

The majority of proteins in eucaryotes contain post-translational modifications (PTMs). For example, 60-70% of these proteins are glycosylated, and a similar number are phosphorylated. Current proteomics techniques only identify protein expression level, and ignore PTMs despite their importance in numerous cellular functions. This has led us to pursue alternative approaches to provide information on PTM changes at the proteome level. For example, lectins have a long history of usage in the separation and identification of glycoproteins and glycopeptides, which has led us to use lectin chromatography as the first step in a multidimensional LC approach for proteomics. We have also investigated the ability of reverse phase, size exclusion, and ion exchange to enrich fractions for glycosylated peptides. These glycopeptide pools are then deglycosylated enzymatically. This will serve to further reduce the complexity of this mixture by eliminating glycan heterogeneity and enhances the MS signal. The deglycosylated peptides are then subjected to RPLC-MS/MS to allow the identification of their parent proteins. The use of N-linked glycopeptides has an advantage in that all of these glycopeptides must contain an NXT/S sequon, providing an additional sequence tag to further refine the protein identification step. Our current methodology has allowed us to identify N-linked glycosylation sites on proteins found in human serum and glycoproteins produced by the epimastogote stage of *Trypanosoma cruzi*. Ultimately, procedures will be developed to identify glycoproteins whose abundance, extent of glycosylation, site of glycosylation and/or glycan structure change with the physiological condition, information which is not readily obtainable with any of the current proteomic approaches.