Biological Science

5A, 17A/2007G653

Crystal structure analysis of the BLUF protein, PixD

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Introduction

Light perception is one of the principal signal recognition processes in many organisms. The sensor of Blue Light Using FAD (BLUF) is one of the flavin-binding blue light receptor [1]. We have determined the crystal structure of the BLUF protein from *Thermosynechococcus elongatus* BP-1, PixD, at 2.0 Å resolution, previously [2]. Five PixD monomers are located around the non-crystallographic 5-fold non-crystallographic axis to form a pentamer, and two pentamers related by 2-fold non-crystallographic symmetry form a decameric assembly. The monomer consists of two domains, the BLUF domain at the N-terminal region and the C-terminal domain (Fig. 1A). The BLUF domains are located at the outer part of the 5-fold double ring, and the C-terminal domains are at the inside. The isoalloxazine ring of the FAD is located between two helices at the outer part of the double ring. The structure revealed that many conserved residues are located in close contact around the isoalloxazine ring (Fig. 1B). BLUF domains are known to show the reversible red-shift of the absorption spectrum upon light illumination that recovers in the subsequent dark-adaptation within seconds to minutes. As the photoreaction of wild type PixD, 10nm red-shifted of flavin absorption was observed upon blue light illumination [3], and it is considered that structural change of the FAD-binding residues might be associated with the photoreaction. To elucidate the photoreaction cycle of PixD, we started crystal structure analysis of PixD protein upon the blue light illumination under the cryo condition.

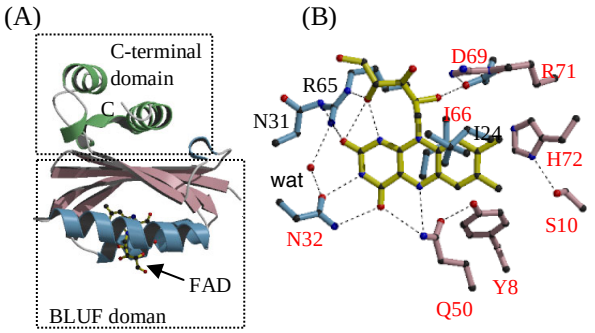

Fig. 1 (A) Monomer of PixD. (B) FAD binding site of PixD. Completely conserved residues were written in red letters.

Materials and Methods

The purified PixD sample was concentrated and crystallized using PEG400 reagent as a precipitant [2].

The crystals belong to the space group of $P2_12_12_1$ with the cell dimensions of a=89.5Å, b=109.9Å, and c=169.9Å. The crystals were obtained from both methods, vapour-diffusion method and batch method. Moreover, the crystals from the isopropanol solutions as a precipitant reagent were also obtained. The space group and the cell dimensions from the isopropanol crystals were the same as the crystals from PEG400. The crystals were mounted under the blue light illumination in the dark room to collect the data of the "blue-light excited state crystal". The data collection in the dark room was also carried out for the PixD crystals incubated in the dark box to collect the data of the "dark-state crystal". On the other hand, the crystallization under the blue light illumination was also carried out to obtain the "blue-light excited state crystal", however, no crystals were obtained.

Results and Discussions

The crystals from PEG400 or isopropanol were used for diffraction studies under the blue light illumination, respectively. The diffraction results indicate that many of the crystals from PEG400 were polycrystals, and the crystals from isopropanol showed only low-resolution diffraction data. Only a few diffraction data were obtained as those of single crystals, and they were employed to the structure analyses. The crystal structures of the "blue-light excited state crystal " and the "dark-state crystal" were determined by the molecular replacement method using the coordinates of PixD (PDB code: 1x0p) as a search model. The comparing of these structures indicates that there are no significant differences around the FAD binding site. It is not known whether the polycrystalline diffraction as described above relates to the blue-light illumination or not. Considering that, together with the fact that no crystals were obtained under the blue light illumination, the motion of the side-chain atoms under the blue light illumination might disturb the crystal packing.

References

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