

GLYCOSYLTRANSFERASE MECHANISMS: INSIGHTS FROM STUDIES OF PHOSPHORYLASES

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Phosphorylases constitute a special group of glycosyltransferases (GTs) that catalyze glucosyl transfer to and from phosphate. Unlike the majority of GTs that use a nucleotide or lipid-activated donor sugar for carbohydrate biosynthesis, phosphorylases are catabolic enzymes that fulfil a physiological function in the degradation of glycosides in the presence of inorganic phosphate. The sequence-based classification of GTs reveals that family GT4 contains among various other enzymes, fungal trehalose phosphorylases (TP). Family GT35 contains glycogen (α -glucan) phosphorylases (GP) from different organisms and cell types. Both TP and GP are retaining enzymes which convert their respective α -glucosidic substrate into α -D-glucose 1-phosphate. GP and probably TP belong to clan IV of GTs and have a GT-B fold. The mechanism of retaining GTs is not well understood, and evidence from crystal structures and kinetic analysis of wild-type and mutant enzymes have not clearly supported a classical two-step reaction scheme which involves two configurationally invertive steps and the formation of covalent β -glycosyl enzyme intermediate. The paper reports on results obtained with two phosphorylases, TP from the wood-rotting basidiomycete fungus *Schizophyllum commune* and GP from soil bacterium *Corynebacterium callunae* which we have chosen as model enzymes to explore the mechanism of α -retaining glucosyl transfer. Structure-guided site directed mutagenesis and detailed analysis of kinetic consequences in purified point mutants was used to assign roles in catalysis to certain key residues in the active sites of the two phosphorylases [1]. Findings are discussed in the context of plausible reaction mechanisms.

[1] Schwarz, A.; Pierfederici, F.M.; Nidetzky, B. *Biochem. J.* **2005**, *387*, 437-445.