GlycoPOD

Abstract

Recently, carbohydrate chains occupy truly significant positions in various fields of life sciences and biotechnology. Accordingly, an increasing number of students and researchers are conducting experiments on carbohydrate chains. However, we sometimes encounter a common beginner's feeling that experiments in glycoscience are not so easy to perform satisfactorily. In molecular biology, most of the reagents are commercially available as a series of kit and all you need is to follow the operating manual provided with the distributors of the reagents. In glycoscience, in contrast, Materials and Methods in a professional journal may not be enough to provide beginners with sufficient information about the experimental procedures of their interest.

GlycoPOD (GlycoScience Protocol Online Database) project started in May 2009 as a part of the program of the Japan Consortium for Glycobiology and Glycotechnology Data Base (JCGGDB), aiming to fill the gaps between the beginners and experts by delivering the accumulated knowledge and skills of the experts to the beginners through internet communication under the catch-phrase of "Follow the flow chart and you will get the goal directly". Now, more than 150 experts (JCGG members) have been involved in the GlycoPOD activity so far.

Features of GlycoPOD

As of March 1, 2017, GlycoPOD (*see* Figure 1) provides 262 protocols.

As an on-line protocol, GlycoPOD has a unique architecture. It consists of three main parts, "Introduction", "Protocol" and "References" (*see* Figure 4 & 10). In "protocol", all the experimental procedures are documented in flow charts with comments, which contain precious tips for success.

If you choose "Authors" at the top page, you will see the name of all authors listed in alphabetical order (*see* Figure 2).

If you click "`KeyWords" button, all the experimental procedures listed in "protocol" are broken down into individual experimental elements (parts) and

they are reorganized in alphabetical order (*see* Figure 3). "KeyWords will be useful in case you cannot find the exact protocol you need in "Protocols (main text)", or you want to modify the experimental conditions of the original protocol and others.

Here, we would like to introduce a general outline of GlycoPOD.

1. Introduction (*see* Figure 5 & 11)
This section contains a summary of the method or outline of the major procedures involved in the protocol.

2. Protocol (*see* Figure 4 & 10)
"Protocols" has 11 sections, Category, Protocol Name, Authors, Keyword, Reagents & Instruments, Methods (Comments), Note, Initial Amounts, Discussion, and Figure & Legend.

2-1. Category (17 categories)
All the protocols (216 as of March 1, 2017) are organized into 17 categories of the following:

Isolation & structural analysis of glycans
N-Glycans
O-Glycans
Glycolipids and related compounds
Glycosaminoglycans
Glycosyltransferases & related proteins
Nucleotide sugar transporters
Sugar binding proteins
Matrices & cellular trafficking
Biosynthesis & Metabolism
Glycogene transgenic animals
Glycosidases & related proteins
GPI anchored proteins
Large scale preparation of glycans, glycoproteins & glycolipids
Roles of glycans during microbial infection

Plant cell Wall analysis
Glyco-proteomic mass spectrometry protocols

2-2. Key Words
A few important key words in protocols are listed in this section.

2-3. Reagents & Instruments
Recommendable reagents or instruments for the experiment are itemized with supplier/manufacturer names.

2-4. Methods (Procedures)
This is the main section of the protocol and explains in detail the individual steps necessary to carry out the experiment.

2-5. Comments (for each procedure) (*see* Figure 7 & 13)
Any hints and tips that help improve your method are described.

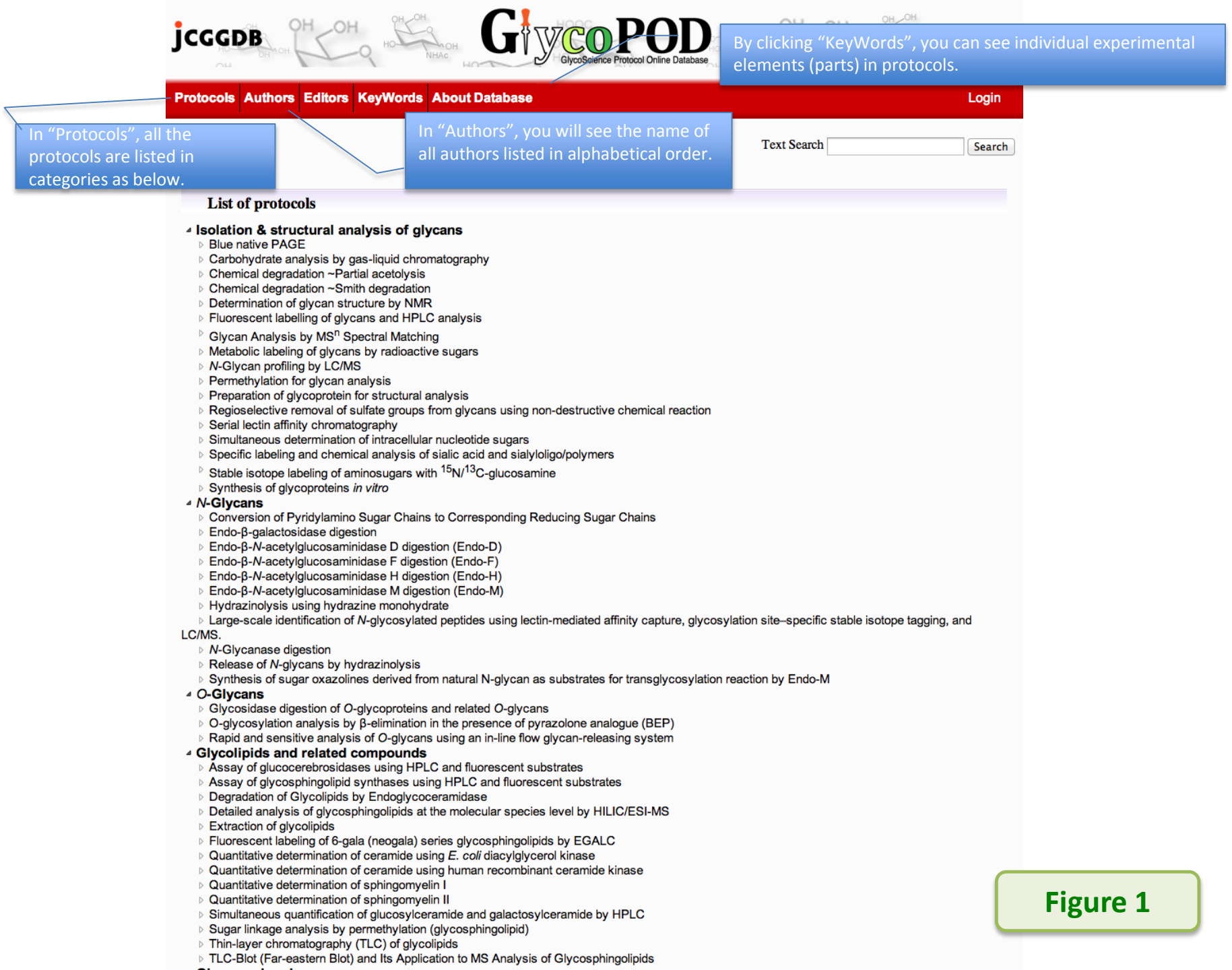
2-6. Notes (*see* Figure 14)
Overall comments or any other special instructions for the method are described in this section.

2-7. Initial Amount
The amount of samples required for analysis is described here.

2-8. Discussion
The interpretation of the results are explained in this section.

2-9. Figure & Legend

3. Reference
All references of figures, tables, or text passages cited in protocols are listed in this section. PubMedIDs are indicated at the end of each reference, and clicking the ID links the Pubmed pages (*see* Figure 8 & 15).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Carbohydrate analysis by gas-liquid chromatography
Chemical degradation ~Partial acetolysis
Chemical degradation ~Smith degradation
Determination of glycan structure by NMR
Fluorescent labelling of glycans and HPLC analysis
Glycan Analysis by MSn Spectral Matching
Metabolic labeling of glycans by radioactive sugars
N-Glycan profiling by LC/MS
Permethylation for glycan analysis
Preparation of glycoprotein for structural analysis
Regioselective removal of sulfate groups from glycans using non-destructive chemical reaction
Serial lectin affinity chromatography
Simultaneous determination of intracellular nucleotide sugars
Specific labeling and chemical analysis of sialic acid and sialyloligo/polymers
Stable isotope labeling of aminosugars with 15N/13C-glucosamine
Synthesis of glycoproteins in vitro
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Conversion of Pyridylamino Sugar Chains to Corresponding Reducing Sugar Chains
Endo-β-galactosidase digestion
Endo-β-N-acetylglucosaminidase D digestion (Endo-D)
Endo-β-N-acetylglucosaminidase F digestion (Endo-F)
Endo-β-N-acetylglucosaminidase H digestion (Endo-H)
Endo-β-N-acetylglucosaminidase M digestion (Endo-M)
Hydrazinolysis using hydrazine monohydrate
Large-scale identification of N-glycosylated peptides using lectin-mediated affinity capture, glycosylation site–specific stable isotope tagging, and LC/MS.
N-Glycanase digestion
Release of N-glycans by hydrazinolysis
Synthesis of sugar oxazolines derived from natural N-glycan as substrates for transglycosylation reaction by Endo-M
O-Glycans
Glycosidase digestion of O-glycoproteins and related O-glycans
O-glycosylation analysis by β-elimination in the presence of pyrazolone analogue (BEP)
Rapid and sensitive analysis of O-glycans using an in-line flow glycan-releasing system
Glycolipids and related compounds
Assay of glucocerebrosidases using HPLC and fluorescent substrates
Assay of glycosphingolipid synthases using HPLC and fluorescent substrates
Degradation of Glycolipids by Endoglycoceramidase
Detailed analysis of glycosphingolipids at the molecular species level by HILIC/ESI-MS
Extraction of glycolipids
Fluorescent labeling of 6-gala (neogala) series glycosphingolipids by EGALC
Quantitative determination of ceramide using E. coli diacylglycerol kinase
Quantitative determination of ceramide using human recombinant ceramide kinase
Quantitative determination of sphingomyelin I
Quantitative determination of sphingomyelin II
Simultaneous quantification of glucosylceramide and galactosylceramide by HPLC
Sugar linkage analysis by permethylation (glycosphingolipid)
Thin-layer chromatography (TLC) of glycolipids
TLC-Blot (Far-eastern Blot) and Its Application to MS Analysis of Glycosphingolipids

Figure 1


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Abe, Hiroko (1)
Akama O., Tomoya (1)
Akiyoshi, Sayaka (1)
Amano, Junko (1)
Angata, Kiyohiko (1)
Angata, Takashi (1)
Aoki, Kazuhiro (1)
Aoyama, Naoki (1)
Ariki, Shigeru (1)
Asano, Masahide (1)
Ashida, Hisashi (1)
Chisada, Shin-ichi (1)
Denda-Nagai, Kaori (1)
Dobson M, Christina (1)
Dong, Weijie (1)
Endo, Tamao (1)
Fang, Meng (1)
Fujihira, Haruhiko (1)
Fujinawa, Reiko (1)
Fujita, Kiyotaka (4)
Fujita, Morihisa (3)
Fukuda N., Michiko (1)
Fukuda, Tomohiko (1)
Furukawa, Jun-ichi (1)
Furukawa, Keiko (3)
Furukawa, Koichi (10)
Goda M., Hatsumi (1)
Goto-Inoue, Naoko (1)
Goto, Satoshi (1)
Goto, Yoshiyuki (1)
Gu, Jianguo (1)
Guo, Chao-Tan (1)
Habuchi, Hiroko (1)
Habuchi, Osami (2)
Haga, Yoshimi (2)
Harada, Yoichiro (3)
Hashimoto, Yasuhiro (1)
Hayashi, Yasuhiro (2)
Hirabayashi, Jun (2)
Hirayama, Hiroto (1)
Honke, Koichi (2)

Figure 2


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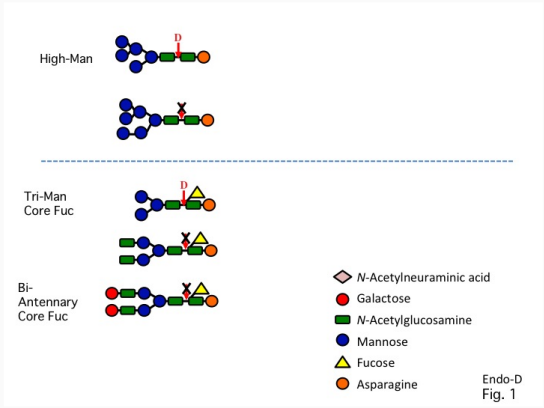
% body weight
α 1-6 fucose (core fucose)
α(1,2)-fucose
α-1,2-Galactosyltransferase
α1-6 fucose (core fucose)
β-N-Acetylgalactosaminidase
β-N-Acetylglucosaminidase
β-elimination
β-galactosidase
β-galactoside
1-amino-1-deoxy derivative
2-O-sulfotransferase
5D4 monoclonal antibody
6-gala (neogala) series GSLs
Acetohydrazide
Acetylation
Acidic GSLs (ganagliosides)
Aggregation chimera
Alkaline phosphatase
Annexin
Antibody
Apoptosis
Arylazide
Asialoglycoprotein receptor
Asparaginyl-oligosaccharides
Bacteria
Baculoviruses Overexpression system
Behavior
Blood group antigen
Branch analysis
C.elegans
C4ST
C6ST
CD3ζ
CL-P1
CMP-Neu5Ac
CMP-Neu5Gc
COX-2
Carbohydrate mediated endocytosis
Cations
Cell growth factor receptors
Cell wall
Cellulose
Ceramide
Ceramide kinase
Chemical reaction
Choline oxidase
Chondroitin sulfate
Climbing assay
Cmah
Collectin
Competitive ELISA
Complement activation assay
Conversion
Cy3 fluorescence labeling
DAB (3,3'-Diaminobenzidine tetrahydrochloride; DOJINDO)
DMB
DSS (dextran sodium sulfate)
Dendritic cell
Desulfation
Detergent resistant membrane microdomains (DRM)
DiI Labeling
Diacylglycerol kinase (DGK)
Diacylglycerol kinase (DGK) assay
Drosophila
Drosophila melanogaster
EDEM (ER degradation enhancing α-mannosidase-like protein)
EGALC
EGCase
ELISA
EMARS
ENGase
ER (endoplasmic reticulum)
ER-associated degradation (ERAD)
ER-to-Golgi transport
ERAD
ERAD (ER associated degradation)
ERQC (ER quality control)
EXT gene family
Ebola virus
Embryonic lethality
Endoglycosidase
Endoplasmic reticulum-associated degradation (ERAD)
Endothelial cells
Enrichment
Envelope glycoprotein
Enzyme assay
Enzyme-linked lectin binding assay
Epidermal Growth Factor
Extra-embryonic tissue
Extracellular matrix
Extraction of glycosphingolipids (GSLs)
F5F8D
FLP/FRT system
FRET
Feeding RNAi
Ficolin-2
Flight assay
Flow cytometry
Fluorescent label
Fractionation
Fucosylation
Functional Glycomics
GFP
GLC
GPI anchor
GPI-anchored proteins
Gal 6-O-sulfotransferase(Gal6ST)
Gal/GalNAc specific lectin
GalNAc transferase
GalNAc4S-6ST
Galectin
Ganglioside
Ganglioside complex
Gas chromatography /

Figure 3

Endo-β-*N*-acetylglucosaminidase D digestion (Endo-D)

Authors : Kiyotaka Fujita, Kenji Yamamoto (Ver 1.0) GET IN CONTACT

Category	*N*-Glycans
Protocol Name	Endo-β-*N*-acetylglucosaminidase D digestion (Endo-D)
Authors	***Kiyotaka Fujita*** Faculty of Agriculture, Kagoshima University ***Kenji Yamamoto*** * Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University *To whom correspondence should be addressed.
KeyWords	asparagine-linked glycan endoglycosidase N-glycan endo-β-N-acetylglucosaminidase
Reagents	• Endoglycosidase D from *Streptococcus pneumoniae* (formerly known as *Diplococcus pneumoniae*). Enzymes purified from culture filtrate are commercially available from Seikagaku Biobusiness Corp. (Tokyo, Japan) and United States Biological (Swampscott, MA). • Neuraminidase, β-galactosidase, and β-*N*-acetylglucosaminidase are commercially available from Sigma-Aldrich (St. Louis, MO) and others. • 5× Reaction buffer : 500 mM citrate phosphate buffer (pH 6.0) • 2× SDS-PAGE sample buffer: 0.125 M Tris-HCl buffer (pH 6.8), 10% β-ME, 4% SDS, 10% sucrose, 0.004% Bromophenol blue
Instruments	• Reaction incubator or water bath (37°C) • SDS-PAGE system
Methods	**1. Release of oligosaccharides from glycoproteins by using Endo-D.** 1) Transfer 20 μL of glycoprotein sample (10 μg/μL), 10 μL of 5× reaction buffer, 2.5 μL of 400 mU Endo-D, and 10 μL of deionized water into a microtube. If complete removal of oligosaccharides is required, 2.5 μL of 800 mU neuraminidase, 2.5 μL of 400 mU β-galactosidase, and 2.5 μL of 400 mU β-*N*-acetylglucosaminidase should be added with Endo-D. Comment 1 2) Incubate at 37°C for 20 h. Comment 0 3) To examine the release of oligosaccharide, mix 5 μL of the reaction sample and 5 μL of 2× SDS-PAGE sample buffer, and then heat at 100°C for 3 min. Comment 0 4) Load 10 μL of the sample mixture on SDS-PAGE gel and run the electrophoresis. Perform either Coomassie blue staining or silver staining. Comment 0
Figure & Legends	**Figure & Legends**  **Fig. 1. Specificity of Endo-D.**
Copyrights	

Date of registration : 2016-01-21 11:34:01

Figure 4

Endo-β-*N*-acetylglucosaminidase D digestion (Endo-D)

Authors : Kiyotaka Fujita, Kenji Yamamoto (Ver 1.0) GET IN CONTACT

Introduction | **Protocol** | **References** | **Credit lines**

Endoglycosidases cleave at defined sites within an oligosaccharide chain of glycoproteins/glycolipids. They are the most easy-to-use enzymes for elucidating the function and structure of oligosaccharides, because they can separate both intact oligosaccharide chains and proteins/lipids from glycoconjugates under mild conditions without causing damage.

Endo-β-*N*-acetylglucosaminidase (EC 3.2.1.96) catalyzes the hydrolysis of the *N*, *N*'-diacetylchitobiose moiety in the core region of asparagine-linked oligosaccharides of various glycoproteins. The enzyme has a characteristic to remain one *N*-acetyl-D-glucosamine residue on the protein. On the other hand, the deglycosylation method using PNGase F cannot remove oligosaccharides unless the protein is denatured. Thus, only endo-β-*N*-acetylglucosaminidases can be used for deglycosylation of native glycoproteins.

Endo-β-*N*-acetylglucosaminidase D (Endoglycosidase D or Endo-D) is the first to-be-reported endo-β-*N*-acetylglucosaminidase that releases N-linked oligosaccharides from glycoproteins [1]. This enzyme can hydrolyze tri-mannose core fucosyl oligosaccharide and $Man_5GlcNAc_2Asn$, but not $Man_6GlcNAc_2Asn$, hybrid-type and complex- type oligosaccharides (Fig.1)[2]. Thus, the presence of unsubstituted α-mannosyl residue at the C-3 position of the innermost β-mannosyl residue is essential for the action of Endo-D[3]. Endo-D has been used for the structural study of IgG glycopeptides[4] and for the removal of oligosaccharides from glycoproteins (*e.g.*, IgG, fetuin, transferring, etc)[5].

Figure 5

Endo-β-*N*-acetylglucosaminidase D digestion (Endo-D)

Authors : Kiyotaka Fujita, Kenji Yamamoto (Ver 1.0) GET IN CONTACT

Introduction | **Protocol** | References | Credit lines

Category	*N*-Glycans
Protocol Name	Endo-β-*N*-acetylglucosaminidase D digestion (Endo-D)
Authors	***Kiyotaka Fujita*** Faculty of Agriculture, Kagoshima University ***Kenji Yamamoto*** * Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University *To whom correspondence should be addressed.
KeyWords	asparagine-linked glycan endoglycosidase N-glycan endo-β-N-acetylglucosaminidase
Reagents	• Endoglycosidase D from *Streptococcus pneumoniae* (formerly known as *Diplococcus pneumoniae*). Enzymes purified from culture filtrate are commercially available from Seikagaku Biobusiness Corp. (Tokyo, Japan) and United States Biological (Swampscott, MA). • Neuraminidase, β-galactosidase, and β-*N*-acetylglucosaminidase are commercially available from Sigma-Aldrich (St. Louis, MO) and others. • 5× Reaction buffer : 500 mM citrate phosphate buffer (pH 6.0) • 2× SDS-PAGE sample buffer: 0.125 M Tris-HCl buffer (pH 6.8), 10% β-ME, 4% SDS, 10% sucrose, 0.004% Bromophenol blue
Instruments	• Reaction incubator or water bath (37°C) • SDS-PAGE system

Figure 6

Methods	**1. Release of oligosaccharides from glycoproteins by using Endo-D.** 1) Transfer 20 µL of glycoprotein sample (10 µg/µL), 10 µL of 5× reaction buffer, 2.5 µL of 400 mU Endo-D, and 10 µL of deionized water into a microtube. If complete removal of oligosaccharides is required, 2.5 µL of 800 mU neuraminidase, 2.5 µL of 400 mU β-galactosidase, and 2.5 µL of 400 mU β-*N*-acetylglucosaminidase should be added with Endo-D. 2) Incubate at 37°C for 20 h. 3) To examine the release of oligosaccharide, mix 5 µL of the reaction sample and 5 µL of 2× SDS-PAGE sample buffer, and then heat at 100°C for 3 min. 4) Load 10 µL of the sample mixture on SDS-PAGE gel and run the electrophoresis. Perform either Coomassie blue staining or silver staining.	Comment 1 Comment 0 Comment 0 Comment 0

Comment

Step : Transfer 20 µL of glycoprotein sample (10 µg/µL), 10 µL of 5X reaction buffer, 2.5 µL of 400 mU Endo-D, and 10 µL of deionized water into a microtube. If complete removal of oligosaccharides is required, 2.5 µL of 800 mU neuraminidase, 2.5 µL of 400 mU β-galactosidase, and 2.5 µL of 400 mU β-*N*-acetylglucosaminidase should be added with Endo-D.

Comment

List of Comment

User: All **Type:** All

RCG 2013-10-31 11:32:35
Native glycoprotein samples can be used for the reaction.

Clicking the Comment, you can see notices for each step.

Figure 7

Endo-β-*N*-acetylglucosaminidase D digestion (Endo-D)

Authors :

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Introduction | Protocol | References | Credit lines

1. Muramatsu, T. (1971) Demonstration of an endoglycosidase acting on a glycoprotein. *J. Biol. Chem.* **246**, 5535-5537 [PMID : 4108054]
2. Muramatsu, T. (1978) Endo-beta-N-Acetylglucosaminidase D from *Diplococcus pneumoniae*. *Methods Enzymol.* **50**, 555-559 [PMID : 26846]
3. Tai, T., Yamashita, K., Ogata-Arakawa, M., Koide, N., Muramatsu, T., Iwashita, S., Inoue, Y. and Kobata, A. (1975) Structural studies of two ovalbumin glycopeptides in relation to the endo-beta-N-acetylglucosaminidase specificity. *J. Biol. Chem.* **250**, 8569-8575 [PMID : 389]
4. Tai, T., Ito, S., Yamashita, K., Muramatsu, T. and Kobata, A. (1975) Asparagine-linked oligosaccharide chains of IgG: a revised structure. *Biochem Biophys Res Commun.* **65**, 968-974 [PMID : 239717]
5. Muramatsu, T., Koide, N. and Maeyama, K. (1978) Further studies on endo-beta-N-acetylglucosaminidase D1. *J Biochem.* **83**, 363-370 [PMID 75885]

Clicking the PMID links to show the PubMed page.

Abstract ▾ Send to: ▾

J Biochem. 1978 Feb;83(2):363-70.

Further studies on endo-beta-N-acetylglucosaminidase D1.

Muramatsu T, Koide N, Maeyama K.

Abstract

The purification procedure for endo-beta-N-acetylglucosaminidase D was improved to yield an enzyme preparation which was homogeneous upon gel electrophoresis. The molecular weight of the enzyme as estimated by Sephadex G-200 column chromatography was 280,000, while SDS-gel electrophoresis after reduction with 2-mercaptoethanol gave a value of 150,000. The purified enzyme did not show any chitinase, hyaluronidase or lysozyme activity. In the presence of exoglycosidases removing peripheral sugars, the endoglycosidase acted on serum glycoproteins such as transferrin and fetuin. The enzyme also hydrolyzed an oligosaccharide, (Man)5(GlcNAc)2, indicating that the peptide portion of substrates does not have much effect on susceptibility to the enzyme.

PMID: 75885 [PubMed - indexed for MEDLINE] Free full text

Figure 8

Authors

Nobuko Kawasaki (8)

Click this icon to access the protocol.

Contributor

Name	Nobuko Kawasaki
Affiliation	Research Center for Glycobiotechnology , Ritsumeikan University
Protocols	***N*-Glycanase digestion** (Ver.1.0) *Nobuko Kawasaki* 2015-03-26 **Isolation of mannan-binding protein from human serum (plasma) ~Method 3** (Ver.1.0) *Nobuko Kawasaki* 2015-03-26 **Assay method for the lectin activity of mannan-binding protein ~ELLBA-3** (Ver.1.0) *Nobuko Kawasaki* 2015-03-26 **Assay method for the lectin activity of mannan-binding protein ~ELLBA-1** (Ver.1.0) *Nobuko Kawasaki* 2015-03-26 **Isolation of mannan-binding protein from human serum (plasma) ~Method 2** (Ver.1.0) *Nobuko Kawasaki* 2015-02-25 **Isolation of mannan-binding protein from human serum (plasma) ~Method 1** (Ver.1.0) *Nobuko Kawasaki* 2015-02-25 **Determination of mannan-binding protein by enzyme-linked immunosorbent assay (ELISA)** (Ver.1.0) *Nobuko Kawasaki* 2015-02-25 **Assay method for the lectin activity of mannan-binding protein ~ELLBA-2** (Ver.1.0) *Nobuko Kawasaki* 2015-02-25

Figure 9

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Figure 10

N-Glycans

N-Glycanase digestion

Authors : Nobuko Kawasaki (Ver 1.0) GET IN CONTACT

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Introduction | Protocol | References | Credit lines

N-glycanase [peptide-N^4-(*N*-acetyl-β-D-glucosaminyl) asparagine amidase, EC 3.5.1.52], also called peptide *N*-glycanase, PNGase, glycopeptidase and glycoamidase, is a kind of amidase that acts on the glycosylamide linkages between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex (bi, tri-, or tetraantennary) oligosaccharide chains from *N*-linked glycoproteins (Fig.1). *N*-glycanase F (PNGase F) (Plummer TH Jr et al. 1984) from *Flavobacterium meningosepticum* is assumed to hydrolyze the glycosylamine linkage by a mechanism similar to that of the analogous almond enzyme (glycopeptidase A) (Takahashi N, Nishibe H. 1978), and the aspartylglycosylamine amidohydrolase (EC3.5.1.26), (Makino M et al. 1966), which was first found in blood serum and tissues cleaving the acid-amide linkage only in a small molecules consisting of one Asn linked to one GlcNAc residue.

Figure 11

N-Glycanase digestion

Authors : Nobuko Kawasaki (Ver 1.0) GET IN CONTACT

Create a new version for this protocol

Category	*N*-Glycans
Protocol Name	*N*-Glycanase digestion
Authors	***Nobuko Kawasaki*** Research Center for Glycobiotechnology, Ritsumeikan University Address：1-1-1 Nojihigashi, Kusatsu, Shiga 525-8577, Japan Phone：+81-77-561-3452 Fax：+81-77-561-3452 Email address：14v00048@gst.ritsumei.ac.jp
KeyWords	peptide N-glycanase glycopeptidase asparagine-linked glycan amidase PNGase F N-glycan N-glycanase glycoamidase
Reagents	• PNGase F (from *Flavobacterium meningosepticum*, molecular weight 35.5k) is commercially available from a number of sources. Enzymes purified from culture filtrate, New England BioLabs Inc., Ipswich, MA, Sigma-Aldrich, St. Louis, MO (proteomics grade) etc. Recombinant enzymes (expressed in *E. coli)* from Roche Diagnostics GmbH, Mannheim, Germany (*N*-glycosidase F), Sigma-Aldrich, Takara Bio Inc., Otsu, Japan etc. • Glycopeptidase A (from almond emulsion), also called glycoamidase A is available from Seikagaku Corp., Tokyo Japan, Sigma-Aldrich, and Roche Diagnotics GmbH. • 1% SDS • 7.5% Nonidet P-40 (NP-40) • 0.2 M sodium phosphate buffer, or 0.2 M Tris-HCl buffer, pH 7.5–8.5 • 1% PMSF dissolved in 2-propyl alcohol • dithioerythreitol • iodoacetamide • 50% acetonitrile • 50 mM–100mM ammonium bicarbonate buffer, pH 8.5 • C18 Sep-Pak cartridge (Waters Corp., Milford, MA) • pepsin (alternatively, trypsin and chymotrypsin)
Instruments	• Reaction Incubator or water bath (100°C, 37°C)

Figure 12

Methods

1. *In solution* deglycosylation of *N*-linked glycoprotein with PNGase F.

1) Heat a glycoprotein sample (10–50 μL, 1–2 mg/mL) in 0.2 M sodium phosphate buffer or 0.2 M Tris-HCl buffer, pH 7.5–8.5, containing 0.5% SDS, 50 mM 2-mercaptoethanol at 100°C for 3 min. Comment 1

2) Put 10 μL of the denatured glycoprotein sample solution into a microtube. Comment 1

3) Add 10 μL of 0.2 M sodium phosphate buffer or 0.2 M Tris-HCl buffer, pH 7.5, containing 2 mM EDTA and 0.03% PMSF. Comment 1

4) Add 5 μL of 7.5% NP-40 (the concentration of NP-40 is 7 fold excess that of SDS in the reaction mixture). Comment 1

5) Add 5 μL of PNGase F solution to give a final enzyme concentration of 0.5–10 U (IU) /mL. Comment 1

6) Incubate at 37°C for 18 h. Comment 0

7) Stop the digestion by boiling the reaction mixture for 5 min. Comment 0

8) Analyze aliquots of the reaction product.⇒SDS-I

9) Add 3 volumes of ice-cold ethanol.⇒Centrifuge
oligosaccharides.

2. Releasing the *N*-glycans from the glycopeptide w

1) Dissolve glycopeptide(s) sample (~100 μg) in 20(

2) Add 2 μL of PNGase F (2 mU).

3) Incubate at 37°C overnight.

4) Separate the *N*-glycans from de-*N*-glycosylated p
mL).

5) Wash the cartridge with 5% acetic acid.

6) Elute the cartridge with 3 mL each of 20 and 40% propanol in 5% acetic acid (a third 60% propanol fraction can be collected but usually glycopeptides will elute earlier. The alternative water/acetonitrile solvent system can also be used). Comment 0

7) Pass through (*N*-glycans)⇒ Comment 0
Analyze the *N*-glycan structures by MS analysis, etc.

8) Eluate (de-*N*-glycosylated peptides)⇒ Comment 0
Identify the respective glycosylation sites in the de-*N*-glycosylated peptides by subjecting
to automated nanoLC-ESI-MS/MS analysis etc. (by digestion with PNGaseF, Asn in the original glycosylated peptides changed to Asp in de-*N*-glycosylated peptides (*see* Fig.1)).

Clicking the Comment, you can see notices for each step.

Comment

Step : Heat a glycoprotein sample (10–50 μL, 1–2 mg/mL) in 0.2 M sodium phosphate buffer or 0.2 M Tris-HCl buffer, pH 7.5–8.5, containing 0.5% SDS, 50 mM 2-mercaptoethanol at 100°C for 3 min.

Comment

List of Comment

User: All Type: All

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The pH optimum for PNGase F activity is 8.6, but the enzyme is at least 80% active between pH 7.5 and 9.5. PNGase F is compatible with a wide variety of inorganic and organic buffers (0.1 M), including sodium phosphate, lithium carbonate, ammonium bicarbonate, Tris-HCl, glycylglycine, N-2-hydroxyethylpiperazine-N'- 2-ethansulfonic acid (HEPES), and triethylamine acetate, but sodium borate is inhibitory (Tarentino L et al. 1994)

Figure 13

Notes	Two different *N*-glycanases, PNGase F and glycopeptidase A are now commonly used as a tool for liberating native oligosaccharides from the original glycoprotein molecule. The specificity of PNGase F is well established and the enzyme hydrolyzes asparagine-linked glycans representing all major oligosaccharides classes, but both the amino and carboxyl groups of the asparagine residue have to be in peptide linkage. PNGase F preferred a tripeptide or longer (Fan JQ, Lee YC. 1997). Asparagine-linked oligosaccharides containing an α(1→6)-fucose substituted on the asparagine-proximal GlcNAc residue are easily hydrolyzed by PNGase F, but the corresponding core α(1→3)-fucose substituent, found in glycoproteins from plants, insects and other lower animals completely blocks deglycosylation. This appears to be the only known structural feature of an oligosaccharide moiety that confers resistance to PNGase F. In contrast, glycopeptidase A can hydrolyze asparagine-linked oligosaccharides containing an α(1→3)-, as well as α(1→6)-fucose on the asparagine-proximal GlcNAc residue. However, for glycopeptidase A to work well, the size of the peptides must be in the range of 3–40 amino acids and protease digestion of the glycoprotein is required (*see* Comment in Step 4 of Procedure 4. Thus PNGase F is the most versatile and widely used tool to de-*N*-glycosylate glycoproteins and/or glycopeptides for further analyses, provided the glycoprotein sources are not from lower animals or plants known or suspected to carry core α(1→3)-fucosylation. In the latter cases, the use of Glycopeptidase A, either directly on glycopeptides or following a prior digestion with PNGase F, is required to obtain both pools of N-glycans with and without core α(1→3)-fucosylation. PNGases are used as tools for ① investigating whether a glycoprotein has *N*-glycans or not. ② investigating structure -function studies of biologically active glycoproteins. ③ analyzing the structures of the glycans liberated from the original glycoprotein. Standard conditions for case ① ; use 1mU(IU) PNGaseF for 25 μg of the denatured glycoprotein (*see* comment 1), and incubate at 37°C overnight; for case ②; use 10 mU(IU) PNGaseF for 25 μg of the undenatured glycoprotein and incubate at 37°C overnight. Susceptibility of the glycoproteins to PNGaseF are different depending on the glycoprotein sample, check the deglycosylation of an aliquots of the sample, and add the enzyme and incubate for one more night; for case③; use 1mU(IU) PNGaseF for 25 μg of the denatured glycoprotein overnight. In case the detergents added for the denaturation may disturb further structural analysis, deglycosylate the sample in non-denatured condition.
Initial amount	
Produced amount	
Discussion	

Figure 14

N-Glycans

N-Glycanase digestion

Authors : Nobuko Kawasaki (Ver 1.0) GET IN CONTACT

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asparagine- linked glycans; purification,

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Abstract

J Biochem. 1978 Dec;84(6):1467-73.

Some characteristics of a new glycopeptidase acting on aspartylglycosylamine linkages.

Takahashi N, Nishibe H.

Abstract

A new type of glycopeptidase hydrolyzing beta-aspartylglycosylamine linkages was partially purified from almond emulsin by chromatography on Sephadex G-200 and DE 52. The enzyme degraded stem bromelain glycopeptide, Asn-Asn(Man3,Xyl1,Fuc1,GlcNAc2)-Glu-Ser-Ser, to yield equimolar amounts of intact oligosaccharide, peptide (Asn-Asp-Glu-Ser-Ser), and ammonia. The Km value for the stem bromelain glycopeptide was 4 mM, and the optimum pH was 5.2. The enzyme was markedly inhibited by 10 mM Cu2+, Fe3+, and Zn2+. Thiol inhibitors and actinomycete protease inhibitors had no effect. The glycopeptides used as substrates were prepared from stem bromelain, ovalbumin or ovotransferrin. The enzyme hydrolyzed glycopeptides with 3-11 amino acid residues, whereas it did not hydrolyze glycopeptides with 1-2 amino acid residues. Furthermore, Asn-oligosaccharide was not inhibitory to the enzyme.

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Figure 15