A Glycan of Ψ-Factor from Dictyostelium discoideum Contains a Bisecting-GlcNAc, an Intersecting-GlcNAc, and a Core α-1,6-Fucose

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A secretory glycoprotein named Ψ-factor that we have purified and cloned from Dictyostelium discoideum is prespore cell-inducing factor. To address its functional significance, it is necessary to examine the attached sites and structures of its glycans as well as its protein structure. Here we identified and isolated a tryptic glycosylated peptide with the 71st to 89th amino acids of Ψ-factor that contained the consensus amino acid sequence for an N-linked glycan (N-T-T). MALDI-TOF mass spectrometry indicated that the major protonated molecular ions, [M + H]+, of the glycopeptide were present at m/z 3,806, the minor m/z 3,603 and 3,400 ions corresponding to the loss of one and two N-acetylhexosamines respectively. Digestion of it with N-glycosidase F gave a molecular mass of 1,766.9 for the whole glycan moiety, which accounts for its composition of five hexoses, four N-acetylhexosamines, and a deoxyhexose. Further digestion experiments on the basis of the substrate specificity of α-mannosidase and β-N-acetylhexosaminidase allowed us to elucidate the unique structure of the glycan, which contains a bisecting and an intersecting GlcNAc and a core α1,6-fucosyl moiety.

Key words: bisecting-GlcNAc; intersecting-GlcNAc; Dictyostelium discoideum; glycopeptide

The slime mold Dictyostelium discoideum is a eukaryote that has the unique potential of differentiating into spore or stem cells during the life cycle, depending on environmental conditions. Researchers use this organism as a model system to investigate the molecular mechanisms of differentiation. Some secretable differentiation factors that induce stem cells were known, and their properties have been studied in some detail. In contrast, no factors that induce spore cells had been identified until recently we purified, cloned, and characterized a prespore cell-inducing factor, which we named psi-factor (Ψ-factor).1–3) This factor, purified from culture media, is a glycoprotein that induces prespore cells from undifferentiated cells. Its full-length cDNA sequence is unique overall, but contains the PA14 domain, a putative β-barrel sugar binding site, which is found in a variety of proteins, e.g., bacterial glycosidases, glycosyltransferases, proteases, and toxins such as anthrax protective antigen (PA). Dictyostelium morphology drastically changes from unicellular to multicellular about 10 h after the onset of starvation, and gene expression is accordingly switched to that required to form cell aggregates 6–14 h after the nutritional change.4) We have found that Ψ-factor comes to be expressed after about 9 h of the initiation of starvation by transcript analysis,3) suggesting that the Ψ-factor plays a role in preparing cells to entering the multicellular stage of the life cycle. In clarifying the link between the Ψ-factor’s structure and its function, the sugar chain structures of glycopeptides are an important target. Protein attached sugars take part in cell adhesion,5,6) are ligands for lectins such as discoids I and II, which are greatly expressed during the cell aggregation stage,7) and hence are relevant to the mechanism of slime-mold differentiation.

As for D. discoideum, there have been many structural and functional studies of the glycans of its glycoproteins and unique modifications of the glycans as compared with mammalian counterparts have been reported, including sulphated mannose,3) methylphosphorylated mannose,8) and intersecting-GlcNAc.9,10–12) N-Glycan core fucosylation to the innermost GlcNAc is specifically regulated at differentiation stages: α-1,6-fucosylation is maximal during growth, and in contrast α-1,3-fucosylation occurs almost exclusively during differentiation.13) In this study we analyzed a glycan structure in Ψ-factor using a combination of mass spectrometry and specific glycosidase digestion to achieve a more insight into the complex structure of slime-mold glycans and a basis for functional analysis of them.

Materials and Methods

Materials. α-Mannosidase (from Jack bean) and β-N-acetylhexosaminidase (from Turbo cornutus) were purchased from Seikagaku corporation (Tokyo). N-Glycosidase A (from almond), N-glycosidase F (Flavobacterium meningosepticum, a recombinant from E. coli) and endoglycosidase H (from Streptomyces pilatus, a recombinant from E. coli) were from Roche (Mannheim, Germany). A standard PA-sugar chain, PA-055, was from Masuda Chemical (Kagawa, Japan). Sequencing-grade modified trypsin was from Promega (Madison, WI). 2,4-DHB and CHCA were purchased from Waters (Milford, MA) and used for MALDI matrices. SuperQ Toyopearl, TSKgel G3000SWTM, and TSK ODS-80TM were from Tosoh (Tokyo). ResourceRPC was from GE Healthcare Japan (Tokyo), and PLRP-S

Abbreviations: Ψ-factor, prespore cell-inducing factor; MALDI-TOF, matrix assisted laser desorption/ionization time-of-flight; 2,4-DHB, 2,4-dihydroxybenzoic acid; CHCA, α-cyano-4-hydroxycinnamic acid; gp, glycopeptide; WGA, wheat germ agglutinin; Endo H, endoglycosidase H; MS, mass spectrometry

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1000 Å 8 µm (150 × 2.1 mm) column (Polymer Laboratories, Shropshire, UK) was from AMR (Tokyo).

Purification of Ψ-factor. Amoebae of D. discoideum strain V12M2 were grown with Klebsiella aerogenes on a modified SM agar.\(^1\) The conditioned medium (CM) was obtained from the culture medium by filtration using a membrane filter of 0.2 µm pore size. Then the pooled CM was applied to SuperQ Toyopearl-TSKgel G3000SWxL tandem chromatography by a method reported previously.\(^2\) Fractions were monitored by SDS-PAGE using a 7.5% T gel,\(^14\) and the fractions containing 106 kDa protein were collected. The pooled fractions were further purified by Resource RPC chromatography, as reported previously.\(^2\)

Protein assay. The quantity of purified Ψ-factor was estimated using a Protein Assay Kit (Bio-Rad, Hercules, CA), with BSA as standard.\(^11\)

Proteolytic digestion by trypsin of Ψ-factor. Purified Ψ-factor (30 µg) was carboxymethylated with iodoacetamide and then incubated with excess trypsin (at a weight ratio of 1 to 100) at 37°C.\(^2\) After 1 h, the same quantity of trypsin was added to the mixture, and the mixture was incubated for a further 1 h. Then the mixture was boiled for 15 min to inactivate trypsin.

Reverse phase HPLC of Ψ-factor fragments. The trypsin-treated mixture was divided into two parts. One was subjected to reverse-phase chromatography without treatment, and the other was incubated with N-glycosidase A (250 µU) for 24 h at 37°C and then injected into a PLRP-S 1000 Å 8 µm reverse-phase column. Reverse-phase chromatography was performed with a pulseless HPLC system (Omniseparo-TJ, Hyogo, Japan). The column was pre-equilibrated with 0.02% v/v trifluoroacetate (TFA) in water. Peptides were eluted with a linear gradient of 0.1% TFA for 10 min, 20–50% over the next 40 min, 50–100% over the next 10 min and 100–100% over the next 10 min at a flow rate of 0.1 mL/min. The eluate was monitored at 214 nm.

Treatment of glycopeptides with endoglycosidases. An aliquot containing 300 fmol of the glycopeptide was used following each digestion experiment, and solvents were evaporated with a MicroVac MV-100 centrifugal evaporator (Tomy Seiko, Tokyo). The resulting residues were treated with 20 units of N-glycosidase F in 20 µL of 100 mM sodium phosphate (containing 25 mM EDTA, pH 7.2) for 72 h at 37°C, or with 5 µL of Endo H in 20 µL of 50 mM sodium phosphate (pH 5.0) for 72 h at 37°C.

Treatment of glycopeptides with exoglycosidases. Glycopeptide aliquots (300 fmol) were incubated with 2 µmol of β-N-acetyl-Ο-hexosaminidase in 20 µL of 100 mM ammonium acetate buffer, pH 4.0, for 24 h at 37°C,\(^16\) with 400 µmol of α-mannosidase in 20 µL of 50 mM ammonium acetate buffer, pH 4.0, for 72 h at 37°C, or with 6 µmol of α-mannosidase and 2 µmol of β-N-acetyl-Ο-hexosaminidase in 20 µL of 100 mM ammonium acetate, pH 4.0, at 37°C overnight.

Sample preparation for mass spectrometry. After incubation with the various glycosidases, the reaction mixture was applied to a ZipTip C18 cartridge (Millipore, Billerica, MA) pre-equilibrated with 0.1% TFA for desalting and concentration. The products were eluted with 0.05% TFA in 50% acetonitrile from the cartridge, and the eluates were evaporated using a MicroVac MV-100 centrifugal evaporator. Then 10 µL of water and 1 µL of matrix solution (10 mg/mL of CHCA: 20 mg/mL of 2,4-DHB=1:1) were mixed with the dried materials. In the case of the standard PA-sugar chain, only 2,4-DHB was added. The various mixtures were spotted on a sample plate and then the solvent was immediately evaporated with an air dryer.

MALDI-TOF mass spectrometry. All measurements were done using a Voyager DE-PRO mass spectrometer (Applied Biosystems, Carlsbad, CA). The accelerating voltage was 20 kV and the delay time for delayed extraction was set at 100 nsec. Angiotensin II (Sigma-Aldrich, St. Louis, MO) was used for mass calibration in all analyses.

Lectin blotting of glycopeptides. An aliquot (7.88 pmol) of the glycopeptide was spotted onto an Immobilon-P PVDF membrane (Millipore) pre-equilibrated with water in an ATTO dot-blotter (AE-6190, ATTO, Tokyo). Spots were washed 3 times with PBS for 10 min and 5 times with PBS containing 0.05% Tween-20 for 10 min. Then 1 µg each of biotinylated lectin (WGA-Biotin, LCA-Biotin, or PHA-E-Biotin, J-Ohl Mills, Tokyo) in 50 µL of PBS containing 0.05% Tween-20 was reacted with the glycopeptides on the membrane in the wells for 90 min at room temperature. After removal of the lectin solutions by suction, spots were washed 5 times by PBS containing 0.05% Tween-20. The membrane was taken from the blottter and washed 5 times with PBS containing 0.05% Tween-20 for 10 min, then washed with PBS for 15 min. It was treated with a VECTASTAIN Elite ABC kit (Vector Laboratories, Burlingame, CA), and was visualized with peroxidase products using Konica Immnostain HRP-1000 (Konica Minolta Health Care, Tokyo).

Analysis of α-mannosidase specificity using 2-amino pyridine-derivatized glycan. To examine effects of the presence of bisecting-GlcNAc in a glycan on the α-mannosidase reaction, a standard PA-sugar, PA-055, was treated with the enzyme. Ten picomoles of PA-055 were incubated with 6.2 µmol of α-mannosidase in 20 µL of 50 mM ammonium-acetate (pH 4.0) for 72 h at 37°C. After incubation, one was analyzed by MALDI-TOF/MS and the other was subjected to TSK-GEL ODS-80TM (4.6 × 250 mm) reverse phase chromatography.

Chromatographic solvent A was 10 mM ammonium-acetate (pH 4.0) in water, and solvent B was solvent A containing 1% 1-buthanol. The column was pre-equilibrated with solvent A/solvent B at 85:15. PA-sugars with and without treatment by α-mannosidase were eluted with a linear gradient of solvent B from 15–15% over 5 min, 15–50% over 65 min, and then 50–50% over 10 min at a flow rate of 0.5 mL/min at 55°C. The eluate was excited at 320 nm and we monitored emission at 400 nm with a Shimadzu RF-535 Fluorescence HPLC monitor (Shimadzu, Kyoto).

Results

Identification, isolation, and amino acid sequence of a trypsic glycopeptide

A glycopeptide from Ψ-factor was identified by peptide mapping on a PLRP-S column with and without pre-treatment of trypsic digests with N-glycosidase A. Glycosidase treatment caused the loss of a single peak observed on the untreated mapping, as indicated by an arrow in Fig. 1A (Fig. 1A, arrow). The peak material was analyzed with an Applied Biosystems 4770A amino acid sequencer, and the peptide sequence was determined to be Y\(^7\)FFAPNQISX\(^8\)TTFNCGR\(^9\), which exactly matches the 71st to 87th amino acids as deduced from the cDNA sequence of Ψ-factor,\(^3\) except for the unidentified 80th amino acid (X). The deduced amino acid sequence indicates that the missing amino acid is the asparagine in the consensus sequence (NTT) for the N-linked glycan, consistently with the presence of a sugar chain at Asn\(^80\).

MALDI-TOF mass spectrometry of the isolated glycopeptide

The molecular mass of the N-glycosidase A-sensitive glycopeptide was determined with a Voyager DE-PRO MALDI-TOF mass spectrometer (Applied Biosystems, Carlsbad, CA). The mass spectrum (Fig. 1B) contained a prominent peak, peak 1, of protonated molecular ions ([M + H]\(^+\)) at m/z 3,805.7, and three minor associated peaks, 0, 2, and 3, at m/z 3,967.3, m/z 3,603.2, and m/z 3,400.3 respectively. The m/z value of the peak 0 ions was larger than that of the major ions by 162, which corresponds to a hexose residue. The latter two minor ions (peaks 2 and 3) appeared to have formed by the loss
Fig. 1. Peptide Mapping on HPLC and Mass Spectrometry of the N-Glycosidase A-Sensitive Fraction.

A. Tryptic peptides from N-glycosidase A-treated (broken line) and untreated (solid line) ψ-factor were subjected to PLRP-S reverse phase chromatography. The column was equilibrated with 0.02% v/v TFA. Peptides were eluted with a linear gradient of 0.018% v/v TFA in 95% v/v acetonitrile, from 0–20% over 100 min, 20–50% over next 40 min, 50–100% over next 10 min, and 100–100% over the next 10 min at a flow rate of 0.1 mL/min. The eluate was monitored at 214 nm. The arrow indicates the position of the most prominently different peak between two profiles. B. The N-glycosidase A-sensitive fraction was analyzed using a Voyager DE-pro MALDI-TOF mass spectrometer (Applied Biosystems, Carlsbad, CA). Peaks 0, 1, 2, and 3 correspond to m/z 3,967.3, m/z 3,805.7, m/z 3,603.2, and m/z 3,400.3 ions respectively.

Structural determination of the gp3806 glycan by mass spectrometry after digestion with glycosidases of the defined substrate specificity

The molecular mass and core structure of the gp3806 glycan

Isolated gp3806 treated with N-glycosidase F was analyzed by MALDI-TOF mass spectrometry. The glycan of gp3806 was a substrate of N-glycosidase F. The peak intensities of gp3806 and the associated minor ions decreased greatly, but a strong peak was newly observed at m/z 2,037.9. The molecular mass of the m/z 2,037.9 ions was identical to that of the protonated peptide containing the 71\(^{\alpha}\) to 89\(^{\beta}\) amino acids of ψ-factor (Fig. 2A). These results in combination with the known structural characteristics of N-linked glycans allowed us to estimate the composition of the sugar chain as follows: five mannoses, four N-acetylglucosamines and one fucose. The gp3806 glycan had no β-galactose, because treatment of it with β-galactosidase produced no products (data not shown).

Next, the effect of Endo H on the glycans was tested by MALDI-TOF/MS. Cleavage of gp3806, 3603, and 3400 by Endo H gave two peaks corresponding to protonated N-acetyl-d-glucosaminy1 peptide ions (m/z 2,240.3) and protonated deoxyhexosyl-N-acetyl-d-glucosaminy1 peptide ions (m/z 2,386.2) (Fig. 2B), consistently with the known specificity of Endo H. This suggests that the deoxyhexose of gp3806 is a fucose that binds to the innermost GlcNAc. Furthermore, this fucose appears to be attached to GlcNAc through the 3-position of GlcNAc. The sugar composition of gp3806 and the results of the Endo H digestion experiments suggest that the gp3806 glycan is a high mannose-type N-linked glycan (Fig. 2B, inset).
Man were digested with GlcNAc(s) on mannosidase action, the glycopeptides were examined to determine the configuration of peripheral sugars from the gp3806 (Fig. 4A). In contrast, we subjected gp3400. This indicates that the gp3806 glycan has a bisecting GlcNAc residue from gp3603 and/or N-acetylmuramyl residues respectively from peak 0 ions. "X" indicates an unidentified peak.

To complete the structural determination of the gp3806 glycan, the configuration of peripheral sugars consisting of one mannose and two N-acetylmuramylenaminas was examined using the reactivity of exoglycosidas, as explained below.

**β-N-Acetyl-d-hexosaminidase treatment**

Isolated glycopeptides were incubated with β-N-acetyl-d-hexosaminidase, the resulting products were subjected to mass spectrometry, and a single prominent peak at m/z 3,399.9 was observed. This ion can be formed by the loss of two N-acetyl-d-hexosaminyl residues from protonated gp3806 ions, suggesting the presence of two N-acetyl-d-hexosaminyl triamino plus four successive losses of mannosyl residues, yielding the core glycan, Man–GlcNAc–GlcNAc (Fuc)–amine. About 300 fmoles of the glycopeptides were digested with β-N-acetyl-d-hexosaminidase and α-mannosidase was analyzed using a Voyager DE-pro MALDI-TOF mass spectrometer (Applied Biosystems, Carlsbad, CA). The peak indicated by "X" at m/z 2,239.3 was unidentified. To determine the configuration of the fourth GlcNAc, we next investigated the reactivity of the glycan with α-mannosidase.

**α-Mannosidase treatment**

α-Mannosidase treatment scarcely released mannose residues from the gp3806 (Fig. 4A). In contrast, we observed m/z 3,442.0 ions formed by the loss of a mannosyl residue from gp3603 and m/z 2,752.5 ions formed by losses of four mannosyl residues from gp3400. This indicates that the gp3806 glycan has structural characteristics causing inhibition of α-mannosidase. To examine the effects of peripheral GlcNAc(s) on mannosidase action, the glycopeptides were digested with α-mannosidase and β-N-acetyl-d-hexosaminidase. This time, complete digestion of the peripheral residues of the gp3806 glycan occurred in turn, for two successive losses of N-acetyl-d-hexosamine plus four successive losses of mannosyl residues, yielding the core glycan, Man–GlcNAc–GlcNAc (Fuc)– (Fig. 4B). This suggests that the presence of peripheral GlcNAc(s) in the glycan has profound effects on the substrate specificity of α-mannosidase.

**Lectin blotting of glycopeptides**

To confirm these configurations of sugar residues, lectin blot analysis was done. As Fig. 4C shows, WGA and LCA bound to glycopeptides, but PHA-E did not. The specificity of these lectins is in general as follows: WGA recognizes bisecting-GlcNAc, LCA core α-1,6-fucose, and PHA-E GalNAc. This indicates that the structures of glycopeptides contain both bisecting-GlcNAc and core α-1,6-fucose, but not GalNAc.

**Reactivity of α-mannosidase for a standard sugar chain containing bisecting-GlcNAc**

To determine how the presence of bisecting-GlcNAc in a substrate affects the mannosidase action, a Masuda chemical, PA-055 ([M + H]⁺, m/z 1,741.7), a standard sugar chain containing a bisecting-GlcNAc (IVa), was similarly digested with α-mannosidase. To specify a particular residue on the sugar sequence, Roman numerals are used hereafter, counting from the aspar-
that spatial positioning of GlcNAc relative to a mannose (Fig. 5A).

Mass spectrometry indicated that the most intense peak, at $m/z$ 1,574.2, corresponded to the ammonium adduct ion $[M+NH_4]^+$ arising from the loss of a mannosyl residue, respectively. B, Elution profiles of α-mannosidase-treated (solid line) and untreated (broken line) PA-sugars. The standard PA-sugar (PA-055, 5 pmoles) alone and the reaction mixtures with α-mannosidase (including 5 pmoles PA-sugar) were subjected to HPLC on a TSK-GEL ODS-80TM reverse phase column. Arrows 8–11 indicate the elution positions of PA-glucose oligomers.

HPLC analysis of the reaction products confirmed that α-mannosidase can cleave a glycoside bond between $V_6$ and $IV_4$ that binds with the central mannose $III_4$ of the trimannosyl core through the $1\rightarrow6$ linkage, but not the other $V_3$–$IV_6$ linkage (Fig. 5). This can be explained by steric hindrance of a bulky bisecting-GlcNAc ($IV_4$) adjacent to the Man ($V_3$) residue. In contrast, the other $V_6$–$IV_4$ linkage was readily hydrolyzed by α-mannosidase regardless of the presence of bisecting-GlcNAc ($IV_4$) (Fig. 5). Moreover, α-mannosidase completely released all eight mannosyl residues from a high mannose-type standard PA-sugar composed of nine mannoses under the same conditions as for PA-055 hydrolysis, described above (data not shown), further exemplifying α-mannosidase specificity. This indicates that spatial positioning of GlcNAc relative to a mannose is crucially important in determining α-mannosidase selectivity. Almost complete resistance of the glycan of gp3806 against the α-mannosidase action suggests that every peripheral GlcNAc is present adjacent to mannose residues, and hence the fourth GlcNAc should bind with a mannose by the $1\rightarrow4$ linkage at the intersecting position ($V_3$ in Fig. 6). Consistently, gp3603 and gp3400 that lack one or two GlcNAc were substrates of α-mannosidase (Fig. 4A).

The structure of gp3806 we determined is shown in Fig. 6. It is unique, with a bisecting-GlcNAc ($IV_4$), an intersecting-GlcNAc ($V_4$), and an α1,6-fucose ($II_6$) bound to the innermost GlcNAc.

**Discussion**

We were the first to report that prespore-cell-inducing factor (Ψ-factor) in *D. discoideum* is a glycoprotein with six consensus sequences (N-X-T/S) for N-linked glycans. To clarify the role of its sugar chains, we started analyzing the sugar chain structure. An N-glycosidase A-sensitive glycopeptide was identified and isolated from tryptic fragments of the purified Ψ-factor by differential chromatography of the glycosidasesdigests. The glycopeptide was analyzed by MALDI-TOF/MS in combination with digestion patterns by glycosidases of defined substrate specificity. From these results, we estimated the unique structure of gp3806, with a bisecting-GlcNAc, an intersecting-GlcNAc, and an α1,6-fucose bound to the innermost GlcNAc (Fig. 6).

*Dictyostelium discoideum* with a simple life cycle including mono- and multi-cellular states is a useful tool for studying the molecular mechanisms of cell differentiation and cell-cell interaction, and researchers have used it in experiments on these lines. Its genome sequence was completely determined in 2005, and the roles of many gene products have been successfully estimated. However, genome information in itself is not enough fully to understand the precise mechanisms of slime mold differentiation, because post-translational modifications, including N-glycosylation of proteins, are believed to take part in these mechanisms critically. The organisms produce N-glycans with unique modifications: *i.e.*, phosphomannosyl- and methoxyphosphomannosyl-modification or intersecting-GlcNAc residues. This study adds a new structure to the structural variety of the slime mold glycans.
Assignment of the intersecting GlcNAc residue to the mannosyl residue IV\(_6\) is not based on mass spectrometry, but relies on the fact that glycans with the peripheral GlcNAc residue are resistant to jack bean \(\alpha\)-mannosidase. We have not yet succeeded in acquiring structurally informative post-source decay spectra with the Voyager mass spectrometer that is currently available to us. More accurate determination must await assignment by NMR, which sample limitation has hindered. A previous study indicated that \(\alpha\)-mannosidase is inactive as to bovine rhodopsin glycans that contain a GlcNAc at the non-reducing end of the sugar chain linked to the 3 position of the core mannose (III\(_4\)) of high mannose type oligosaccharides, but in the present study \(\alpha\)-mannosidase did react with PA-055, which has one of these rhodopsin glycan structures plus a bisecting GlcNAc at core mannosyl residue III\(_4\), and it released only a mannosyl residue (V\(_6\)) linked \(\alpha\)1,6 to IV\(_6\) linked \(\alpha\)1,6 to the core III\(_4\) (Fig. 5A), as confirmed by the elution order on reverse phase HPLC. Tomiya et al. have reported the elution properties of PA-sugars with different numbers of glucose units on a ODS column. The PA-sugar H5.1 (the same as PA-055) shows a G.U. of 8.7, and PA-sugar H4.1, lacking the V\(_6\) residue of H5.1, exhibits a G.U. of 9.5. The difference between these G.U. values is 0.8, the same as that between those of PA-055 and the product of the reaction of PA-055 with \(\alpha\)-mannosidase (Fig. 5B). Based on these results, \(\alpha\)-mannosidase clefts only the \(\alpha\)1,6 linkage between mannosyl residues IV\(_6\) and V\(_6\). Another \(\alpha\)1,6 linkage, on mannosyl residue IV\(_6\), is more resistant to the enzyme action than the \(\alpha\)1,6 linkage, due to its configurational properties and steric hindrance by a bisecting GlcNAc. We used an approximately 1,000 times greater enzyme concentration than the previous study, which may be the reason for this difference in the \(\alpha\)-mannosidase action. If GlcNAc attaches \(\alpha\)1,3 to a mannosyl residue of the trimannose core, gp3806 is a hybrid sugar chain with a bisecting-GlcNAc and the innermost fucosylated GlcNAc. The glycopeptide of such a structure should be cleaved by \(\alpha\)-mannosidase as PA-055, but we did not observe any fragments arising from gp3806 under \(\alpha\)-mannosidase treatment.

In *D. discoideum*, the presence of an intersecting GlcNAc\(^{10–12}\) has been reported, and a variety of other unique N-glycan structures have been determined.\(^{25,28}\) Moreover, Sharkey and Kornfeld\(^{12}\) have reported N-glycans with both bisecting and intersecting-GlcNAc in *D. discoideum* on the basis of the results of N-acetyl-\(\beta\)-hexosaminidase digestion experiments. Recently, Schiller, et al. reported that the N-glycan (Man\(_3\)GlcNAc\(_4\)) containing both a bisecting-GlcNAc and an intersecting-GlcNAc, serves as a substrate for a core fucosyltransferase, leading to \(\alpha\)1,3-fucosylation on its innermost GlcNAc.\(^{29}\) The structure of this glycan is the same as that of gp3806 from \(\Psi\)-factor, except for the position of a central fucosyl residue. The selectivity of N-glycosidase F in removing core \(\alpha\)1,6-, but not \(\alpha\)1,3-, fucosylated glycans indicates that gp3806 contains a fucosyl residue linked \(\alpha\)1,6 to the innermost GlcNAc. Core \(\alpha\)1,3-fucosylation, which generally occurs in N-glycans of plant origin, increases at the stage of tips and fruiting bodies in the development of *D. discoideum*. In contrast, \(\Psi\)-factor expression starts about 9 h after initiation of starvation. During this latent period, a dramatic transition occurs in gross morphology and the expression of glycosyltransferases, including fucosyltransferases, and then cell-type divergence begins in *Dictyostelium*.\(^{4,13}\)

The unicellular-to-multicellular transition, occurring as a turning point 8–12 h after the onset of development, leads to a shift in fucosyltransferase expression. The activity of core \(\alpha\)1,6 fucosyltransferase decreased with a contrasting increase in that of \(\alpha\)1,3 fucosyltransferase. The significance of core \(\alpha\)1,6 fucosylated, bisecting, and intersecting glycans or of switching of core fucosylation sites during the transition inducing cell type divergence remains to be clarified.

In this report we describe a unique glycan with a bisecting-GlcNAc (Fig. 6, IV\(_4\)), an intersecting-GlcNAc (Fig. 6, V\(_4\)), and the innermost \(\alpha\)1,6-fucosylated (Fig. 6, VI\(_4\)) GlcNAc in \(\Psi\)-factor of *D. discoideum*. The function of this glycan has not yet been defined. Further physiological studies on the glycan are in progress.

### References


