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Contribution of Arg52 to the electrostatic property of PYP M intermediate

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INTRODUCTION

Photoactive yellow protein (PYP) derived from purple photosynthetic bacteria, *Ectothiorhodospira halophila*, is a small water-soluble photoreceptor protein and is presumed to be involved in the negative phototaxis of the bacteria. PYP is composed of 125 amino acids and a unique chromophore, *p*-coumaric acid, and has a α/β-fold structure. PYP has several intermediates during the photoreaction cycle. Among these intermediates, M state is presumed to be active form, which would bind the interacting protein to transduce the signal. Therefore, in order to understand the molecular recognition mechanism between PYP and its transducer, it is essential to study the structure and the surface property of the M intermediate.

We have already investigated the interaction between M and various anions, which differ in size and electrostatic property, using UV-VIS spectroscopy and small-angle x-ray solution scattering (SAXS). Consequently, it is showed that some citrate molecules are bound to the M intermediate and the bound state causes the larger conformational change than that in the non-bound state, which is observed in the presence of acetate molecules. We presumed that the positive charge cluster would appear at the protein surface by the conformational change. Therefore, in order to find the binding site of citrate molecules, we measured the solution structure and the property of the M intermediate of the site-directed mutant, R52Q, because Arg52 is proposed to expose into the solution during the photocycle [1].

MATERIALS & METHODS

Small-angle x-ray solution scattering (SAXS) measurements were performed with SAXES installed at BL10C. R52Q mutant was expressed in *E.coli* and purified by an ordinary method. The purified R52Q was dissolved into 10mM MOPS buffer, 100mM sodium acetate in 10mM MOPS buffer or 50mM sodium citrate in 10mM MOPS buffer at 5°C, pH7. The sample was irradiated with a 1kW slide projector (HILUX-HR, Tokyo Master) through a glass optical filter (Y43, Toshiba).

RESULTS & DISCUSSION

We examined the difference in SAXS profile between dark state and illuminated state (M intermediate) of R52Q. Although the Rg value under illumination is larger than that under dark, the increase in Rg obtained by extrapolation to 0 concentration are independent of buffers and the value is 0.8Å. Moreover, the I(0)/c value in R52Q is also independent of buffers (Figure 1). Figure 2 shows the SAXS profiles between dark state and illuminated state in each buffer. There are no effects on buffer systems for the scattering profile of the M intermediate of R52Q as well as that under dark. These M profiles are similar to those of wild type in acetate buffer. The absorption spectrum of M state of R52Q in each buffer is also identical. These indicate that the M intermediate of R52Q cannot bind citrate molecules. Thus, Arg52 is involved in the binding site and it is presumed that Arg52 plays an important role in the alteration of charge distribution in the protein surface of PYP.

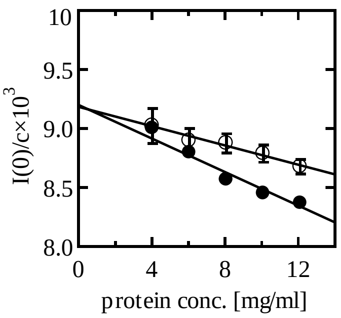

Figure 1)

The I(0)/c value under dark (close) and under illumination (open) in R52Q with 50mM sodium citrate

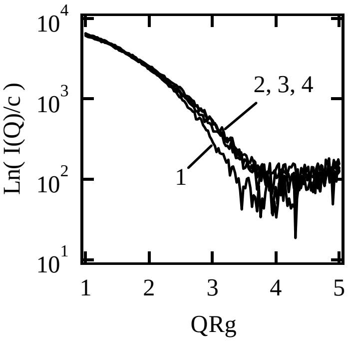

Figure 2)

Scattering profiles of R52Q under dark (curve 1) and under illumination in 3 buffer systems (curve 2, 3, 4)

Reference
[1] Genick, U. K. et al., *Science*, 275, 1471-1475 (1997)

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