In Vitro N-Glycosylation of Peptides Using PNGase F and Determination of N-Glycosylated Sites by Acid Hydrolysis

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Abstract

N-linked glycosylation is very complex process that begins with the transfer of a lipid-linked oligosaccharide moiety ($Glc_3Man_9GlcNAc_2$-P-P-Dolichol, where Glc is glucose, Man is mannose, GlcNAc is *N*-acetylglucosamine and P is phosphate) to asparagine residues of nascent polypeptide chains by the oligosaccharyltransferase[1]. Although glycosylation is intricate steps that undergo changes with a number of glycosyltransferases, it can be overcome by the reverse reaction using peptide-*N*-glycosidase F (PNGase F) at a time.

In vitro glycosylation of a pentapeptide, RKDVY, with PNGase F caused some nonenzymatic glycosylation, known as the Maillard reaction, as well as enzymatic glycosylation[2].

In this work, we improved the possibility of glycan attachment using enzymes for industrial applications, and we found that acid hydrolysis of N-glycosylated peptides is an effective method for the identification of unexpected N-glycosylation locus. In addition, A glycan, *N,N'*-diacetylchitobiose (two N-acetylglucosamines) was identified to be frequently attached to Arg and Lys residues in peptides through the nonenzymatic glycosylation.

Key Words : N-linked glycosylation, peptide-*N*-glycosidase F, acid hydrolysis

Reference

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