

OW8 Characterisation of a new post-translational modification, O-linked N-acetylglucosamine-phosphorylation, on a synaptic protein.

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A specific type of glycoposphorylation was observed for the first time.

A novel post translational modification, O-linked N-acetyl glucosamine phosphorylation (O-GlcNAc-P), was found at Thr-310 of assembly protein 180 (AP180). Using a QTOF configuration mass spectrometer, a reporter ion at m/z 284 was the first clue to the existence of this labile modification. The reporter ion was fragmented in a pseudo MS³ experiment. The fragmentation observed in the pseudo MS³ spectrum was nearly identical to an MS² spectrum of synthetic GlcNAc-6-P, although the position of the phosphate could not be determined. To confirm the site of modification and rule out the possibility of a gas phase reaction between GlcNAc and phosphate ions, the modified peptide was analysed by electron transfer dissociation ion trap mass spectrometry. A difference of 384 units between the c5 and c6 ion confirmed the presence of Thr-GlcNAc-P at Thr-310. The Thr-310 was occupied by either O-GlcNAc-P or simply O-GlcNAc, but not phosphorylation alone. This implied a stepwise modification of Thr-310. AP180 is a brain specific component of clathrin coated vesicles and participates in synaptic vesicle endocytosis (SVE) by interacting, through specific domains and motifs, with the membrane, clathrin and the adaptor AP-2 to assemble vesicles. The rapid de-phosphorylation of AP180 occurs immediately prior to SVE. This new modification could be a new dynamic signalling mechanism.