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Title:	Structure-guided mutational evidence and postulates explaining how a glycohydrolase from Pyrococcus furiosus functions simultaneously as an amylase and as a 4-α-glucanotransferase
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Keywords:	Glucanotransferase Amylase Loop-based transfer Glucose GH57 PF0272
Issue Date:	2019
Publisher:	Elsevier
Citation:	Biochemical and Biophysical Research Communications, 509(4),pp. 892-897.
Abstract:	Pyrococcus furiosus exoamylase-cum-4-α-glucanotransferase (4-α-GTase; PF0272; PfuAmyGT) is reported to both (i) act upon starch, and (ii) catalyze 'disproportionation' of maltooligosaccharides (with glucose as the smallest product). PfuAmyGT shares ~65% sequence identity with a homo-dimeric Thermococcus litoralis 4-α-GTase, for which structures are available in complex with a non-hydrolysable analog of maltotetraose (acarbose) bound to one subunit and maltose (of unknown origin) bound to the other subunit. We structurally transposed the maltose onto the acarbose-bound subunit and discovered that the two molecules lie juxtaposed in what could be perfect 'acceptor' and 'donor' substrate-binding sites, respectively. We also discovered that there is a loop between the two sites which could use an available aspartate to excise a glucose from the donor, and an available tryptophan to transfer the glucose to the non-reducing end of the acceptor glucan. We derived a structure for PfuAmyGT through homology-based modeling, identified the potential donor site, acceptor site, glucan-transferring loop, and catalytically important residues, and mutated these to alanine to examine effect(s) upon activity. Mutation D362A abolished creation of shorter, or longer, maltooligosaccharides. Mutation W365A abolished creation of longer oligosaccharides. Mutation H366A had no effect on activity. We propose that D362 facilitates glucose excision, and that W365 facilitates its transfer, either (a) directly into solution (allowing PfuAmyGT to act as an exoamylase), or (b) by glycoside bond formation with an acceptor (allowing PfuAmyGT to act as a 4-α-glucanotransferase), depending upon whether the acceptor site is vacant or occupied in a reaction cycle.
URI:	https://www.sciencedirect.com/science/article/pii/S0006291X19300270 (https://www.sciencedirect.com/science/article/pii/S0006291X19300270) http://hdl.handle.net/123456789/2215 (http://hdl.handle.net/123456789/2215)
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