



Characterization of N-Linked Protein Glycosylation

Purpose

The purpose of this project was to characterize glycoforms and identify the site(s) of N-linked glycosylation for a protein sample. Characterization is important for demonstrating control of the process to reproducibly manufacture the protein. Wolfe Laboratories utilized rapid and scalable methods that are suitable for characterization during early biotherapeutic development, and for comparability and lot release studies.

Experimental Design

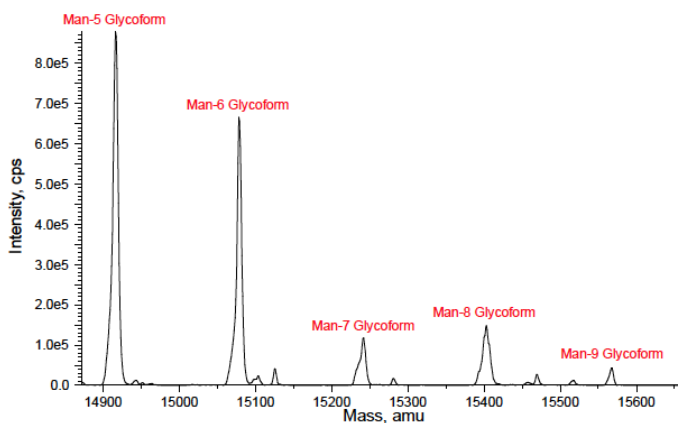
Wolfe Laboratories used mass spectrometry to examine glycoforms of the intact protein. Wolfe Laboratories identified the sites of glycosylation by tryptic peptide mapping (enzymatic proteolysis followed by LC-MS/MS analysis) of the glycosylated and unglycosylated proteins. N-Linked glycans were released from RNase B, purified, and labeled with 2-AB (2-aminobenzamide) before analysis by UPLC equipped with a fluorescence detector. Peaks were identified by comparison to authentic 2-AB labeled standards.

Conclusions

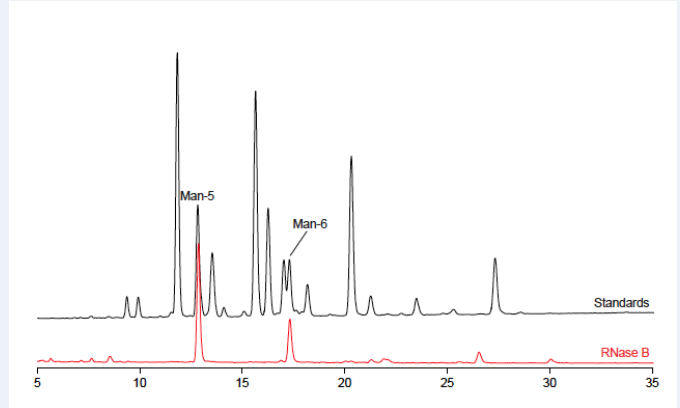
Wolfe Laboratories confirmed that Asn34 was the single site of glycosylation on RNase B. The five glycoforms (Man-5, Man-6, Man-7, Man-8, and Man-9) were characterized by multiple, orthogonal methods, in agreement with scientific literature. Fluorescence spectroscopy shows that Man-5 and Man-6, identified by comparison to authentic standards, were the predominant glycoforms for this sample.

1. Joao, H.C., and Dwek, R.A., Eur. J. Biochem., 218, 239-244 (1993).

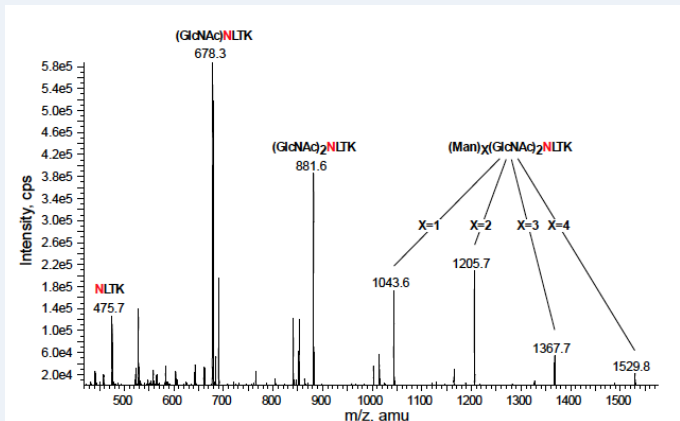
Deconvoluted mass spectrum of the intact RNase B shows Man-5 through Man-9 glycoforms.



Fluorescence spectra of 2-AB glycans show primarily Man-5 and Man-6. Minor peaks are consistent with predicted retention times for Man-7 to Man-9.



LC-MS total ion current chromatograms show unglycosylated (RNase A) and glycosylated (RNase B) tryptic peptides.



LC-ESI/MS/MS product ion spectrum (15.1 min, m/z 846.5+2 amu) confirms the site of glycosylation, Asn34, and has a fragmentation pattern consistent with NLTK-Man-5.

