Enhanced Protein N^{α}-acetylation Analysis by SCX and Dimethyl Labeling and Its Application to Discrimination of Protein Isoforms

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Protein N-terminal acetylation is one of the most common modifications occurring co- and post-translationally on either eukaryote or prokaryote proteins. However, compared to other post-translational modifications (PTMs), the physiological role of protein N-terminal acetylation is relatively unclear. To explore the biological functions of protein N-terminal acetylation, a robust and large-scale method for qualitative and quantitative analysis of this PTM is required. Enrichment of N^{α}-acetylated peptides or depletion of the free N-terminal and internal tryptic peptides prior to analysis by mass spectrometry are necessary based on current technologies. This study demonstrated a simple strong cation exchange (SCX) fractionation method to selectively enrich N^{α}-acetylated tryptic peptides via dimethyl labeling without tedious protective labeling and depleting procedures. This method was introduced for the comprehensive analysis of N-terminal acetylated proteins from HepG2 cells under oxidative damage by *tert*-butyl peroxide (*t*-BHP). Several hundreds of N-terminal acetylation sites were readily identified in a single SCX flow-through fraction and the protein N-terminal acetylation patterns with and without oxidative damage were simultaneously determined when the stable isotope dimethyl labeling was introduced. Moreover, the N^{α}-acetylated peptides of some protein isoforms were simultaneously observed in the SCX flow-through fraction, which indicated that this approach can be utilized to discriminate protein isoforms with very similar full sequences but different N-terminal sequences. Compared to other methods, this method is relatively simple and can be directly implemented in a two-dimensional separation (SCX-RP)-mass spectrometry scheme for quantitative N-termini proteomics using stable-isotope dimethyl labeling.