Experimental report for ESRF Expt. MX-1813, proposer Clemens Grimm, University of Wuerzburg, Beamline ID30B

Overview

Enzyme engineering has been established as a major tool where chemical synthesis reaches its limits. The industrially important Sucrose Phosphorylases have been modified, usually by deleting large residues for the synthesis of several structurally diverse products. We report on the creation of a new multifunctional acceptor binding site via a domain shift (Fig. 1A) constituting an unprecedented mechanism for protein design. The domain shift is triggered by a single amino acid exchange (Q345F) and is responsible for remodeling the active site of *Bifidobakterium adolescentis* sucrose phosphorylase (BaSP) into a polyphenol binding site (1). Fig. 1B and C. The engineered variant is capable of the glucosylation of a wide variety of flavonoids, including quercetin and the synthesis of rare disaccharides (2). During this experimental session we solved the crystal structures of engineered sucrose phosphorylase in complex with its product, resveratrol-3-α-D-glucosid and of the same enzyme in complex with the rare sugar nigerose. These structures explain the mode of substrate binding of this tailored enzyme and reveal hotspots for future modifications towards the next generation of engineered biocatalysts.

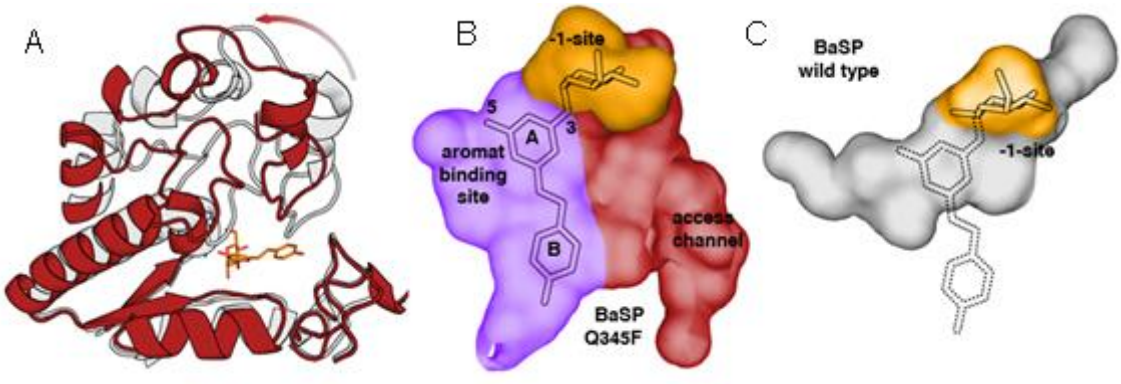

Fig. 1: (A) Domain rotation responsible for the creation of a novel active site. Red: BaSP Q345F, grey: BaSP wildtype (PDB ID 2gdv chain B). (B) Active site dimensions and substrate positioning in BaSP Q345F. (C) Active site dimensions and substrate positioning in BaSP wild type,

Evaluation and results

An inactive variant of the engineered BaSP enzyme was prepared by a single amino acid exchange in its active center. Diffraction quality crystals were produced and soaked with diverse products generated by engineered BaSP, mainly glycosylated polyphenols and several rare sugars.

A B

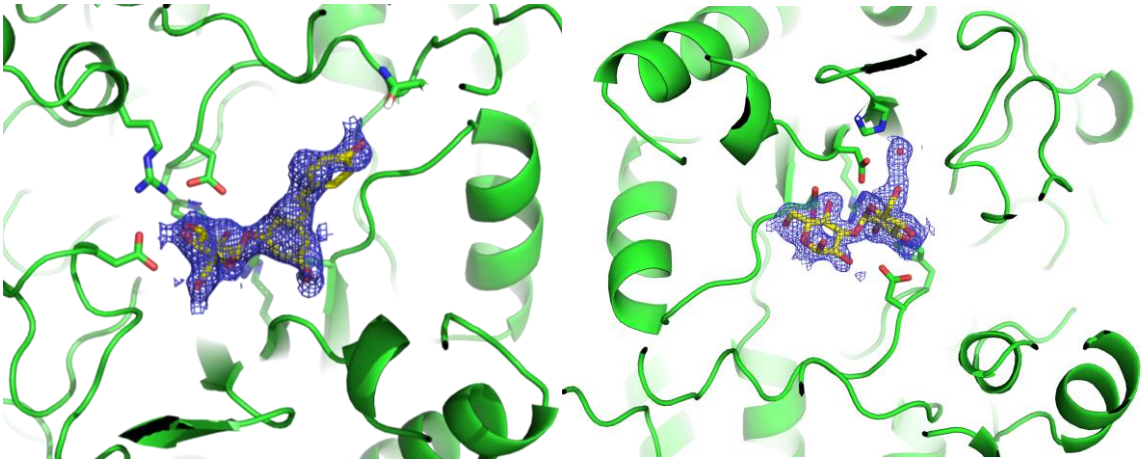

Fig. 2: Active site of BaSP with bound products. For the ligand, the simulated annealing omit map is shown as a mesh on 1.0 sigma contour level. (A) BaSP in complex with resveratrol-3-α-D-glucosid at 2.7 Å resolution (B) BaSP in complex with nigerose at 1.8 Å resolution.

From a total of 90 crystals more than 100 datasets were measured and examined for the presence of bound ligands. The majority of the structures solved from these datasets featured a single glucose residue bound in the active site, indicative of residual enzymatic activity leading to product hydrolysis. However, two structures solved from crystals soaked with resveratrol-3-α-D-glucosid and nigerose displayed clear density for the bound product (Fig. 2A and 2B, respectively). Furthermore, the entirety of the solved crystal structures belonging to a total of three different crystal forms document reliably and in detail the domain shift responsible for the formation of the enlarged pocked within the active center (compare Fig. 1).

References

11. M. Kraus, C. Grimm, J. Seibel, Redesign of the Active Site of Sucrose Phosphorylase through a Clash-Induced Cascade of Loop Shifts. *Chembiochem* **17**, 33-36 (2016).

14. M. Kraus, J. Görl, M. Timm, J. Seibel, Synthesis of the rare disaccharide nigerose by structure-based design of a phosphorylase mutant with altered regioselectivity. *Chem Commun (Camb)* **52**, 4625-4627 (2016).