It All Starts with a Sandwich: Identification of Sialidases with Trans-Glycosylation Activity

Sialidases (3.2.1.18) may exhibit trans-sialidase activity to catalyze sialylation of lactose if the active site topology is congruent with that of the Trypanosoma cruzi trans-sialidase (EC 2.4.1.-). The present work was undertaken to test the hypothesis that a particular aromatic sandwich structure of two amino acids proximal to the active site of the T. cruzi trans-sialidase infers trans-sialidase activity. On this basis, four enzymes with putative trans-sialidase activity were identified through an iterative alignment from 2909 native sialidases available in GenBank, which were cloned and expressed in Escherichia coli. Of these, one enzyme, SialH, derived from Haemophilus parasuis had an aromatic sandwich structure on the protein surface facing the end of the catalytic site (Phe168; Trp366), and was indeed found to exhibit trans-sialidase activity. SialH catalyzed production of the human milk oligosaccharide 3'-sialyllactose as well as the novel trans-sialylation product 3-sialyllactose using casein glycomacropeptide as sialyl donor and lactose as acceptor. The findings corroborated that Tyr119 and Trp312 in the T. cruzi trans-sialidase are part of an aromatic sandwich structure that confers trans-sialylation activity for lactose sialylation. The in silico identification of trans-glycosidase activity by rational active site topology alignment thus proved to be a quick tool for selecting putative trans-sialidases amongst a large group of glycosyl hydrolases. The approach moreover provided data that help understand structure-function relations of trans-sialidases.

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