

Sublancin is not a lantibiotic but an S-linked glycopeptide

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Sublancin, an antimicrobial peptide produced by *Bacillus subtilis* 168, was discovered and classified as a lantibiotic more than a decade ago. Interestingly, a search of the recently published *B. subtilis* 168 genome did not reveal genes for any of the four known classes of lantibiotic biosynthetic enzymes. Intrigued by the possibility of a fifth pathway to generate lanthionine containing peptides, we further investigated the biosynthesis of sublancin. We have characterized the structure of sublancin using proteomic technologies including enzymatic digestion, peptide fingerprint mapping, and peptide sequencing using tandem mass spectrometry. Sublancin is shown to be an unusual S-linked glycopeptide containing a glucose attached to a Cys residue, establishing a new post-translational modification. A gene encoding for a putative glycosyl transferase is located within the sublancin biosynthetic gene cluster. The gene was cloned and heterologously expressed, and the activity of the S-glycosyl transferase, named SunS, was reconstituted *in vitro*. The enzyme is shown to have relaxed substrate specificity allowing the preparation of analogs of sublancin. This is the first example of the reconstitution of an S-glycosyl transferase. Glycosylation is essential for the antimicrobial activity of sublancin. A search of the protein databases suggests that sublancin is a member of a larger family of S-linked glycopeptides, further expanding the already impressive structural diversity of post-translationally modified peptide natural products.