proteomics

PNGase F Enzyme: Efficient N-Deglycosylation in Glycoprotein Analysis

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Application Notes

- Convenient, stable, freeze-dried formulation with a long shelf life
- Enzyme formulation is detergent-free and stabilizer-free and won't interfere with MS analysis
- Resulting protein and glycan samples are compatible with MS analysis
- Deglycosylated tryptic peptides ionize more readily, yielding additional MS peaks and increased overall sequence coverage

Introduction

Glycosylation is one of the most common post-translational modifications of proteins in eukaryotic cells. These glycoproteins are involved in a wide range of biological functions such as receptor binding, cell signaling, immune recognition, inflammation and pathogenicity. Mammalian glycoproteins contain three major types of oligosaccharides (glycans): N-linked, O-linked and glycosylphosphatidylinositol (GPI) lipid anchors. N-linked glycans are attached to the protein backbone via an amide bond to an asparagine residue in an Asn-Xaa-Ser/Thr motif, where X can be any amino acid, except Pro. O-linked glycans are linked via the hydroxyl group of serine or threonine. Variation in the degrees of saturation at available glycosylation sites results in heterogeneity in the mass and charge of glycoproteins.

To study the structure and function of a glycoprotein, it is often desirable to remove all or just a select class of glycans. This approach allows the assignment of specific biological functions to particular components of the glycoprotein. The removal of N-linked glycans from glycoproteins eliminates heterogeneity in MALDI-TOF mass spectrometric (MS) analysis. Also, removal of glycans may enhance or reduce the blood clearance rate and/or potency of a glycoprotein therapeutic. Although sites of potential N-glycosylation can be predicted from the consensus sequence Asn-Xaa-Ser/Thr, it cannot be assumed that a site will actually be glycosylated. Therefore the sites of glycan attachment must be identified and characterized by analytical procedures.

Peptide-N-glycosidase F (PNGase F) is one of the most widely used enzymes for the deglycosylation of glycoproteins. The enzyme releases asparagine-linked (N-linked) oligosaccharides from glycoproteins and glycopeptides. A tripeptide with the glycan-linked asparagine as the central residue is the minimum substrate for PNGase F. The glycan can be a high-mannose, hybrid or complex type. However, N-glycans with fucose linked α1,3 to the Asn-bound N-acetylglucosamine are resistant to the action of PNGase F.

MALDI-TOF MS is a widely used technique for rapid identification of proteins separated by gel electrophoresis. Glycopeptides, however, suffer from signal suppression during MS analysis due to the microheterogeneity of the attached glycans. In this application note, we have used PNGase F enzyme (Product Code P 7367) for the deglycosylation of a model glycoprotein, α1-antitrypsin, and have identified and localized N-glycosylation sites by MALDI-MS peptide mapping. The ProteoProfile Enzymatic In-Gel N-Deglycosylation Kit (Product Code PP 0200) conveniently provides quality-tested reagents and a detailed protocol for in-gel protein deglycosylation.

Efficient and convenient in-gel protein deglycosylation

A specific example demonstrating the use of the enzyme is the deglycosylation of the glycoprotein α1-antitrypsin. After reduction and alkylation (using tributylphosphine and iodoacetamide contained in the ProteoPrep Reduction and Alkylation Kit, Product Code PROT-RA), samples are mixed with SDS-PAGE sample buffer and separated on 10% BisTris NuPAGE® gel (Invitrogen Corp., Carlsbad, CA) using MOPS running buffer. The gel is stained with 0.1% Coomassie blue stain and destained.

The glycoprotein band from the 1D gel (or a spot from a 2D gel) is carefully cut out and sliced into sections. The pieces are destained, dried and incubated with PNGase F. The liquid surrounding the gel pieces is removed and either discarded or retained for gylcan analysis if required. The dried gel pieces are incubated with trypsin (Product Code T 6567) and the surrounding liquid containing the tryptic peptides is removed for further analysis.

Facile sample preparation for mass spectrometry

Sample preparation is simplified since the enzyme formulation does not contain detergents and stabilizers that could interfere with analysis by MS. The solution of tryptic

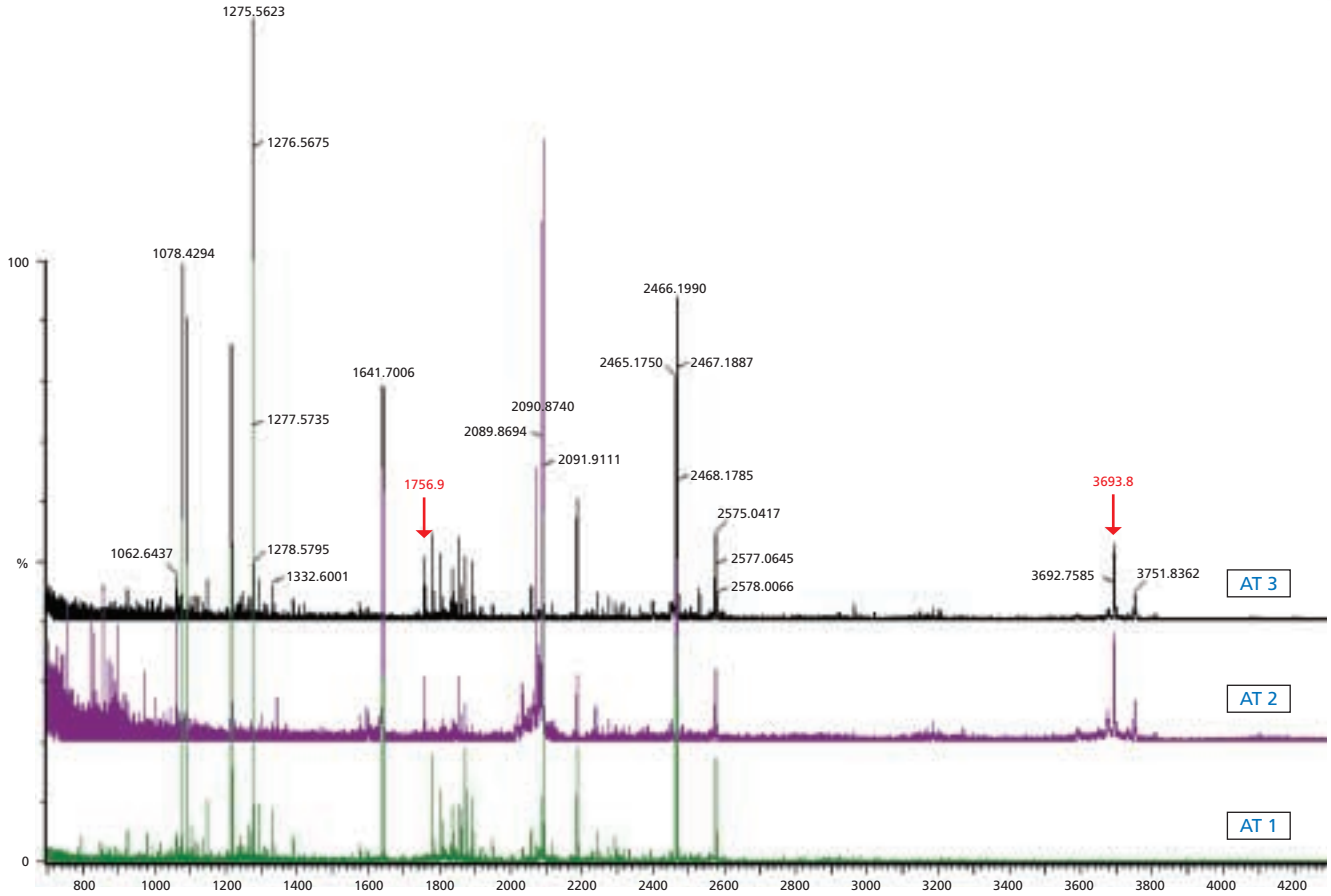

Figure 1. MALDI-TOF MS analysis of native and deglycosylated α1-antitrypsin. *Peptide mass profile of control (AT1), in-gel deglycosylated (AT2) and in-solution deglycosylated (AT3) human α1-antitrypsin. All proteins were subjected to trypsin digestion prior to MS analysis. The signals at m/z 1756 and 3693 in the PNGase F-treated sample (absent in the control sample) correspond to peptide fragments 268-283 and 94-125, respectively.*

peptides is concentrated and desalted on a C_{18} ZipTip® (Milliopore Corporation, Billerica, MA) prior to analysis. For the results reported here, MS analysis was carried out on a MALDI-TOF instrument fitted with a reflectron and a 337-nm UV laser. Peptides were analyzed in positive ion reflector mode using α-cyano-4-hydroxycinnaminic acid as the matrix. Spectra were acquired over a mass range of 4000 m/z with matrix suppression set at 800 mass units. Data analysis was carried out using ProteinLynx™ software (Water Corporation, Milford, MA).

Peptide mass profiles of in-gel and in-solution deglycosylated α1-antitrypsin are shown in Figure 1. The shift in MW on deglycosylation in solution can be observed by SDS-PAGE analysis, as illustrated in Figure 2.

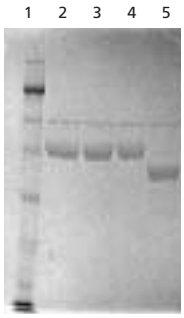

Figure 2. SDS-PAGE analysis of native and PNGase F-treated α1-antitrypsin. *The test sample (Lane 5) was deglycosylated in solution with 5 units of PNGase F for 1 hour at 37 °C prior to separation on SDS-PAGE. Note the shift in the mobility of the band upon deglycosylation.*

Lane 1: Molecular weight markers;
Lanes 2, 3, 4: Control, native α1-antitrypsin;
Lane 5: In-solution deglycosylated α1-antitrypsin.

Summary

PNGase F is an effective enzyme for the release of N-linked glycans from glycoproteins, in gel or in solution. The formulation employed for PNGase F (Product Code P 7367) provides a convenient, stable, freeze-dried product that is compatible with MS analysis. This enzyme is also included in the In-Gel and In-Solution Deglycosylation Kits that provide Proteomics grade, high purity enzymes and reagents for N-deglycosylation and tryptic digestion.

Ordering Information

Product	Description	Unit
P 7367	PNGase F, Proteomics Grade, Lyophilized	50 units 300 units
PP0200	ProteoProfile Enzymatic In-Gel N-Deglycosylation Kit	1 kit
PROT-RA	ProteoPrep Reduction and Alkylation Kit	1 kit
T 6567	Proteomics Grade Trypsin	5 x 20 µg
PP0201	ProteoProfile Enzymatic In-Solution N-Deglycosylation Kit	1 kit
PP0100	Trypsin Profile IGD Kit	1 kit

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