Title:	Exploring the novel mechanistic aspects of function of a hyperthermophile two-site exo-amylase-cum-glucanotransferase displaying substrate versatility.
Authors:	Sarkar, Arpita (/jspui/browse?type=author&value=Sarkar%2C+Arpita) Guptasarma, Purnananda (/jspui/browse?type=author&value=Guptasarma%2C+Purnananda)
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Abstract:	We have recently described an exo-amylase-cum-glucanotransferase, PfuAmyGT, from Pyrococcus furiosus which uses disproportionation to generate a pool of glucose and small malto-oligosaccharides (MOGs) from starch, or from any single MOG (or a combination of MOGs). PfuAmyGT appears to use a combination of exo-amylase action and excision-and-transfer of glucose from donor to acceptor MOGs, using a unique and novel mechanism involving two catalytically-active sites (i.e., separate donor and acceptor sites, with a loop transferring the excised glucose) in place of the more common single site (at which the donated glucose is excised, and left, by the departing donor MOG, for being picked up by the acceptor MOG). We demonstrate that there are five residues (three glutamates and two aspartates) that are essential for activity, suggesting that different acidic side chains act at the donor and acceptor sites. We also demonstrate that PfuAmyGT acts upon substrates possessing different monomeric units and different types of glycosidic bonds (e.g., pectin, xylan or cellulose), indicating an unprecedented level of versatility, given that only one other glycosyl hydrolase (a neo-pullulanase) has previously been reported to act upon more than one type of glycosidic bond (1,4 and 1,6). Our bioinformatics and biochemical analyses suggests that the donor site of PfuAmyGT is processive, while the acceptor site releases and rebinds MOGs in each duty cycle. We have identified a total of six residues in the vicinity of a catalytic aspartate (D362) that potentially function to give rise to the said substrate versatility. Four of the six residues are also catalytically important for the functioning of PfuAmyGT on different substrates.
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