The two N-glycans present on bovine Pofut1 are differently involved in its solubility and activity.

O-Fucosylation is a post-translational glycosylation in which an O-fucose is covalently attached to the hydroxyl group of a specific serine or threonine residue. This modification occurs within the consensus sequence C2X(4-5)(S/T)C3 present on epidermal growth factor-like repeats of several proteins, including the Notch receptors and their ligands. The enzyme responsible for the addition of O-fucose to epidermal growth factor-like repeats is protein O-fucosyltransferase 1. Protein O-fucosyltransferase 1-mediated O-fucosylation is essential in Notch signaling, folding and targeting to the cell surface. Here, we studied the expression pattern of protein O-fucosyltransferase 1 in cattle and showed that the active enzyme is present in all tissues examined from embryo and adult as a glycoprotein with two N-glycans. By comparing protein O-fucosyltransferase 1 sequences available in databases, we observed that mammalian protein O-fucosyltransferase 1 enzymes possess two putative N-glycosylation sites, and that only the first is conserved among bilaterians. To gain more insight regarding the significance of N-glycans on protein O-fucosyltransferase 1, we substituted, by site-directed mutagenesis, bovine protein O-fucosyltransferase 1 N65, N163 or both, with L or Q. We demonstrated that the loss of N-glycan on N163 caused a slight decrease in protein O-fucosyltransferase 1 activity. In contrast, glycosylation of N65 was crucial for protein O-fucosyltransferase 1 functionality. Loss of glycosylation at N65 resulted in aggregation of protein O-fucosyltransferase 1, suggesting that N-glycosylation at this site is essential for proper folding of the enzyme.