西 裕季³、黒木 良太²、新村 信雄³、片岡 幹雄^{1,2}
¹奈良先端大・物質創成、²原研、³茨大院・理工・応用粒子線
Neutron crystallographic study of photoactive yellow protein
Shigeo Yamaguchi (1), Hironari Kamikubo (1), Kazuo Kurihara (2), Yoichi Yamazaki (1), Yuki Ohnishi (3), Ryota Kuroki (2), Nobuo Niimura (3) and Mikio Kataoka (1, 2). (1: Graduate School of Materials Science, Nara Institute of Science and Technology; 2: Japan Atomic Energy Agency; 3: Graduate School of Science and Engineering, Univ of Ibaraki)

Hydrogen atoms play important roles for many biochemical reactions during protein function. In the case of photoactive yellow protein (PYP), proton transfer from Glu46 to the chromophore and the following rearrangements in the hydrogen bond network are proposed to be closely related to the conformational changes. Therefore, to understand the molecular mechanism of PYP photoreaction, it is essential to determine the exact positions of hydrogen atoms. Neutron crystallography is one of the most effective methods to determine the hydrogen positions. However, neutron studies additionally require a quite large single crystal ($> 1 \text{ mm}^3$), because the brilliance of the neutron beam is quite low. We have surveyed crystallization conditions for PYP, resulting in the optimum condition. A fairly large crystal ($2.89 \times 0.85 \times 0.79 \text{ mm}^3$) of PYP can be obtained by using ammonium sulfate solution including a moderate amount of sodium chloride as a protein precipitant in D_2O [1]. We have performed neutron diffraction experiments with BIX-4 in the JRR-3 reactor at JAEA, and have acquired the neutron diffraction data to 1.6 Å resolution. We are analyzing the crystal structure of PYP based on the obtained neutron diffraction data. At the conference, we will present the neutron crystallographic structure and discuss the roles of some important hydrogen atoms in PYP.
[1] S. Yamaguchi, H. Kamikubo, N. Shimizu, Y. Yamazaki, Y. Imamoto & M. Kataoka, (2007). Photochem. Photobiol. 83. 336-338.

- 3P253 Ala/Val スキャニングによる Photoactive Yellow Protein の構造・機能に対する重要なアミノ酸残基のマッピング
○永田 大介¹、山崎 洋一¹、上久保 裕生¹、今元 泰²、片岡 幹雄¹
¹奈良先端大・物質、²京大・院理
Mapping of the essential amino acid residues for the structure and function of photoactive yellow protein by Ala/Val-scanning mutagenesis
Daisuke Nagata(1), Yoichi Yamazaki(1), Hironari Kamikubo(1), Yasushi Imamoto(2), Mikio Kataoka(1).(1:Graduate School of Materials Science, Nara Institute of Science and Technology;(2)Graduate School of Science, Kyoto University)

Photoactive yellow protein (PYP), a photoreceptor protein from a purple phototrophic bacterium *Halorhodospira halophila*, is a soluble protein composed of 125 amino acid residues. PYP has been well studied as a structural prototype of PAS domain superfamily, many of which act as the sensor proteins or domains. In this study, relationship between the primary structure and function of PYP was investigated by an Ala/Val-scanning mutagenesis, in which all non-Ala residues were replaced by Ala, whereas Ala residues were replaced by Val, with the exception of Met1 and Cys69. 123 possible substitution mutants were prepared and characterized. UV-visible absorption spectra of all mutants were measured. The absorption maxima of 16 mutants were significantly different from that of wild type. Mutations in the N-terminal cap segment did not affect the absorption maxima, whereas the significant shifts of the absorption maxima were observed for the mutants in PAS-core region, 84-85 loop, and chromophore binding loop. Half of the mutation sites that affect the absorption maximum are located near the chromophore. The others are distant from the chromophore. Furthermore, some residues near the chromophore were tolerant for the substitution. These facts suggest that the color tuning of PYP is brought by both direct interaction and indirect interaction of chromophore and protein moiety. The mapping of the essential amino acid residues are compared with the tertiary structure of PYP, and the roles of individual amino acid residues of PYP will be discussed.

- 3P255 イエロープロテインの光反応サイクルにおける構造変化メカニズム
○今元 泰¹、Kovaleva Valentina²、笹山 裕晃²、針貝 美樹³、片岡 幹雄²
¹京大・院理・生物物理、²奈良先端大・物質、³京大・霊長研
Structural switch of photoactive yellow protein during the photocycle
Yasushi Imamoto (1), Valentina Kovaleva (2), Hiroaki Sasayama (2), Miki Harigai (3) and Mikio Kataoka (2)(1: Graduate School of Science, Kyoto Univ; 2: Graduate School of Materials Science, Nara Inst. of Science and Technology; 3: Primate Research Institute, Kyoto Univ)

Photoactive yellow protein (PYP) is a water-soluble photosensor protein isolated from a purple photosynthetic bacterium. A photon is absorbed by a *p*-coumaroyl chromophore, and PYP undergoes the photocycle. In the latter stage, two bleached intermediates are formed. They are in the pH-dependent equilibrium and assume the significantly different structures. The acidic form, that is formed earlier than the alkaline form in the photocycle, assumes more compact structure. Thus the mechanism of the structural switch between acidic form and alkaline form should be revealed to understand the light-induced structural change of PYP. Our recent study has demonstrated that the interaction between β -sheet and N-terminal region is responsible for the conformational change. Thus we noted His108, which belongs to the β -sheet and possibly forms the hydrogen bond with N-terminal region via a water molecule. His108 was replaced by Ala, Gln, Ser, or Phe and the light-induced protein conformational changes of the mutants were characterized by Fourier transform infrared spectroscopy (FTIR). For H108A and H108F, pH-dependent equilibrium of bleached intermediates was observed like wild-type PYP. However, the difference FTIR spectra were pH-independent for H108Q and H108S, showing the formation of a single bleached intermediate. These results suggest that the amino acid side chain having oxygen atom at position 108 suppresses the structural switch by a strong hydrogen bond.