ICAT^{TM}, ICRAP, ICROC: Evolving methods for cysteine capture and stable isotope incorporation for relative protein quantitation

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Our initial experiments using the isotope coded affinity tag (ICAT^{TM}) reagent revealed a few problems that were overcome using another strategy. This alternative, termed isotope coded reducible affinity proteomics (ICRAP), employed a cysteine reactive biotin label with a reducible disulfide linker, which was used to trap cysteine-containing peptides on an avidin column. The peptides were gently eluted from the avidin column using a reducing agent, and the cysteine thiols were isotope tagged using either N-ethyl iodoacetamide or d_5-N-ethyl iodoacetamide. Both of these alkylating reagents can be prepared in a single step using ethylamine (or its deuterated counterpart) and iodoacetic anhydride. The advantages of this method over ICAT^{TM} were that we observed fewer non-specifically bound peptides (i.e., peptides lacking cysteine), the ICRAP isotope tag was of much lower mass, and it did not affect the charge state of the peptides in electrospray ionization. Furthermore, in contrast to ICAT^{TM} labeled peptides, we observed no highly abundant tag-specific fragment ions in the tandem mass spectra. One problem with the ICRAP method was that the biotinylation reagent had poor solubility, which made it difficult to force the reaction forward. Hence, an alternative strategy was devised called isotope coded reduction off of a chromatographic support (ICROC). This method is similar to ICRAP, except that thiol reactive beads are employed, rather than a biotinylation reagent. The cysteine containing peptides are attached to a solid support via a reducible disulfide linker, the beads are washed, and the cysteine-containing peptides are eluted using a reducing agent. The isotope tagging uses the aforementioned N-ethyl iodoacetamide reagents. Validation experiments were successfully performed, and the ICROC method was subsequently applied to the study of cell surface proteolysis. New and previously known cell surface metalloprotease substrates were identified.