

CHARACTERISATION OF INTACT PROTEINS AND ANTIBODIES BY LC-AND CZE-ESI-TOF MS

Matthias Pelzing¹, Christian Neusuess²

¹ Bruker Daltonics, Melbourne, Australia and ² University of Applied Sciences, Aalen, Germany.

Fast and information-rich Glycoprotein characterization by ESI-TOF MS after appropriate separation by LC or CZE is presented.

An increasing number of drugs are based on recombinant proteins. Particularly antibodies are expected to be of significant importance in future biopharmaceutical developments. However, there is a lack of fast methods, which are appropriate for both quality control and for development support of these complex molecules. This is primarily due to the often observed microheterogeneity of posttranslational modified proteins. Especially glycosylation often leads to a broad range of isoforms. These complexities in addition to the observed charge envelope in ESI MS prevent often a direct analysis with mass spectrometry. Thus, separation is required, though difficult to achieve due to variable properties of proteins. An orthogonal-accelerated ESI-TOF MS at a resolution of 15000 is used for obtaining spectra of different glycoproteins as well as intact antibodies of various sources, applied both intact and reduced. Separation is achieved in various matters: an LC-based approach using a C8 column at 60°C has been developed in order to obtain clear spectra for intact antibodies as well as for the separation of light and heavy chain after reduction. A CZE approach using acetic acid as background electrolyte and a new dynamic polyacrylamide-based coating has been applied to separate glycoforms of proteins like Ribonuclease B, fetuin, alpha-acid glycoprotein or erythropoietin. Furthermore, a fast CZE-MS method for the characterization of non-derivatized glycans has been developed to support the protein data. Based on the separation and efficient online desalting clear spectra for these intact proteins could be obtained. The mass accuracy was determined for the charge-deconvoluted spectra: accuracy for the isotopically resolved small proteins (= 15-17kDa) is better than 5 ppm. For non-isotopically resolved mass spectra a reproducibility of better than 1 Da was achieved. Even intact antibodies (140-150kDa) could be characterized with a reproducibility of a few Dalton allowing the determination of glycosylated isoforms. The LC separation enabled both the sensitive characterization of intact antibodies. Furthermore, it is easily possible to separate light and heavy chain and obtain their accurate mass. The CZE-MS approach allows the separation of glycoforms, differing in its sialic acid content. Moreover, even small mass/size changes without introducing a charge (as repeats of hexose-N-acetylhexosamines) could be separated applying a new dynamic coating material.

Based on the carbohydrate information on the glycan level an integral carbohydrate composition for each intact glycoform can be assigned with high confidence. Thus, the CZE-ESI-MS analysis of both intact glycoproteins and released glycans is an excellent method, for the development of therapeutically improved glycoproteins like erythropoietin. This approach or similarly the LC-ESI TOF MS method enables a fast and information-rich quality control of heterogenic intact proteins.