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Algorithm Development for Analysis of O-GlcNAcylated Protein using LC-MS/MS

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O-GlcNAcylation is a post-translational modification that occurs at residues of a protein Ser/Thr by O-GlcNAc transferase (OGT), but the O-GlcNAcylation is regulated by O-GlcNAcase (OGA). The O-GlcNAcylation is involved in various cellular processes and diseases including diabetes, cancer and Alzheimer's disease. We suggest a new method for identification of O-GlcNAcylated proteins with LC-MS/MS. After protein extraction and digestion from B cell lymphoma cell line, HILIC fractionation and LC-MS/MS analysis combined with HCD, triggered HCD and ETD method were performed. Using the fractionated 10 raw files of LC-MS/MS, we developed identification algorithm coded by python 3.9 as follows: 1. Selection of spectra with oxonium ions from raw MS data was obtained using LC-MS/MS; 2.Spectral matching was performed with 20 ppm against a tryptic and O-GlcNAcylated peptide DB generated from Uniprot fasta DB; 3.In the protein candidates, matching score was calculated between b/y and c/z theoretical ion m/z list, and experimental HCD and ETD spectra, respectively; 4.The best calculated score was selected in each O-GlcNAcylated peptide spectrum; 5.The manual validation was performed with evaluating a model using ROC curve comparing search results.