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Distinguishing of fucose linkages in *N*-linked complex glycans at the ultrastructural level

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Fucosylation is an oligosaccharide modification on proteins found in invertebrates, plants, bacteria as well as in vertebrates. This study is aimed to distinguish the different linkages of fucose in *N*-linked glycoproteins in situ. We focused on terminal fucosylation and both possible linkages of core-fucosylation. Core-fucose is linked to the proximal *N*-acetylglucosamine of the pentasaccharide core by either the α1,3-linkage, which is typical for plant, trematode, and arthropod *N*-glycans; or by the α1,6-linkage widespread in mammalian/vertebrate *N*-glycans [1]. Changes in fucosylation pattern are specific hallmarks in several types of cancer cells. For example, α1,6-core-fucose linkage of on the α-1-antitrypsin protein is a cancer specific marker in patient with hepatocellular carcinoma [2].

We used soluble glycoproteins containing fucose in the known type of linkage to oligosaccharide and dissolved them separately in gelatin. We used glycoproteins containing either core-fucose only in α1,6-linkage (porcine thyroglobulin); or core fucose only in α1,3-linkage (horseradish peroxidase type II); or terminal fucose (human α1-acid glycoprotein); or lactoferrin from human milk containing both α1,6- linked core fucose and terminal fucose. After initial formaldehyde fixation, material was prepared using two methods: preparation of cryosections and embedding of the material into LR White resin that was UV polymerized at -20°. Sections of glycoproteins were labeled using fucose-specific lectins and specific antibody directed to α1,3-linked core-fucose (anti-Fuc). Deglycosylation enzymes were used as pretreatment of both cryo- and resin sections prior the specific glycan labeling. Results of gold labeling were quantified and statistically evaluated.

Our results proved the possibility to localize and to distinguish glycoproteins containing both α1,3-core-fucose and/or α1,6-core-fucose from terminal fucosylation in the case of bi- and tri-antennary N-linked complex glycans. α1,6-core-fucosylated glycans were positively labeled with lectins from *Aleuria aurantia* (AAL) and *Lens culinaris* (LCA). Glycopeptidase F (cleaving off the whole N-glycan except of α1,3-core-fucosylated ones) treatment of either cryosections or resin sections lead to negative lectin binding, whereas pretreatment of sections using endoglycosidases F2/F3 (leaving the proximal GlcNAc of N-glycans and the fucoses linked to it) cause negative LCA and positive AAL binding reactions. Generally, the labeling efficiency of fucosylated oligosaccharides was higher in cryosections than in resin sections (Figure 1A); however treatment of resin sections with endoglycosidase increased the AAL labeling of α1,6 core-fucose to values close to the labeling efficiency values of labeled cryosections (Figure 1B). This fact proved that accessibility to the core of the glycosylated glycans is a critical factor in resin embedded material. Efficiency of labeling obtained with antibody against the α1,3-core-fucosylation was 66% in cryosections and was considerable lower in resin sections (16%).

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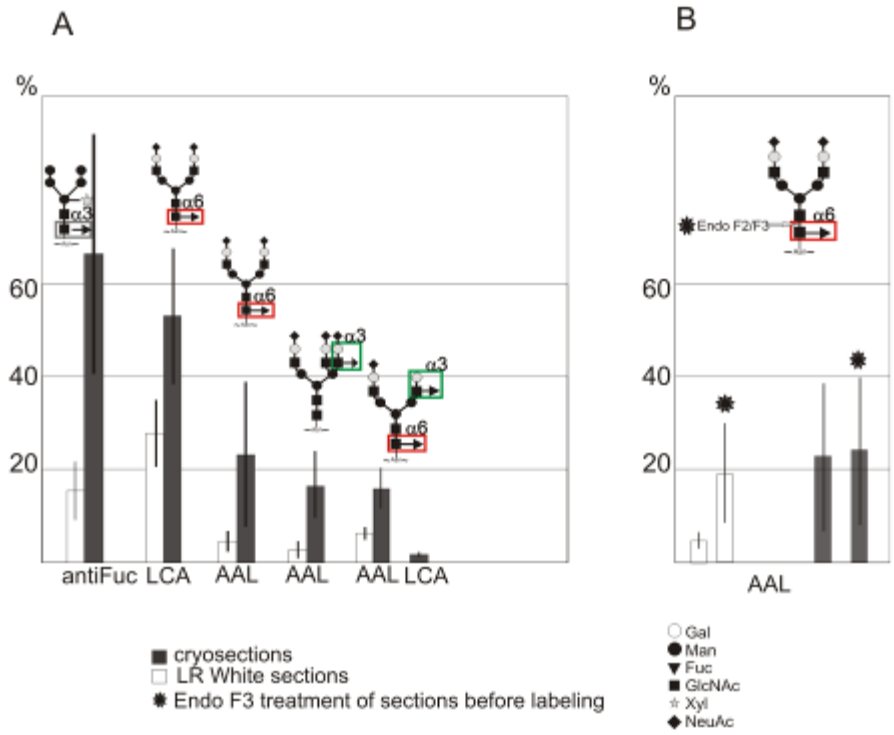

Figure 1. Labeling efficiency of different fucose linkages present in the sections of randomly dispersed soluble glycoproteins (A). Accessibility of α1,6-core fucose is crucial for successful AAL labelling of resin embedded material in contrast to cryosections (B).