A newly discovered multifunctional α2,3-sialyltransferase from *Pasteurella dagmatis* carries a natural Ser-to-Thr substitution

Katharina Schmölzer[1], Doris Ribitsch[1], Tibor Czabany[2], Christiane Luley-Goedl[1], Deja Kokot[2], Andrzej Lyskowski[1], Sabine Zitzenbacher[1], Helmut Schwab[1,3], Bernd Nidetzky[1,2]

[1]Austrian Centre of Industrial Biotechnology, Petersgasse 14, 8010 Graz, Austria
[2]Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Petersgasse 12/I, 8010 Graz, Austria
[3]Institute of Molecular Biotechnology, Graz University of Technology, Petersgasse 14, 8010 Graz, Austria

The newly discovered sialyltransferase from *Pasteurella dagmatis*, in short PdST, is a multifunctional α2,3-sialyltransferase. It was identified from the *P. dagmatis* genome by similarity search with sialyltransferases from glycosyltransferase family GT-80. After sialyltransferase PmST1 from *P. multocida*, PdST is the second member of family GT-80 to display a remarkable catalytic promiscuity. In addition to its α2,3-sialyltransferase activity, purified PdST is alternatively active as α2,6-sialyltransferase and at low pH as α2,3-sialidase and α2,3-trans-sialidase. It also shows CMP-Neu5Ac hydrolase activity when no sialyl acceptor substrate is present in the reaction. The acceptor spectrum of PdST is restricted to β-D-galactosyl substrates. An interesting and peculiar feature of PdST however is a natural Ser-to-Thr interchange in the YDDGS-motif that is otherwise invariant in family GT-80 sialyltransferases. The equivalent serine in PmST1, Ser143 is involved in binding of the CMP-Neu5Ac donor substrate and has an important role in triggering the large closure movement of the N-terminal Rossmann domain towards the C-terminal nucleotide binding domain upon CMP binding to define the acceptor binding site. A T116S-PdST variant was created to reverse the natural mutation. This variant showed marked increase in α2,3-trans-sialidase side activity while the major sialyltransferase activity was lowered. The Michaelis-Menten constant for CMP-Neu5Ac was decreased about 4-fold as compared to wild-type PdST, which indicates that residue 116 of PdST contributes to a delicate balance between substrate binding and catalytic activity. Multifunctionality is supposed to be determined by multifunctionality of important active site residues (Sugiarto et al. 2011). Therefore, the pH-dependencies of the different reactions were examined and site-directed mutants were created

to carve out the different molecular interactions that determine sialyltransferase, sialidase and trans-sialidase activity.

Sugiarto G., Lau K., Li Y., Khedri Z., Yu H., Le D.T., Chen X. (2011) Mol. BioSyst. 7:3021-3027.