Characterizing Deamidation and Oxidation in Adalimumab with Low pH Peptide Mapping and Middle-Up Mass Spec Analysis

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1. Introduction

Non-enzymatic chemical modifications such as deamidation, oxidation and disulfide bond scrambling can affect the stability and efficacy of biotherapeutic proteins. Peptide mapping, the method of choice for site-specific monitoring of these modifications, has a significant drawback which is that the sample preparation methods often induce the same modifications intended to be measured. A primary source of these sample preparation artifacts is the alkaline pH favored by trypsin and other commonly used proteases. To address this problem we have developed a sample preparation procedure in which all steps including proteolytic digestion are performed under acidic conditions. Although trypsin is inhibited at low pH, we have overcome this limitation by supplementing the digestion with a low pH resistant Lys-C protease. Here we describe the utility of this new sample preparation procedure for analysis of deamidation in Adalimumab (Humira). We show that observed deamidation sites appear to be artifacts induced during conventional sample preparation procedures. In addition, we have analyzed peroxide-induced oxidation in Adalimumab, both by peptide mapping and subunit level analysis following digestion with IdeZ protease. An oxidation suppressant added to the peptide mapping protocol reduces oxidation when digestion occurs in the presence of peroxide.

2. Study Design

Deamidation Analysis: Low pH vs Conventional Digestion

Adalimumab was denatured, reduced, silylated and digested into peptides using either a conventional protocol (pH 8) with Trypsin or with a low pH protocol utilizing Trypsin and a low pH resistant Lys-C.

Oxidation Analysis: Suppression of Peroxide-Induced Oxidation

Adalimumab was exposed to peroxide in the presence or absence of an oxidation suppressant while simultaneously being digested at low pH with Trypsin and a low pH resistant Lys-C.

Oxidation Analysis: Middle-Up MS with IdeZ Protease

Adalimumab was oxidized with increasing peroxide concentrations for 45 min, then digested with IdeZ protease for 30 min into F(ab')2 and Fc fragments. Middle-up MS Analysis was performed to monitor oxidation.

3. Analysis of Adalimumab Deamidation

A. Comparison of Conventional and Low-pH Digestion

Dissociation at low pHe produces equivalent sequence coverage

Dissociation at low pHe does not affect C-terminal Lysine truncation, glycosylation or pyroglutamate.

B. XIC Analysis of “PENN” Peptide Deamidation (GFYPSDIAVEWESNGQPENNYK)

C. Deamidation Summary for all Peptides

4. Analysis of Adalimumab Oxidation

A. Oxidation Study of Adalimumab using IdeZ Digestion and Middle-Up MS

B. Addition of an Oxidation Suppressor Reduces Peroxide-Induced Met-Oxidation

5. Conclusions

We have developed a method for peptide mapping at low pH

Key Benefits of this method:

• Robust digestion: Sequence coverage is equivalent to conventional pH digestion.
• Artifact suppression: Deamidation and disulfide bond scrambling are not introduced.
• Oxidation during sample prep can be reduced with an optional oxidation suppressant.

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