Structural Model Construction of the Photointermediate of Photoactive Yellow Protein by High-Angle X-Ray Scattering

Solution X-ray scattering is an effective technique used for characterizing biomolecular structures and their changes in solution. The so-called *ab initio* shape prediction analysis enables us to construct a molecular envelope of a protein from an X-ray scattering profile without any assumptions. Scattering profiles of only up to $Q = 0.3$ Å^{-1}, which corresponds to a resolution of ~20 Å, are usually used in this analysis. High-angle profiles theoretically contain structural information at a high resolution, such as secondary structure packing and tertiary folds. Although the availability of third-generation synchrotron radiation sources with a two-dimensional detector has improved the quality of profiles even in the high-angle region by up to 6 Å^{-1}, high-angle scattering has not yet been applied systematically to derive solution structures. Here, we demonstrate the use of a promising method for analyzing structural changes in a protein by high-angle scattering combined with fluctuation analysis [1].

In this study, a light sensor protein, photoactive yellow protein (PYP), was used as a model protein [2]. PYP is a putative photoreceptor of a negative phototaxis in a purple phototropic bacterium, *Halorhodospira halophila*, which is composed of an N-terminal region and a chromophore-binding domain (see Fig. 1). The isomerization of the chromophore in the chromophore-binding domain upon light absorption triggers the subsequent thermal reaction. The blue-shifted intermediate PYP_M formed during the reaction is assumed to be an active state; therefore, it is essential to analyze the structural changes during PYP_M formation for the understanding of the molecular mechanism of photosignal transduction. The results of our previous studies on small-angle X-ray scattering and CD have revealed that the N-terminal region of PYP_M exhibits a deformation of its tertiary and secondary structures [3]. However, the essence of the structural changes in the chromophore-binding domain has not been clarified.

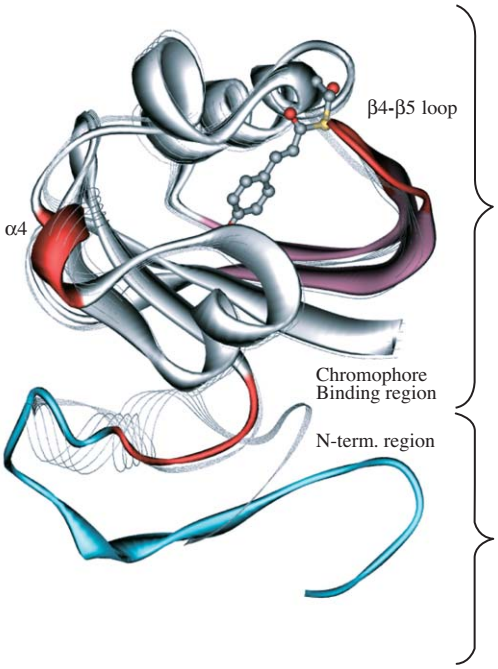

Fig. 1. Schematic model of PYP_M of the intact PYP (solid ribbon model). The crystal structure of the dark state of the intact PYP (line ribbon model) is superimposed on the model. Regions largely deviating from the dark state structure are shown in red color.

We have proposed an analytical method of high-angle scattering to characterize the tertiary structural change of PYP under illumination. In order to achieve the above-mentioned objective, we prepared several N-terminal truncated PYPs, in which the N-terminal 6 and 23 residues were cleaved by chymotrypsin treatment; these N-terminal truncated PYPs are referred to as T6 and T23, respectively. Scattering profiles of the intact PYP and the two truncated variants (T6 and T23) were observed at beamline **BL40B2** (Fig. 2(a)) [1]. The profile of the intact PYP shows two broad peaks at $Q = 0.35$ and 0.55 Å^{-1}, with a valley at around $Q = 0.41$ Å^{-1}. The peak position shifts toward a higher Q value, and concomitantly, the intensity of the peak at a high Q value decreases with an increase in the degree of N-terminal truncation. T23, which is completely devoid of the N-terminal region, exhibits a scattering profile with a single maximum around at $Q = 0.39$ Å^{-1}. These characteristic profiles indicate that the scattering profile observed in the Q region reflects the intramolecular interference. The theoretical profiles of the intact PYP, T6, and T23 well explained their respective observed profiles (Fig. 2(b)), indicating that the structures of T6 and T23 as well as that of the intact PYP can be explained by removing the corresponding residues from the crystal structure.

X-ray scattering profiles of T6 and T23 were measured under continuous illumination. Figure 3 shows the intensity profiles of the PYP_M of the truncated PYP variants compared with those obtained for their dark states. Significant differences between the two states were observed for each truncated PYP. Characteristic profile changes in T23 indicate rearrangements of the secondary structure packing in the chromophore-binding region during the formation of PYP_M. From the obtained profile, we attempted to

generate the plausible conformations of PYP_M from a variety of structures derived from the crystal structure of PYP, using the high-angle X-ray scattering profile as a boundary condition. A structural ensemble comprising 500 structures was constructed by fluctuation analysis. The high-angle scattering profile of each generated structure was calculated. While the profiles of most of the structures were similar to that of the dark state of T23 (a single broad peak at Q = 0.39 $Å^{-1}$), the profiles of some structures exhibited a bimodal shape, which was the same as that observed for the PYP_M of T23. From the structural pool, we selected structures whose scattering profiles were in good agreement with the characteristic features of the PYP_M of T23. Consequently, 51 structures from the 500 structures were selected and averaged to build a structural model of the chromophore-binding domain of PYP_M. According to this model, the loop between β4 and β5 and the α4 helix move away from each other to open the chromophore-binding pocket (Fig. 1). These structural changes agree well with the model predicted by a previous MD simulation study [4]. The schematic structural model for wild-type PYP_M can be obtained by combining the structural model of the chromophore-binding region of the PYP_M intermediate of T23 with the structural fluctuation of the N-terminal region predicted by a previous SAXS study (Fig. 1).

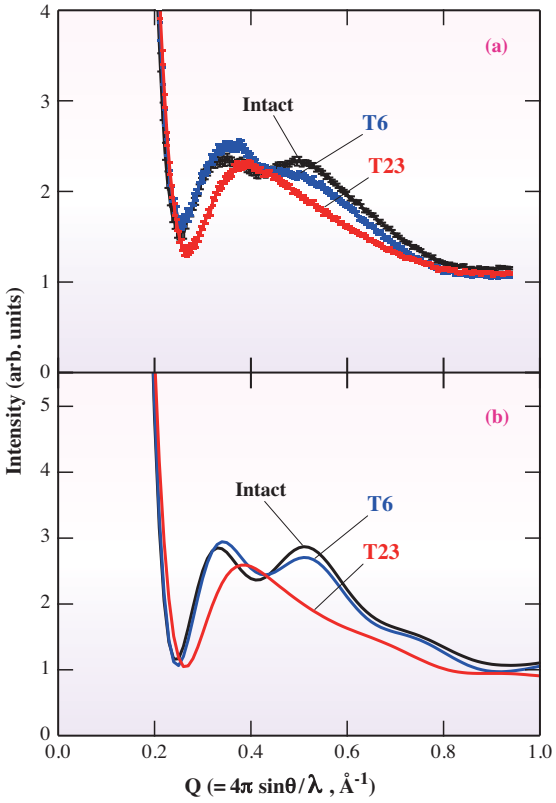

Fig. 2. High-angle X-ray scattering profiles of wild-type PYP, T6, and T23 measured in solution **(a)** and calculated from their respective atomic structural models **(b)**.

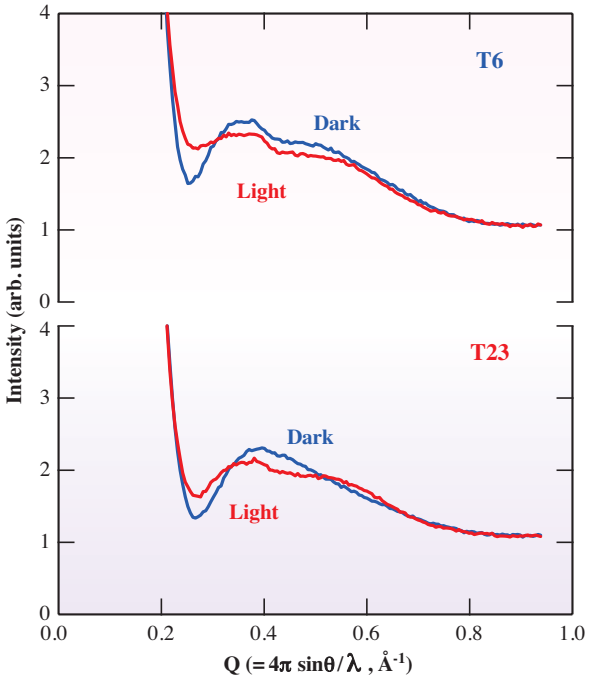

Fig. 3. High-angle X-ray scattering profiles of T6 and T23 under illumination (red). The profiles of the dark states are shown (blue) as reference.

This study confirms that high-angle X-ray scattering is quite effective and useful for determining structural properties under various solution conditions. In addition, it allows us to analyze structural changes in the transient states of proteins as well as their resting structures in solution. Fluctuation analysis combined with high-angle X-ray scattering is a promising technique for the solution structural studies of proteins.

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