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X-ray crystallography of the cell surface antigen CD38 complexed with ganglioside

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The cell surface antigen CD38 is a multifunctional ectoenzyme that acts as an NAD⁺ glycohydrolase, an ADP-ribosyl cyclase, and also a cyclic ADP-ribose hydrolase. The extracellular catalytic domain of CD38 was expressed as a fusion protein with maltose-binding protein, and was crystallized in the complex with a ganglioside, GT1b, one of the possible physiological inhibitors of this ectoenzyme. Two different crystal forms were obtained using the hanging-drop vapor diffusion method with PEG 10,000 as the precipitant. One form of the crystals diffracted up to 2.4 Å resolution using synchrotron radiation at 100 K, but suffered serious X-ray damages. They belong to the space group $P2_12_12_1$ with unit-cell parameters $a = 47.9$, $b = 94.9$, $c = 125.2$ Å. The second form of the crystals is a thin plate, but the data sets were successfully collected up to 2.1 Å resolution by the use of synchrotron radiation at BL41XU. The crystals belong to the space group $P2_1$ with unit-cell parameters $a = 57.4$, $b = 51.2$, $c = 101.1$ Å, and $\beta = 97.9^\circ$. The Rmerge, completeness, and I/s values were 0.052 (0.123), 92.4% (78.7%), and 11.1, respectively (the values at the outer shell are shown in parentheses). However, molecular replacement using the cyclase as a search model failed. The second form crystals were found to be partially twinned and detwinning is quite difficult. Therefore, we are now promoting the crystallographic analysis of the first orthorhombic crystal form.

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X-ray crystallographic study of DNA primase from hyperthermophilic archaeon

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DNA primase synthesizes a short RNA primer using a DNA template in the DNA replication process. Although DNA primase is such a crucial enzyme from bacteria to higher eukaryotes, the mechanism how it produces the short RNA primer has not yet been elucidated. Recently, the crystal structure of *E. coli* DNA primase has been reported, which has significantly different fold as compared to the usual polymerases. Eukaryotic primase has very low sequence similarity and different polypeptide size as compared to prokaryotic primase. The goal of this study is to determine the archaeal DNA primase, which is eukaryotic type, to elucidate the mechanism of primer synthesis in eukaryotic cells.

We crystallized DNA primase from hyperthermophile, *Pyrococcus horikoshii*. The crystals belong to a trigonal space group $P3_12_1$ with unit-cell parameters $a = b = 77.362$ Å, $c = 128.84$ Å. The crystals diffract X-ray up to 1.8 Å resolution in the BL41XU. The Rmerge and completeness were 0.051 (0.201) and 94.9% (49.8%), respectively (the values at the outer shell are shown in parentheses). By MAD experiment using SeMet derivative crystals, we could determine the phase and construct the atomic model in the electron density map. Conventional energy minimization and simulated annealing refinement with the program CNS resulted in the final working R factor is 22.2% and the free R factor is 25.8%. The structure determination of the complex with

nucleotide is now in progress.