Analytical Characteristics of ICAT-LC-MS/MS for Quantitative Proteomics Studies

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Introduction: Recent studies have employed isotope-coded affinity tags (ICAT) in concert with tandem mass spectrometry (MS/MS) to assess differential expression of proteins on a proteomic scale and perform unbiased quantitative comparisons between samples from different physiological or disease states. We sought to determine the performance characteristics and analytical profile of ICAT-based quantitative proteomics studies using proteins released by follicular lymphoma (FL)-derived cells as a model system. **Materials and Methods:** FL-derived cells were subjected to mitogenic stimulation for 12 hours in serum-free media. A control sample was grown in serum-free media without stimulation. Proteins precipitated from the media of both samples were ICAT-labeled, subjected to trypsin digestion, and analyzed by LC-MS/MS. The ICAT-labeled fraction and the flow-through fraction were both analyzed in triplicate and the obtained spectra were assessed with cross correlation and delta correlation scores. **Results:** A total of 244 peptides meeting standard criteria, corresponding to 227 proteins, were identified in the ICAT-labeled samples. Twenty-five percent of the peptides contained labeled cysteine residues and had quantitative results. An additional 50 peptides were identified from the flow-through sample. In the combined analysis of all samples, only 5 proteins were identified with multiple unique peptides; the remainder of the proteins were identified using a single peptide. Peptides with quantitative data demonstrated a quantitative range from 0.01-20-fold differential expression indicating a dynamic range of greater than three orders of magnitude. **Conclusions:** ICAT is a convenient approach for analysis of quantitative changes on a proteome-wide scale. ICAT-based studies are advantageous because of reduction of sample complexity and selection of cysteine labeled peptides for quantification. Analysis of flow-through material consisting of non-ICAT tagged peptides, or replicate analyses of ICAT-labeled peptides do not contribute significantly to quantitative information. ICAT-LC-MS/MS protein quantifications are largely based on single peptides and therefore confirmation by other methods might be necessary in some instances.