An acceptor-substrate binding site determining glycosyl transfer emerges from mutant analysis of a plant vacuolar invertase and a fructosyltransferase

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Glycoside hydrolase family 32 (GH32) harbors hydrolyzing and transglycosylating enzymes that are highly homologous in their primary structure. Eight amino acids dispersed along the sequence correlated with either hydrolase or glycosyltransferase activity. These were mutated in onion vacuolar invertase (acINV) according to the residue in festuca sucrose:sucrose 1-fructosyltransferase (saSST) and vice versa. acINV(W440Y) doubles transferase capacity. Reciprocally, saSST(C223N) and saSST(F362Y) double hydrolysis. SaSST(N425S) shows a hydrolyzing activity three to four times its transferase activity. Interestingly, modeling acINV and saSST according to the 3D structure of crystallized GH32 enzymes indicates that mutations saSST(N425S), acINV(W440Y), and the previously reported acINV(W161Y) reside very close together at the surface in the entrance of the active-site pocket. Residues in- and outside the sucrose-binding box determine hydrolase and transferase capabilities of GH32 enzymes. Modeling suggests that residues dispersed along the sequence identify a location for acceptor-substrate binding in the 3D structure of fructosyltransferases.

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