Structural and functional investigations into Glycocin-Glycosyltransferases

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Antimicrobial resistance is on the rise. Thus, there is an urgent need to discover novel antimicrobials and determine their mechanism of action.
An underexplored class are bacteriocins: Short ribosomally synthesized and post-translationally modified peptides (RiPPs) that often show high potency, and low toxicity. [1, 2]

One subclass requires a glycosylation to show antimicrobial activity and thus have been termed glycoactive bacteriocins, Glycocins [3].
Their challenging synthesis, *in vitro* or *in vivo*, renders the elucidation of their mechanism and their broader use difficult. Reasonable yields of authentic glycocins could only be obtained by the use of the cognate glycosyltransferase that is able to either glycosylate a serine or threonine, resulting in canonical *O*-glycosylation or a cysteine, resulting in a rare type of *S*-glycosylation. [4-6]

Insights into the function of Glycocin-Glycosyltransferases (GGTs) will help to understand the unusual specificity of these transferases for cysteine and/or serine and threonine and the high selectivity for its glycosylation site. Understanding the key elements of the catalytic site may allow to use the transferases as valuable tools to synthesise glycocins, glycopeptides and neo-glycopeptides in general. We identified several putative Glycocins and their cognate GGTs using bioinformatics. The recombinant production and purification of five selected GGTs from *Bacillus subtilis*, *Enterococcus faecalis, Gottfriedia acidiceleris*, *Laceyella sacchari* and *Streptomyces platensis*, was established.
The GGTs were characterised in regard of the metal ion dependency and the carbonucleotide specificity. Using SPPS we synthesised some glycocins and proved their selective glycosylation by the cognate GGT. To investigate the molecular determinants for the observed differences in specificity in terms of preferred sugars and their *S*/*O*-selectivity, we use X-ray crystallography as method of choice. For three GGTs crystals could be obtained and the structure for the *Bs*GGT could be solved to 2.6 Å resolution.

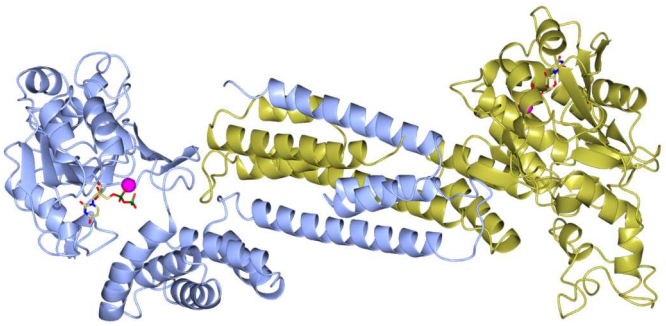

Figure 1. Crystal structure of the crystallographic dimer of *B. subtilis* GGT at 2.6 Å

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