Abstract:

Our laboratory studies the influence of glycosylation on protein architecture and the biological activity of fungal hexosaminidases. I studied the enzyme from *Penicillium oxalicum* which has an advantage of quite a homogenous glycosylation. The propeptide of this enzyme is naturally glycosylated only at two places – asparagine 11 and serine 66. Catalytic subunit and propeptide of the hexosaminidase enzyme from *Aspergillus oryzae* were separated from each other for reconstitution experiments. In the propeptide part of the enzyme separated from the *Penicillium oxalicum* hexosaminidase, asparagine 11 and serine 66 were swapped for cysteine by targeted mutagenesis. The newly synthesized propeptide modified with cellobiose, which is a compound most similar to naturally occurrency saccharide chitobiose, promised a significant chance of restoration of enzyme activity. The glycosylated propeptide was separated from the nonglycosylated. In reconstitution experiments we observed the influence of glycosylation on the enzyme structure and activity using different glycosylated propeptides. The highest efficiency occurred during the reconstitution by the original propeptide. Next we used combinations of artificially glycosylated propeptides of which the highest reconstitution efficiency had cellobiose(Asn^{11})-mannose(Ser^{66}) and N-acetylglucosamine(Asn^{11})-mannose(Ser^{66}). Other variants achieved a lower enzyme activity. (In Czech)