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Grant Title: Synthetic machinery of bacterial glycosphingolipids and their applications

Abstract

Glycosphingolipids (GSLs) are amphiphilic compounds consisting of a hydrophobic ceramide moiety and a hydrophilic sugar moiety. GSLs are ubiquitous in eukaryotes but are also found in certain bacteria, mainly belonging to the order Sphingomonadales of α-proteobacteria. As bacterial GSLs are identified as exogenous ligands for CD1d and activate natural killer T (NKT) cells in mice and humans, they are attractive anticancer drug candidates and as tools to aid immune mechanism research. To explore the health implications of bacterial GSLs, the reaction mechanism and substrate recognition mechanism of bacterial GSL synthases was investigated and a production platform for bacterial GSLs using bacterial GSL synthase genes in eukaryotic cells was established.

First, the α-glucuronosylceramide (GlcACer) synthase of a Gram-negative bacterium, *Sphingobium yanoikuyae* was crystalized, achieving a 3.0 Å resolution crystal structure. The overall structure belonged to the glycosyltransferase-B structural group with the putative substrate binding cleft located in the center of the structure. Substrate docking models and mutational analyses revealed amino acids critical for recognition of the sugar donor, UDP-glucuronic acid and sugar acceptor, ceramide. Furthermore, based on the structural information and amino acid alignment of bacterial glycosyltransferases, GlcACer synthase substrate specificity was successfully changed from UDP-glucuronic acid to UDP-glucose. These results contribute to a great understanding of the molecular mechanism directing bacterial GSLs enzymatic synthesis and the physiological function of bacterial GSLs.

To produce the bacterial GSLs, the bacterial α-galactosylceramide (α-GalCer) synthase gene was expressed in eukaryotic microorganisms including budding yeast, *Saccharomyces cerevisiae* and thraustochytrid, a marine microorganism. As a result, α-GalCer synthase activity, not normally exhibited in the host, was detected in *S. cerevisiae* and thraustochytrids, expressing the α-GalCer synthase gene. In α-GalCer synthase gene expressing strains, hexosylceramide production was also confirmed using liquid chromatography-tandem mass spectrometry. Additionally, lipid fractions, extracted from strains expressing the α-GalCer synthase gene were found that activated NKT cell hybridomas. These results support this as the first successful example of inducing bacterial-type GSL production in eukaryotes, facilitating research into bacterial GSLs and their potential applications.