Method for the identification of novel glycoproteins modified with ribitol-5-phosphate

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Alpha-dystroglycan (αDG) is an O-mannosylated protein, part of the dystrophin-glycoprotein complex present on the membrane of mammalian cells, whose function is to give rigidity to tissue through binding laminin in the extracellular matrix[1]. αDG has a unique O-glycan, essential for its function, made of a core M3 elongated with two ribitol-5-phosphate (Rbo5P) residues. Terminal Rbo5P acts as a linker to a long chain of alternating xylose and a glucuronic acid named matriglycan. Rbo5P is found in mammalian cells uniquely on αDG[2-3]. Enzymes responsible for the synthesis of the alditol donor CDP-Rbo and for Rbo5P transfer onto the O-mannosyl glycans have been identified[4]. A whole pathway is dedicated to this modification therefore we hypothesize that other mammalian proteins might have glycans with Rbo5P and share a similar function to αDG. Novel glycoconjugates carrying Rbo5P will be identified through the chemical tagging of Rbo5P. In particular, Rbo5P is susceptible to oxidation with sodium periodate which generates an aldehyde on the glycoprotein.

The aldehyde can be tagged using a bioorthogonal conjugation with a biotin probe and purified with a streptavidin column. Glycoproteins carrying Rbo5P enriched this way can then be digested into peptides which are identified by mass spectrometry.

Bibliographic references:

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