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Structure and Function Analysis of Geobacillus Starch Antistaling Enzyme

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Glycosyltransferase from Geobacillus sp. (SAS) is expected to see wide use as a starch antistaling enzyme in food including bread and rice products. The enzyme is thought to transfer maltotriose (G3) unit into non-reducing ends of sarch with unknown likage except for usual alpha-1,4 linkage. SAS was crystallized by sitting drop vapar diffusion method in 14～28% PEG4000 (w/v), 10mM CaCl2, 0.1M NaAC at pH 4.6 and 20˚C for 1 month. The obtained crystals belong to a space group of P6522 with cell dimensions of a = b = 112 and c = 320 Å. The crystals were soaked in various oligomaltosaccharides (G1, G2, G3, G4, G5 and G6) for 15 min before flash cooling. The diffraction data of each complex were collected at beam-lines of BL26B1, BL38B1 and BL44XU in SPring-8. The crystal data were collected with 97-99 % completeness and Rmerge of 0.07-0.09 up to 1.6-2.3 Å resolution. The structures were determined by molecular replacement with cyclodextrin glucanotransferase (CGTase, PDB 1CYG) as a search model and were refined with PHENIX. The refined models of SAS/sugars contain one molecule of SAS comprising 733 amino acid residues, 5-8 calcium ions, 543-1141 water molecules and several sugars with R = 0.15-0.19 and Rfree = 0.16-0.23 for the data up to 1.6-2.3 Å resolution. SAS has almost the same overall structure with the CGTase except for several loops in the catalytic domain A. They share a similar active site except for subsite +3 where the non-reducing ends of the oligosaccharides bind. G1 bound to subsite +3, indicating +3 site has the highest affinity to G1. Only G3 was found to bind at subsites +3 ~ +1 when G3, G5 and G6 were soaked, whereas G4 bound at subsites +3 ~ -1 when G4 was soaked. From the clear density map of the bound G4, the bound glucose residue at subsute -1 is found to have alpha-1,6 linkage, indicating the product of this transglucosidase.
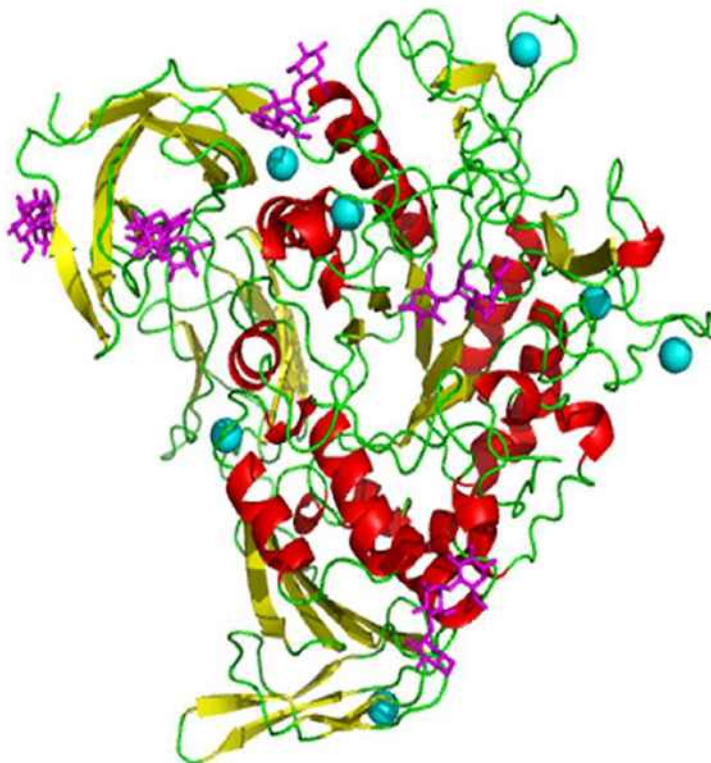
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