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Grant Title: S-acylation of glycosyltransferases: characterization and functional relevance

Abstract

Ganglioside glycosyltransferases (GGTs) are type-II membrane proteins bearing a short N-terminal cytoplasmic tail, a transmembrane domain (TMD), and a luminal catalytic domain. The expression and activity of these enzymes largely determines the quality of the glycolipids that decorate mammalian cells membranes. Many glycosyltransferases (GTs) are themselves glycosylated and this is important for their proper localization, but few if any other post-translational modifications of these proteins have been reported. In this context, the general objective of the project was to investigate the existence of S-acylation in GTs. By using biochemical, bioinformatics, and cellular and molecular techniques, we demonstrated that the GGTs, ST3Gal-V, ST8Sia-I and β 4GalNAcT-I are S-acylated at conserved cysteine residues located close the cytoplasmic border of their TMDs. ST3Gal-II, a GT that sialylates glycolipids and glycoproteins, is also S-acylated at a conserved cysteine located in the N-terminal cytoplasmic tail. Many others GTs also possess cysteine residues in their cytoplasmic regions suggesting that this modification occurs on these GTs as well (1,2). S-acylation, commonly known as palmitoylation, is catalysed by a family of palmitoyltransferases (PATs) that are mostly localized at the Golgi complex but also at the endoplasmic reticulum (ER) and the plasma membrane. Using GT's ER-retention mutants, we found that S-acylation of β 4GalNAcT-I and ST3Gal-II takes place at different compartments, suggesting that these enzymes are not substrates of the same PAT. Finally, we found that cysteines that are target of S-acylation on β 4GalNAcT-I and ST3Gal-II are involved in the formation of homodimers through disulphide bonds. We observed an increase of ST3Gal-II dimmers in the presence of the PAT inhibitor 2-bromopalmitate. Therefore, we propose that, under certain cellular conditions in which there is a redox imbalance, disulphide formation could regulate the amount of S-acylated GTs and thus impact on GTs function.



1.- Ganglioside glycosyltransferases are S-acylated at conserved cysteine residues involved in homodimerization. Sabrina Chumpen Ramirez, Fernando M. Ruggiero, Jose Luis Daniotti and Javier Valdez Taubas. *Biochemical Journal*. In revision to answer reviewers' comments.

2.- The role of S-acylation in protein trafficking. Jose L. Daniotti, Maria P. Pedro and Javier Valdez Taubas. *Traffic*. In revision to answer reviewers' comments.