

## BROADENING THE BIOCATALYTIC PROPERTIES OF RECOMBINANT SUCROSE SYNTHASE 1

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The present paper describes the modification of the biochemical and biocatalytic characteristics of the recombinant glycosyltransferase sucrose synthase 1 (SuSy1, EC 2.4.1.13) from potato by expression in *S. cerevisiae* or *E. coli* and by site-directed mutagenesis of the putative phosphorylation site S11, respectively. SuSy1 catalyzes the reversible conversion of sucrose and UDP to UDP-glucose and D-fructose. In our previous work SuSy1 was produced in *S. cerevisiae* as a biocatalyst with a broad substrate spectrum leading to NDP-glucoses and a variety of sucrose analogues, respectively [1-4].

Non-phosphorylated SuSy1 expressed in *E. coli* revealed differences in biochemical and biocatalytic characteristics concerning the oligomeric organisation and inhibitors as well as an extended acceptor substrate spectrum. D-tagatose, D-ribulose, D-xylose, L-arabinose, L-glucose, and L-rhamnose were found as novel acceptor substrates. To the best of our knowledge, this is the first report on extending the biocatalytic potential of an enzyme by expression in different host systems. It may be deduced that the differences are caused by the lack of phosphorylation at the highly conserved S11 in the prokaryotic host; on the other hand the eukaryotic microbial expression system produces a biocatalyst which should be phosphorylated depending on the specificities of the protein kinases.

Consequently, we decided to mutate the highly conserved phosphorylation site S11. A phosphorylated serine should be imitated in *E. coli* by exchanging serine (S) against aspartic acid (D) and substitution of serine by alanine (A) should result in a non-phosphorylated mutant expressed in yeast. Both S11 mutants showed lower specific activity and stability than the native enzyme. However, affinities for sucrose and UDP (SuSy1-S11A) and D-fructose and UDP-Glc (SuSy1-S11D) were increased 10-fold. Further experiments indicated that the acceptor substrate spectrum has been also changed. The S11A mutant expressed in yeast shows higher activity for D-xylulose, D-psicose, L-glucose, and L-rhamnose, whereas the S11D mutant expressed in *E. coli* had an increased activity for D-psicose and lost its activity for L-arabinose.

In summary, our results demonstrate that the choice of the expression system and mutations in the highly conserved phosphorylation site S11 alter the substrate spectrum of SuSy1.

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