ABSTRACT

Title: Studies on bacterial protein glycosyltransferases of GT-2 family and their use in development of a microbial system for directed evolution of glycosylated peptides and proteins

Part I

O-GlcNAcylation of proteins at Serine/ Threonine residues play vital roles in eukaryotes as well as in prokaryotes. The enzyme that transfer O-GlcNAc on to protein is called O-GlcNAc transferases (OGT). Orthologues of human OGT (hOGT) are present in lower eukaryotes as well as bacteria.O-GlcNAcylated proteins, especially in pathogenic bacteria, are implicated in immunogenicity, proteolytic stability, cell adhesion, biofilm formation, and host-pathogen interactions. GmaR is the first O-linked GlcNAc transferase ever identified in bacteria, namely *Listeria monocytogenes* a foodborne pathogenic Gram-positive bacteria. According to the CAZy database, GmaR belongs to the GT-2 family and is involved in glycosylation of flagellar protein. It is a bifunctional enzyme having an additional role in temperature-dependent regulation of transcription of flagellar motility genes. Accordingly, it acts as a protein thermometer. While genetic evidence for GmaR activity is known, the biochemical and biophysical understanding of the protein was lacking. In this study, we provide an in-depth characterization of glycosyltransferase activity, donor substrate specificity, and enzyme kinetics and the solution structure of GmaR. Using small-angle X-ray scattering (SAXS), the impact of metal ion and substrate binding on the enzyme is studied. Based on these studies, solution structures of the apo form of GmaR, Mg^{2+} bound form of GmaR, the complex of GmaR with Mg^{2+} and UDP-GlcNAc are deciphered. GmaR harbors three different domains in solution, wherein upon binding of

Mg^{2+} metal ion into the active site at the N-terminal, the opening of the loop like structure is observed. Accordingly, from SAXS studies we conclude that the catalytic activity of GmaR may involve an overall shape change mediated through conformational change upon binding of Mg^{2+} and UDP-GlcNAc to the enzyme.

To summarize, the study provides the first evidence of the solution structure of a GT 2 family protein glycosyltransferase. The study also provides optimized conditions for in vitro glycosyltransferase activity of GmaR for application purpose (e.g. O-GlcNAcylation of therapeutic proteins like filgrastim) and finally proposes that flagellar glycosylation could be a co-translational rather than post-translational event

Part II

Bacteriocins are antimicrobial peptides produced by bacteria. The glycosylated bacteriocins are called glycocins. Discovered in firmicutes, glycocins have a wide range of antimicrobial spectrum and stability over a wide range of temperatures and pH. Accordingly, glycocins may have applications in food preservation and or drug discovery. This study describes a hitherto the unavailable recombinant microbial system for the heterologous expression, screening and directed evolution of glycocins in *E. coli*. Using a minimal construct in fusion with the MCH tag, a one-step antimicrobial activity screening assay is achieved and optimized. The system (SELECT-Glycocin) is then suitably exploited for the generation of -O/S- linked mutant libraries of enterocin 96 and screening of glycoactive variants. Enterocin 96 is a di-glycosylated glycocin active against *L. monocytogenes*, a food born pathogen. Among 16 glycoactive variants obtained, at least two improved variants namely C13T and PedioEnt96 could be corroborated and characterized in detail in comparison with Nisin, the only FDA approved food preservative

bacteriocin. Further, we describe a dual vector system optimized for improved yield of glycosylated bacteriocins in a recombinant host.

To summarize, SELECT-Glycocin provides a system and technique a) to screen and evolve known glycocins and b) discovery of novel glycocins especially from bacteria that cannot be cultured under laboratory conditions. For expanding the application of antimicrobial peptides in therapeutics, cosmetics, probiotics, and agriculture, the heterologous microbial systems described here provide application ready glycoengineering tools.