Improved immunoaffinity enrichment method for ubiquitinated peptides with high sensitivity, specificity, and robustness

Mass Spectrometry

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Ubiquitination is an important post-translational modification in eukaryotic cells, triggering protein degradation or cell signaling events. A widely used method for ubiquitination site detection involves tryptic cleavage of ubiquitinated proteins generating a di-glycine remnant (K-GG) at sites of ubiquitination followed by immuno-affinity enrichment using an anti-K-GG antibody and liquid chromatography-mass spectrometry (LC-MS) analysis. Such a method allows identification and quantification of thousands of ubiquitination sites from cells and tissues but typically requires large starting protein amounts and results in co-isolation of many non-specifically binding unmodified peptides. In this study, we introduce an improved immuno-affinity enrichment method for K-GG peptides that overcomes these issues. Our optimized immuno-affinity enrichment method provides 2-fold improved sensitivity, 2-3 fold improved specificity, and ~99% reduction in antibody released from beads upon acid elution. One milligram of mouse liver protein yields nearly 6,000 ubiquitinated peptides identified, numbers typically generated with over 10 mg of starting protein. The decrease in antibody eluted from the beads with K-GG peptides reduces the demand for C18 stage-tip cleaning and increases overall stability of the LC system. In addition, the optimized method uses magnetic beads instead of agarose beads, which speeds and simplifies the procedure. In conclusion, our optimized K-GG peptide immuno-affinity enrichment method significantly improves sensitivity, specificity, and robustness of ubiquitinated peptide identification and quantification.