Mass spectrometry for comparative proteomics of degenerative and regenerative processes in the brain

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Biological processes involve changes at the protein level. Proteomics aims to determine protein changes from a normal state, to measure for instance recovery or disease progression. Mass spectrometry is the most important tool for identification of proteins and determination of post-translational modifications such as glycosylation. Glycoproteins are found to be altered in patients with Alzheimer's disease (AD). Changes in glycosylation levels were quantified after gel separation and glycan structures were determined with mass spectrometry. Protein quantification with mass spectrometric methods is based on stable isotope labeling of proteins. Quantitative proteomics was applied to assess protein expression levels with mass spectrometry in the murine brain after specific neurosurgery to study regenerative processes.

In order to compare glycosylated proteins in cerebrospinal fluid (CSF) from AD patients with control individuals, albumin depleted CSF proteins were separated with narrow pH-range two-dimensional gel electrophoresis followed by multiple staining for quantification of glycoprotein isoforms. Structural site-specific analysis of glycopeptides was performed with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). A decreased glycosylation level for α_1-antitrypsin was found in AD patients. No specific glycoform of the studied proteins could be assigned to AD, emphasizing that further studies should include a larger subject group and cover proteins in various pH intervals. Knowledge of glycoprotein structures may assist in elucidation of the pathogenic process.

In order to study proteins involved in the response of astrocytes and regenerative processes after neurotrauma, a quantitative mass spectrometric method was developed. Astrocytes react to neurotrauma by becoming reactive (reactive gliosis). Mice lacking the intermediate filament proteins GFAP and vimentin (*GFAP*$^{-/-}$*Vim*$^{-/-}$) show attenuated reactive gliosis and enhanced regeneration after neurotrauma. Culture-derived isotope tags (CDIT) and nano-liquid chromatography FT-ICR MS showed that the 14-3-3 epsilon isoform was upregulated in denervated hippocampus in wildtype mice, while this response was attenuated in *GFAP*$^{-/-}$*Vim*$^{-/-}$ mice. Thus, the increase in 14-3-3 epsilon expression in neurotrauma appears to be linked to astrocyte activation. We demonstrated that the CDIT-based quantitative proteomic method is a highly useful approach to assess isoform-specific protein expression levels in defined parts of the brain after neurosurgical interventions.

Keywords: glycoproteomics, quantitative proteomics, protein quantification, glycoprotein, glycosylation, Fourier transform ion cyclotron mass spectrometry, cerebrospinal fluid, Alzheimer's disease, neurodegeneration, hippocampus, astrocytes, neuroregeneration, brain injury, 14-3-3 protein, isoform

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I. **Glycoproteomics of cerebrospinal fluid in neurodegenerative disease.**
Sihlbom Carina, Davidsson Pia, Emmett Mark R., Marshall Alan G. and Nilsson Carol L.
International Journal of Mass Spectrometry (2004) 234, 145-152.

II. **Prefractionation of cerebrospinal fluid to enhance glycoprotein concentration prior to structural determination with FT-ICR mass spectrometry.**
Sihlbom Carina, Davidsson Pia and Nilsson Carol L.
Journal of Proteome Research (2005) 4, 2294-2301.

III. **Structural and quantitative comparison of cerebrospinal fluid glycoproteins in Alzheimer's disease patients and healthy individuals**
Sihlbom Carina, Davidsson Pia, Sjögren Magnus, Wahlund Lars-Olof and Nilsson Carol L.
submitted manuscript

IV. **14-3-3 expression in denervated hippocampus after entorhinal cortex lesion assessed by culture-derived isotope tags in quantitative proteomics**
Sihlbom Carina, Wilhelmsson Ulrika, Li Lizhen, Nilsson Carol L. and Pekny Milos.
submitted manuscript