Poster Presentation

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Novel intersubunit active site of arcchaeal N,N'-diacetylchitobiose deacetylase

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N,N'-diacetylchitobiose deacetylase (Dac) is involved in the archaea-specific chitinolytic pathway. In order to develop a structure-based understanding of the chitinolytic pathway in hyperthermophilic Pyrococcus species, we performed crystallographic studies on Dacs from P. horikoshii (Ph-Dac) and P. furiosus (Pf-Dac). Neither Ph-Dac nor Pf-Dac was expressed in the soluble fraction of Escherichia coli harboring the expression plasmid. However, insertion of the target genes into the chromosome of E. coli yielded the soluble recombinant protein. The purified Pyrococcus Dacs were thermostable up to 95°C. The crystal structures of Ph-Dac and Pf-Dac were determined at resolutions of 2.0 Å and 1.54 Å, respectively. The Pyrococcus Dac forms a hexamer comprised of two trimers. These Dacs are characterized by an intermolecular cleft, which is formed by two polypeptides in the trimeric assembly. In Ph-Dac, catalytic zinc situated at the end of the cleft is coordinated by three side chain ligands from His44, Asp47, and His155, and by a phosphate ion derived from the crystallization reservoir solution. We considered that the bound phosphate mimicked the tetrahedral oxyanion, which is an intermediate of hydrolysis of the N-acetyl group, and proposed an appropriate reaction mechanism. In the proposed mechanism, the Ne atom of His264 (from the adjacent polypeptide in the Ph-Dac sequence) is directly involved in the stabilization of the oxyanion intermediate. These factors give the archaeal Dacs unprecedented active site architecture as a zinc-dependent deacetylase.

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