

Application of LTQ Orbitrap XL ETD™ for Glycopeptides Analysis

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Of all protein post-translational modifications (PTMs), Glycosylation is the most widespread and complex one. Its modifications are highly labile and resulting peptides are most often highly heterogeneous. Characterization of glycopeptides remains a great analytical challenge. LC MS/MS is the most powerful and versatile techniques for glycopeptides structure elucidation. However, commonly used collisional-induced dissociation (CID) has limitations on determining the modification site due to the labile nature of the glycan modifications. The very recent ability to routinely obtain high resolution and high accurate mass measurements of MS and MS/MS fragments combined with Electron Transfer Dissociation (ETD) provides a new and powerful tool that makes the identification of modification site and glycan structure elucidation possible.

Two reasonably well characterized glycoproteins, bovine α 1-acid glycoprotein and human α 1-acid glycoprotein were purchased from Sigma. The proteins were reduced, alkylated and enzymatic digested. The glycopeptides were then introduced onto a graphitic carbon column for nano LC MS/MS analysis. LTQ Orbitrap XL ETD spectrometer was used for glycosylation site determination and glycan structure elucidation.

Graphitic carbon column demonstrated excellent capabilities for glycopeptides analysis especially for short hydrophilic peptides containing bi- or tri-antennary glycan chains without any enrichment. With the high mass accuracy and high resolution of hybrid linear ion trap-Orbitrap MS, the highly heterogeneous glycopeptides were well resolved and accurately measured. Formation of metal adducts on Hypercarb column promotes higher charge species and as a result improves ETD fragmentation of glycopeptides which lead to the successful determination of the glycosylation site. Therefore both the glycopeptides glycosylation site and glycan structure were successfully identified by using the

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combination of porous graphite chromatography and LTQ Orbitrap XL ETD Hybrid FT mass spectrometer in a single LC run.

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