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Association of a novel domain in the active site of the archaic hyperthermophilic maltogenic amylase from Staphylothermus marinus.

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1 Introduction

Staphylothermus marinus maltogenic amylase (SMMA) is a novel extreme thermophile maltogenic amylase with an optimal temperature of 100 °C, which hydrolyzes α(1–4)-glycosyl linkages in cyclodextrins and in linear malto-oligosaccharides [1]. This dual enzyme activity is differs from the classic α-amylases in the glycoside hydrolase 13 (GH13) family [2]. This enzyme has a long N-terminal extension that is conserved among archaic hyperthermophilic amylases but is not found in other hydrolyzing enzymes from the glycoside hydrolase 13 family. The three dimensional structure of SMMA will provides a a molecular basis for the functional properties that are unique to hyperthermophile maltogenic amylases from archaea and that distinguish SMMA from moderate thermophilic or mesophilic bacterial enzymes.

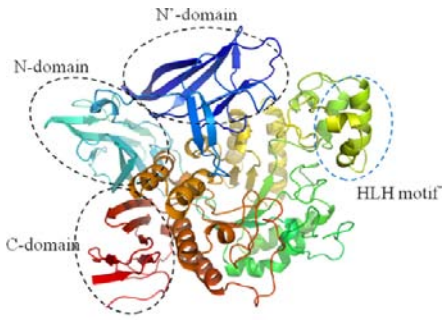
2 Experiment

SMMA crystallization trials were conducted using the sitting drop method at 18 °C. We mixed 1.5 □l of a 14 mg/ml SMMA solution with an equal volume of crystallization reservoir solution containing 12% polyethylene glycol (PEG) 4000, 2% isopropyl alcohol, 0.1 M ADA, pH 6.5, and 0.1 M Li2SO4. Before data collection, rhombus-type crystals were cryocooled to 95Kusing a cryoprotectant consisting of mother liquor supplemented with 25% glycerol. The crystal diffracted to a resolution of 2.28 Å, and the data were collected with a 1° rotation and a total of 340 frames. Diffraction data were processed and scaled using HKL2000 [3].

3 Results and Discussion

The crystal structure revealed that SMMA comprises four domains: the N, catalytic, and C domains, which are observed in most CD-hydrolyzing enzymes, and an additional novel N-terminal domain, the N'-domain, which was first observed in this study (Fig. 1a). Initially, the structure was determined and refined to a 2.28 Å resolution using molecular replacement, with the catalytic and C domains of neopullulanase from *B. stearothermophilus* (Protein Data Bank entry 1J0H) as the template structure. SMMA forms a homodimer via an interaction between the adjacent, novel N'-domains, which have a 2-fold axis perpendicular to the arc shape of the β-strands' interface (Fig. 1a). Each monomer is primarily associated through hydrophobic interactions at the center of the region of aa 5–19 (Ile5 and 19 from one molecule against Ile9 from the other). This interaction is supplemented by salt bridges (Arg181/Asp422 and Arg50/Glu198) at both ends of the strands, which yield a 2140.7 $Å^2$ interface. Most CD-hydrolyzing enzymes form dimers with the N-domain intertwined. However, the SMMA dimer configuration is different from previously reported CD-hydrolyzing enzymes, in that the dimer is arranged with adjacent monomers and an interface unrelated to the active sites. The active site pockets in bacterial CD-hydrolyzing enzymes are generated by the N-domain from the other subunit and yield a groove that is slightly extended between the catalytic domain and the N-domain. In comparison, the SMMA active site pocket is generated by the N'- domain of the same subunit. In the SMMA N'- domain, Phe95, Phe96, and Tyr99 are along the loop, and numerous aromatic residues, such as Tyr43, Phe46, and Phe77, lie on the β -sandwich fold surfaces (Fig 1b). This result suggests that extremophilic archaea that live at high temperatures may have adopted a novel configuration that combines all of the substrate binding components within a monomeric subunit.

a.



b.

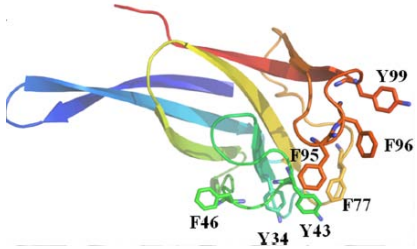


Fig. 1: The overall and N'-domain structure of SMMA. a, a schematic overview of an SMMA monomer that shows the conserved N, catalytic, and C domains in CD-hydrolyzing enzymes with a novel N'-domain. The monomer is colored in a spectrum; the N terminus is in *blue*, and the C terminus is in *red*. b, aromatic residues located near the loop protruding from the N'-domain. The domain has been colored in a spectrum; the N terminus is in *blue*, and the C terminus is in *orange*.

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