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Crystal structures of PCNA from thermophilic archaea *A. pernix*

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1 Introduction

DNA replication, a key event in the cell cycle, involves various proteins, notably DNA-dependent DNA polymerases. Proliferating cell nuclear antigen (PCNA) forms a trimeric ring around DNA, enhancing the activity of replication proteins. Several proteins, including DNA polymerases, flap endonucleases, and DNA ligases, bind to PCNA via the PCNA-interacting peptide box (PIP-box) motif. The sliding clamp acts as a scaffold during DNA replication and is frequently expressed in cancer cells, making it a potential target for disease diagnosis and treatment. Inhibitors of bacterial β-subunits have been explored as novel antimicrobial agents. Structurally, three-dimensional structures of the sliding clamp have been reported in various species, forming a β-clamp dimer in bacteria and a PCNA trimer in eukaryotes. In archael species such as *Aeropyrum pernix*, which inhabits high-temperature environments, heterotrimers of PCNA enhance the polymerase activity. In this study, we elucidated the crystal structures of ApePCNA1, ApePCNA2, and ApePCNA3 from *A. pernix*.

2 Experiment

The *APE_0162*, *APE_0441.1*, and *APE_2182* genes encoding ApePCNA1, ApePCNA2, and ApePCNA3 proteins from *A. pernix* $K1^T$ were amplified by PCR from the genomic DNA of strain NBRC 100138^T. The PCR products were digested with *Nde*I and *Bgl*II, and subcloned into the pET-11a vector. The plasmids were introduced into *E. coli* Rosetta-gami (DE3) cells via electroporation and colonies were selected for primary culture in LB broth with ampicillin and chloramphenicol. Primary cultures were used to inoculate larger LB broth cultures, and APE gene expression was induced with lactose.

The cells were harvested, sonicated, and heat-treated to obtain heat-resistant fractions, followed by precipitation of soluble proteins with ammonium sulfate. The precipitate was dialyzed and loaded onto an ion-exchange column for protein elution. The eluted proteins were concentrated and purified using a gel filtration column. Purified ApePCNA proteins were concentrated to 10 mg/mL and crystallized by hanging-drop vapor diffusion at 298 K.

The ApePCNA1 crystals, shaped like origami shurikens, flat hexagonal ApePCNA2 crystals, and block ApePCNA3 crystals were collected and stored in liquid nitrogen. Diffraction data were collected at the Photon Factory in Tsukuba, Japan and processed using *XDSGUI*. Phases were determined by molecular replacement using the *Pyrococcus furiosus* PCNA model[1]. The structure was built with *ARP/wARP*, refined using *Coot* and *Refmac5*, and validated through *Coot* and wwPDB services.

3 Results and Discussion

The ApePCNA1 crystal structure shows a single subunit in the asymmetric unit, without forming a trimeric ring. The subunit has N-terminal and C-terminal domains linked by an interdomain connecting loop (IDCL), both featuring β-sheets and α-helices. Hydrophobic interactions and cavities are prominent. Similar to the PCNA rings of other species, this structure interacts with negatively charged DNA via inward α-helices. The IDCL and N-terminus regions displayed clear electron density, with the latter interacting with the adjacent molecules. ApePCNA1, resembling the PIP-box consensus sequence at N-terminus, is a monomer in solution, with unverified protein interactions, suggesting an unknown function. The monomer of the asymmetric unit and adjacent monomer interacted with the C-terminal leucine. This situation is similar to the interaction with the N-terminus of ApePCNA1 and with human PCNA:UHRF2^{PIP}[2]. Therefore, this C-terminal region is assumed to be a PIP-box-binding site.

An asymmetric unit of ApePCNA2 crystal contains four monomers which forms “Bridge” or a tetrameric ring. Two sets of dimers linked through hydrogen bonds were joined back-to-back in an asymmetric unit. This structure also formed a tetrameric ring. The double tetrameric rings were alternately stacked in the space group $C222_1$. Although this molecule, which forms a trimer, is unstable as a tetramer, its crystal structure consists of a tetrameric ring.

The asymmetric unit of the ApePCNA3 crystal contains a trimeric ring, which is related to a non-crystallographic three-fold axis, and a monomer that forms a trimeric ring along the three-fold axis of symmetry.

The ApePCNA1-2-3 and ApePCNA2-3 complex structures were simultaneously analyzed by X-ray solution scattering in BL-10C and cryo-EM at SBRC.

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References

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Research Achievements

1. 2020 RCSB Protein Data Bank Poster Prize Winner
   Recipient: T. Yamauchi
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