Crystallographic Analysis of Maltohexaose-producing Amylase

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Maltohexaose-producing amylase (G6-amylase) from an alkalophilic *Bacillus* sp.707 mainly produces maltohexaose (G6) from starch and related α-1,4-glucans. To elucidate the reaction mechanism of G6-amylase, a crystal structure of its complex with G6 was determined at 1.9 Å resolution. The G6-amylase was crystallized by the hanging drop vapor diffusion method using with the reservoir solution containing 50% (v/v) 2-methylpentane-2,4-diol, 100 mM Tris-HCl (pH 8.5) and 200 mM ammonium phosphate and then the obtained crystals were soaked for about 1 hour in the crystallization solution containing 75mM G6. The crystallographic *R* value (R_{free} value) was 0.155 (0.184). The crystal structure revealed that the G6 occupies subsites –7 to –2 like an enzyme-product complex and its structure of the catalytic active site is very similar to that of the pseudo-maltononaose (pG9) complex and not its native structure. As same as in the pG9 complex structure determined previously, an indolyl ring of Trp140 stacks to the glucosyl residues at subsites –6 and –5. Almost α-amylases finally degrade G6 to glucose and/or maltose. This is achived by G6 binding to subsites –1 and +1 at least. However, G6-amylase would little hydrolyze G6 by non-productive binding to G6. Therefore, Trp140 may play an important role on G6 production and hydrolysis preventation to G6.

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