INCREASED TRANS-GLYCOSYLATION ACTIVITY OF HEXOSAMINIDASES FOR SYNTHESIS OF HUMAN MILK OLIGOSACCHARIDES

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It is well known that the composition of human breast milk differs significantly from the one of ordinary bovine milk. Especially the presence of sialylated and fucosylated oligosaccharides contributes to its health and development promoting features for newborn infants. [1] Nevertheless, not all newborns and especially premature infants sometimes cannot be breast fed for different reasons. For those children it is important that they receive a proper balanced formula product containing the above mentioned human milk oligosaccharides (HMOs). With respect to this we are developing new enzymatic routes for synthesis of sialylated and fucosylated oligosaccharides, which can be used as functional ingredient for infant formula.

In a previous work two candidate hexoasaminidases (both belonging to the GH20 family) were identified from a metagenomic library, which were able to synthesize the basic HMO backbone structure, Lacto-N-trose II, from chitobiose and lactose by trans-glycosylation. [2] Since the yields using these enzymes were low (2% for hex1 and 8% for hex2 based on the donor substrate chitobiose) we wanted to increase their trans-glycosylation activity to increase their applicability for a feasible process.

It was decided to follow a rational design approach first to keep the screening effort low. Therefore peptide pattern recognition (PPR) [3] analysis was performed on the whole GH20 CAZy family (approx. 3000 sequences) to identify other enzymes with potential trans-glycosylation activity based on relatedness. By phylogenetic analysis of the group containing the two known enzymes (approx. 1000 sequences) and subsequent alignment of the closely related sequences a loop insertion close to the active site was identified. Homology modelling revealed that introduction of this loop structure into hex1 and hex2 would lead to a significantly narrower active site and therefore contribute to exclusion of water from the active site, which is a well-known strategy to increase trans-glycosylation activity. The proposed loop mutants were then expressed, purified and characterized towards trans-glycosylation activity. For hex2 it turned out that none of the loop mutants showed an improved trans-glycosylation activity compared to the wild-type. But for hex1 three out of four showed an up to seven-fold improved trans-glycosylation activity compared to the wild-type, which refers even to a higher trans-glycosylation activity than previously observed for the hex2 wild-type. [4]

In conclusion we succeeded in engineering an enzyme towards increased trans-glycosylation activity using a custom-made rational approach utilizing available sequence analysis methods.