Structural Comparation of heterotrimer PCNA from Crenarchaeon *Aeropyrum pernix* by solution scattering, Cryo-EM, and Crystallography

T. Yamauchi[1,2], T. Takemori[3], M. Kikuchi[4], Y. Iizuka[1,3], S. Ishikawa[3], M. Tsunoda[1,3,4]

[1]Grad. Sch. of Life Sci. and Tech., Iryo Sosei Univ., 5-5-1 Chuodai-Iino Iwaki, Fukushima Japan,
[2]Dept. of Pharm., Fukushima Rosai Hosp., 3 Numajiri Uchigo-Tsuzuramachi Iwaki, Fukushima Japan,
[3]Fac. of Pharm., Iryo Sosei Univ., 4Grad. Sch. of Sci. and Eng., Iryo Sosei Univ.

df2002@isu.ac.jp

Sliding clamps are ring-shaped proteins that encircle DNA and confer high processivity on DNA polymerases. In bacteria, the β-clamp protein forms a homodimer, whereas in eukaryotes or euryarchaeotes, proliferating cell nuclear antigen (PCNA) proteins form homotrimers. However, PCNA from *Aeropyrum pernix* (*Ap*PCNA), a crenarchaeote species, forms a heterotrimer. The actual structure of *Ap*PCNA-mediated sliding clamps and the mechanism by which they slide along DNA is unknown. Previously, we have analysed the crystal structure of *Ap*PCNA1 from the APE_0162 gene[1], *Ap*PCNA2 from the APE_0441.1, and *Ap*PCNA3 from the APE_2182 genes[2]. The present study aimed to analyse the crystal, solution structure and cryo-electron microscopy (cryo-EM) of the heterotrimeric ring of *Ap*PCNA, examine its interaction with DNA and other proteins, and elucidate the mechanism of PCNA function.

Each *Ap*PCNA molecule, which constitutes a heterotrimer, was expressed using the *Escherichia coli* expression system. The proteins were purified using heat treatment, ammonium sulfate precipitation, and column chromatography. The purified proteins were crystallized using the vapor-diffusion method and the crystals were analysed by X-ray diffraction. To verify the ring shape of *Ap*PCNA2 in solution, the solution structure was analysed using size-exclusion chromatography-small-angle X-ray scattering (SEC-SAXS). A mixture of *Ap*PCNA1-2-3 and *Ap*PCNA2-3 were analysed by SEC-multi-angle light scattering for the presence of a complex, and the solution structure was analysed by SEC-SAXS. The mixture was analysed by cryo-EM, after purified with gel filtration chromatography.

The solution structure of the *Ap*PCNA1-2-3 complex is similar to shape of the British Isles islands. *Ap*PCNA2 and *Ap*PCNA3 interact in a similar manner as the PCNA rings of other organisms; however, *Ap*PCNA1 is located such that it did not form a perfect ring-shaped structure. The scattering curves of the complex and those of the model edited trimeric ring are almost similar with minor differences. The solution structure of *Ap*PCNA2-3 complex was similar to shape of a naan. This particle contains four subunits rather than trimer. The electron density from cryo-EM forms hexagon.

The solution structure was not trimeric ring, containing *Ap*PCNA1-2-3. The N-terminus of *Ap*PCNA1 is approximately 10 residues longer than that of *Ap*PCNA2 and *Ap*PCNA3. This could be why the tripartite complex is not ring shaped. Moreover, Met16 is present downstream of the N-terminal of *Ap*PCNA1. In the future, the effect of N-terminus deletion and binding of the DNA duplex on *Ap*PCNA1 structure should be evaluated. The solution structure of *Ap*PCNA2-3 complex was not trimeric ring too. In crystal structure of *Ap*PCNA3, the C-terminus interacts between adjacent subunits, probably PIP-Box binding site. This interaction may cause *Ap*PCNA2-3 Complex dose not form ring shape. Generally, PCNA rings that consists of homotrimer have 3-fold symmetry, comprise six edges from concave edge between subunits and flat edge that formed PIP-Box binding site. This hexagonal electron density suggests *Ap*PCNA1-2-3 forms trimeric ring in cryo-EM structure. Interestingly, one of the three edges is completely separated. The Fitting model containing *Ap*PCNA1-2-3 hetero subunits suggests, the long N-terminus of *Ap*PCNA1 cause this separated edge.

[1] Yamauchi, T., *et al*., Purification and Crystallization of PCNA from thermophilic archaea. Poster presented at: 138 Annual Meeting of the Pharmaceutical Society of Japan; Mar. 25-28, 2018; Kanazawa, JAPAN.

[2] Yamauchi, T., *et al*., Crystal and Solution structures of Proliferating Cell Nuclear Antigen from Crenarchaeon *Aeropyrum pernix*. Poster presented at: 70 Annual Meeting of the American Crystallographic Association; Aug. 2-7, 2020; Virtual.

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