MS9-P7 Structural Insight of Hyperthermostable Cellulase from The Archaea Pyrococcus horikoshii

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A hyperthermophophilic membrane related β-1,4 endoglucanase (family 5, cellulase) from *Pyrococcus horikoshii* was found to be capable of hydrolyzing crystalline cellulose at high temperatures. This hyperthermophilic enzyme has promise for applications in biomass utilization, but we have no information regarding the substrate-recognition mechanism of the enzyme. To examine its mechanism, we determined the crystal structure of its active domain at a resolution of 1.95 Å. From analysis of its structure and the reaction products, it was clarified that this endocellulase has a unique characteristic of releasing cellobiose from the reducing end of the cellulose substrate. This unique action results from the specific recognition of the reducing end of the substrate by the subsite of the active domain. The enzyme-ligand structure and mutation analysis reveal that the variant residue, Ile162, located at the subsite -1 position may have a hydrophobic role in forming the Michealis complex. The structure of the wild type enzyme-cellobiose complex also provides insight into the retaining mechanism of EGPh

Keywords: cellulase, hyperthermostable, Pyrococcus horikoshii

MS9-P8 Structural basis for ascorbate production by dehydroascorbate reductase in *Oryza sativa L. japonica*

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Ascorbate (ascorbic acid or vitamin C) is a strong antioxidant molecule as well as a cofactor for several enzymatic reactions. Dehydroascorbate reductase (DHAR) catalyzes the GSH-dependent reduction of oxidized ascorbate (dehydroascorbate, DHA) in plants to produce ascorbate. Therefore, DHAR functions in the detoxification of reactive oxygen species (ROS). In previous studies involving transgenic rice, we found that DHAR overexpression resulted in improved growth and increased grain yield. In order to clarify the molecular mechanisms of DHAR, we determined the crystal structures of DHAR from *Oryza sativa* L. *japonica* (OsDHAR) in the native, ascorbate-bound, and GSH-bound forms. These structures represent the first molecular view of the OsDHAR enzyme and the binding sites for ascorbate and GSH within OsDHAR. In addition, the residues involved in ascorbate and GSH recognition were identified at atomic resolution; structural comparisons with other homologous structures (CLIC1 and GSTs) are also discussed. We suggest a detailed enzymatic reaction mechanism for OsDHAR.

Keywords: ascorbate, dehydroascorbate reductase, Oryza sativa L. japonica, X-ray crystallography