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High-resolution structures of *Neotermes koshunensis* β-glucosidase mutants provide insights into the catalytic mechanism and the synthesis of glucoconjugates

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1 Introduction

β-Glucosidases, a heterogeneous group of exo-type glycosyl hydrolases, have been widely used in numerous applications. β-Glucosidases normally catalyze the hydrolysis of glucosidic linkages in disaccharide or glucose-substituted molecules, but transglycosylation under conditions that favour a back reaction can be applied to promote the synthesis of oligosaccharides. *Nk*Bgl, a β-retaining glycoside hydrolase family 1 β-glucosidase (EC 3.2.1.21), was isolated and identified from the salivary glands of the termite *Neotermes koshunensis*.

2 Experiment

The protein was prepared at 25 mg/ml in 50 mM HEPES buffer pH 8.0 containing 100 mM NaCl and 5 mM DTT for crystallization. Crystals were grown from a drop composed of 1.5 μl protein solution and 1.5 μl reservoir solution consisting of 18–21% (w/v) PEG 3350 and 0.1–0.25 M $MgCl_2$ in 0.1 M bis-Tris buffer pH 6.5 equilibrated at 298 K against 250 μl reservoir solution using the sitting-drop vapor-diffusion method.

3 Results and Discussion

The crystal structures of wild-type or mutated *Nk*Bgl in complex with various ligands were determined at 0.97–1.28 Å resolution. The overall structure of *Nk*Bgl is similar to the structures of glycosyl hydrolase family 1 enzymes, exhibiting a classical $(\alpha/\beta)_8$ TIM-barrel fold. The bell-shaped pocket of the *Nk*Bgl active site is approximately 20 Å deep and 3000 $Å^3$ in volume and was located on connecting loops at the C-terminal end of the β-sheets of the TIM barrel.

In the wild-type *Nk*Bgl structures it was found that glucose-like glucosidase inhibitors bind to the glycone-binding pocket, allowing the buffer molecule HEPES to remain in the aglycone-binding pocket. In the crystal structures of *Nk*Bgl E193A, E193S and E193D mutants Glu193 not only acts as the catalytic acid/base but also plays an important role in controlling substrate entry and product release. Furthermore, in crystal structures of the *Nk*Bgl E193D mutant it was found that new glucoconjugates were generated by the conjugation of glucose (hydrolyzed product) and HEPES/EPPS/opipramol (buffer components). Based on the wild-type and E193D-mutant structures of *Nk*Bgl, the glucosidic bond of cellobiose or salicin was hydrolyzed and a new bond was subsequently formed between glucose and HEPES/EPPS/opipramol to generate new glucopyranosidic products through the transglycosylation reaction in the *Nk*Bgl E193D mutant. This finding highlights an innovative way to further improve β-glucosidases for the enzymatic synthesis of oligosaccharides.

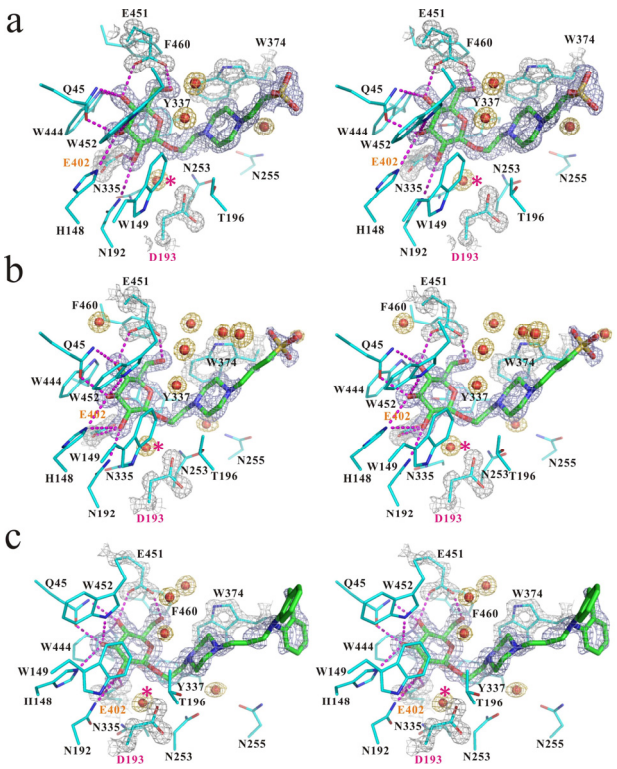

Figure. A stereoview of ligand molecules and the surrounding residues in the active sites of *Nk*Bgl structures. The *Nk*Bgl E193D mutant in complex with HEPES-1-glucoside (a), EPPS-1-glucoside (b) or opipramol-1-glucoside (c).

References

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