

CELL WALL MODIFYING HYDROLASES FROM BARLEY: CRYSTAL STRUCTURES AND CATALYTIC MECHANISMS

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A broad specificity family GH3 β -D-glucan glucohydrolase is a two-domain enzyme consisting of an $(\alpha/\beta)_8$ barrel and an $(\alpha/\beta)_6$ sandwich, connected by a helix-like linker [1]. The active site of the enzyme is positioned in a pocket at the interface of the two domains and contains the catalytic nucleophile Asp285 and the acid/base Glu491, together with substrate-binding residues. A glucose moiety is observed in the active site and represents the product of the hydrolytic reaction [1, 2].

The mechanism-based inhibitors conduritol B epoxide, 2,4-dinitrophenyl 2-deoxy-2-fluoro- β -D-glucoside [2], and transition-state ion-like mimics glucophenylimidazoles [3] displace glucose from the active site. The 'glucose-like' moieties of glucophenylimidazoles adopt a ⁴E conformation, which is critical for the low nanomolar binding of the mimics. The inhibitors and mimics define the multiple steps of the enzyme's catalytic mechanism.

The broad substrate specificity of the β -D-glucan glucohydrolases is explained by crystal structures of the enzyme in complexes with non-hydrolysable *S*-glycoside analogues and glucosyl transfer products [2, 4]. The flexibility of subsite +1, located between Trp286 and Trp434, and the projection of the remainder of the substrates away from the enzyme indicates that the active site pocket accommodates (1,2)-, (1,3)-, (1,4)- and (1,6)-linked β -D-glucosides between two Trp residues at the entrance of the pocket.

The 'exit' of glucose from subsite -1 in the active site serves as a convenient tool for studying mechanisms of a product/substrate interchange. The glucose molecule can be displaced from subsite -1 with 4-deoxy-D-glucose, glycerol, and polyethylene glycol 400; the latter ligand also detects secondary binding sites on the surface of the enzyme. Several interactions, side-chain residues and domain movements are likely to play key roles during the product/substrate interchange. A protein packing in the unit cells of several 1.7 Å resolution crystal structures provides additional information on how glucose could leave the active site.

The β -D-glucan glucohydrolases have been implicated in cell elongation and wall remodelling, and the broad specificity for glycosidic linkage type enables the enzyme to perform diverse roles during plant growth and development [5].

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