**Intact Protein Quantification by Mass Spectrometry**

Wed 8:30 AM - Track 3: Novel Approaches

Mark W. Duncan & David S. Gibson

Division of Endocrinology, Metabolism & Diabetes, Department of Medicine, School of Medicine, University of Colorado Denver, Aurora, Colorado 80045.

 There are few approaches available for the precise and sensitive quantification of proteins. Clinical chemists have relied on immunoassays, but these have significant limitations. Immuno-based strategies require access to antibodies and when these are not available, development time, cost and complexity can be substantial. Further, antibody specificity can limit assay performance. For example, antibodies may not deliver sufficient selectivity to allow independent quantification of all biologically relevant protein variants.

Recent attention has been focussed on mass spectrometric approaches, most notably peptide centric strategies. These begin with sequence specific enzymatic cleavage of proteins to peptides, and subsequent proteotypic peptide quantification, usually by multiple reaction monitoring performed on a triple quadropole mass spectrometer. This approach eliminates the requirement for antibodies and consequently, assay development time is reduced markedly. Nevertheless, the requirement for enzyme-specific proteolysis can itself compromise assay performance parameters, especially specificity.

A third option for protein quantification is MALDI mass analysis of intact proteins. However this strategy must be combined with reproducible and efficient sample clean-up, ideally incorporating some sort of target protein concentration. For example, immuno-based isolation strategies combined with MALDI allow rapid, sensitive and isoform specific quantification. To demonstrate the utility of these approaches we have determined serum amyloid A (SAA) in human plasma samples collected from several groups of subjects where there is reportedly a marked elevation in the levels of this acute phase reactant.

Figure 1 (top) shows the average MALDI mass spectrum of all samples following immune-affinity clean-up of SAA. Significant heterogeneity in SAA is evident and arises because of genetic variability, truncations and other non-enzymatic and enzymatic modifications. This heterogeneity confounds attempts to precisely and accurately measure SAA(s) either by ELISA, or without special attention to the selection of the proteolytic peptides, by peptide centric approaches.