Analysis of highly heterogeneous macromolecular medicines using a novel top-down platform based on native LC/MS/MS

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Characterization of biopharmaceutical products is a challenging task, as it must address not only the covalent structure, but also the conformation and, in some cases, correct assembly of a multi-unit protein. Typically, three different levels of covalent structure modification of therapeutic proteins must be considered: (*i*) enzymatic post-translational modifications (PTMs), such as glycosylation (*ii*) non-enzymatic PTMs, such as oxidation, and (*iii)* "designer" PTM, such as PEGylation or conjugation to a cytotoxic agent. LC/MS is now routinely used to characterize such highly heterogeneous systems. Until recently, the LC method of choice was reversed phase HPLC, which can be readily interfaced with MS. More recently, the advantages of using native MS and MS/MS in combination with non-denaturing separation methods to characterize complex biopharmaceutical products have also been demonstrated [1-3]. Ion exchange chromatography (IXC) is particularly beneficial for the analysis of protein drugs that exhibit significant structural heterogeneity, as both enzymatic and non-enzymatic PTMs frequently produce charge variants that can be resolved by IXC. The use of native ESI MS as an on-line detection tool for IXC provides direct analysis without prior fraction collection or sample manipulation. Despite recent advances, analysis of PEGylated protein drugs still remains a challenging task, as the degree of structural heterogeneity introduced by the synthetic polymer chains is overwhelming for direct ESI MS or even LC/MS analysis. Two recently introduced approaches to MS characterization of highly heterogeneous biopolymers are very promising. First, limited charge reduction induced by reacting mass-selected populations of biopolymer ions with either electrons or anions in the gas phase had been shown to provide meaningful information for a range of highly heterogeneous targets, including such previously intractable systems as intact heparin [4]. An orthogonal approach makes use of collision-activated dissociation (CAD) as a way to remove the polymer chain from the polypeptide to enable meaningful mass measurement for the latter. In this presentation, both of these techniques (used in combination with IXC) will be evaluated as a means of characterizing a stressed PEGylated therapeutic interferon β1a and several other highly heterogeneous biopaharmaceuticals, including mAbs. We will also present examples of using on-line native LC/MS as a means of obtaining meaningful structural information on other extremely heterogeneous macromolecular therapeutics, such as heparin and heparin-based medicines.

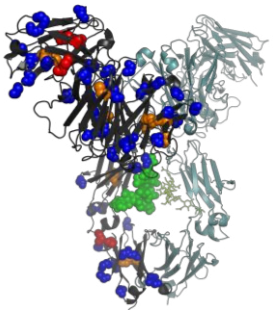

Figure 1. Potential PTM sites within mAb.

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