Tackling the challenge of quantifying co-migrating proteins in two dimensional gel electrophoresis-based proteome analysis.

ABSTRACT

Introduction: When using two-dimensional gel electrophoresis-based proteome analysis technology (2-DE) co-migrating proteins can present a major problem for quantitative protein expression comparison. It has been described that up to six proteins may appear in a single spot. When two or more proteins are identified in a spot differentially displayed between two sample groups, it becomes very time consuming to discover which one is up- or down regulated. To tackle this problem we used the iTRAQ^{TM} labeling technique (Applied Biosystems), originally developed for gel-free proteome analysis, and applied it to an in-gel digest containing 2 proteins in order to distinguish the expression profile of the different proteins present in the spot.

Methods: To optimize the labeling procedure and determine the quantitative accuracy, different amounts of a protein mix (520-2080ng) were loaded onto SDS-PAGE. Paired protein bands were excised and in-gel digested using trypsin. The extracted peptide samples were labeled with either Tag_{114} or Tag_{117}, subsequently desalted and analysed on-line by nano-LC MS/MS. The MS/MS spectra were interpreted manually.

The technique was validated on a second SDS PAGE construct. We therefore combined bands containing the same amount of one protein with bands containing different amounts of other proteins. The samples were digested and purified as described above. For each pair of protein mixtures one situation was labelled with Tag_{114} and the other with Tag_{117}.

Preliminary data: To analyse the quantitative accuracy of this approach we first compared bands from a 1-DE gel containing known amounts of the same protein and labeled the in-gel digests with Tag_{114} or with Tag_{117}. Manual quantitative interpretation of the MS/MS spectra showed ratios matching the known amounts of protein loaded. Both Silver and Sypro stained gels were tested, but no significant differences in label efficiency or quantitative accuracy were found.

We then applied the same approach to in-gel digests containing several proteins of which only one was present in equal amounts. Preliminary data shows that MS/MS spectra with equal tag peak areas derived from this protein can be distinguished from spectra with different tag peak areas derived from the proteins present in variable amounts. This approach will allow us to determine the expression profile of an undefined number of proteins present in a single spot.

We are in the process of validating this approach with 2-DE spots differentially expressed in a proteome analysis known to contain 2 proteins.

Innovation: We have developed a straightforward approach to quantify the expression of co-migrating proteins in a 2-D gel electrophoresis.