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Structural biology of carbohydrate transfer and modification in natural product biosynthesis

AKADEMISK AVHANDLING

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ABSTRACT

Certain organisms, can during periods of limited resources, adapt their metabolism to enable biosynthesis of secondary metabolites, compounds that increase competitiveness and chances of survival. The subjects of this thesis are enzymes acting on carbohydrate substrates during secondary metabolism.

The enzymatic attachment of carbohydrate moieties onto precursors of polyketide antibiotics such as anthracyclines, required for their biological activity, is performed by glycosyltransferases (GT). The anthracycline nogalamycin contains two carbohydrates: a nogalose moiety attached via an *O*-glycosidic bond to C7, and a nogalamine attached via an *O*-glycosidic bond to C1 and an unusual carbon-carbon bond between C2 and C5'' of the sugar. Genetic and functional data presented in this thesis established the roles of SnogE as the GT performing the C7 *O*-glycosyl transfer of the nogalose moiety and SnogD as the *O*-GT attaching the nogalamine moiety onto the C1 carbon. The activity of SnogD was verified *in vitro* using recombinant protein, following establishment of a transglycosylation-like assay. The three-dimensional structure of the homo-dimeric SnogD was determined to 2.6 Å and consists of a GT-B fold. Mutagenesis of two active site residues, His25 and His301, evaluated *in vitro* and *in vivo*, suggested His25 to be the catalytic base, activating the acceptor substrate by proton abstraction from the C1-hydroxyl group. His301 provides a positive charge to stabilise the negative charge formed close to the diphosphate of the leaving group during glycosyl transfer. Genetic, functional and structural data together suggest the involvement of an additional or altogether different enzyme for the C-C bond formation.

The bifunctional enzyme aldose-2-ulose dehydratase (AUDH) from *Phanerochaete chrysosporium* catalyses the dehydration and isomerisation of the secondary metabolites glucosone and 1,5-anhydro-D-fructose (AF) into the antimicrobial compounds cortalcerone and microthecin (Mic), respectively. The three-dimensional structure of the dimeric AUDH was determined to 2.0 Å. The enzyme consists of a seven bladed β -propeller, two cupin folds and a lectin-like domain, in a novel combination. Two structural metal ions, Mg^{2+} and Zn^{2+} , are bound in loop regions. Two additional zinc ions are present at the base of two putative active sites, located in the β -propeller and the second cupin fold. The specific removal of these zinc ions eliminated catalytic activity, proving the metal dependency of the overall reaction. The structure of AUDH in complex with the reaction intermediate ascopyrone M bound at both putative active sites, and a complex of zinc-depleted enzyme with AF bound in the cupin fold have been determined by X-ray crystallography to 2.6 and 2.8 Å resolution, respectively. These observations support the presence of two distinct active sites located 60 Å apart, partly connected by an intra-dimeric channel. The dehydration reaction most likely follows an elimination reaction with the zinc ion acting as a Lewis acid to polarise the C2 keto group of AF. Abstraction of the C3 proton by the suitably located residue His155 would generate an enol intermediate, which is stabilised by the zinc ion. Return of the proton to the C4 hydroxyl group would generate a favourable leaving group.