CONFORMATIONAL STUDIES OF GLYCOPEPTIDES BY ENERGY TRANSFER

INTRODUCTION OF FLUOROPHORE AT SPECIFIC BRANCHES OF BIANTENNARY GLYCOPEPTIDES*

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Biantennary glycopeptides from bovine fibrinogen were fluorescence labeled at each branch specifically for conformational studies by fluorescence energy transfer. Glycopeptides (by Pronase digestion) were separated by anion-exchange chromatography based on the degree of sialylation. The major monosialyl biantennary glycopeptides (see below) were used as substrates for galactose oxidase and periodate oxidation.

Galβ(1–4)GlcNAcb(1–2)Manα(1–6)\[\text{Galb(1–4)GlcNAb(1–2)Mana(1–6)}\]
SAα(2–6)Galβ(1–4)GlcNAcb(1–2)Manα(1–3)\[\text{SAa(2–6)Galb(1–4)GlcNAb(1–2)Mana(1–3)}\]

Galactose oxidase was used to oxidize the terminal Gal6 located on the Manα(1–6)Man branch. The oxidized glycopeptides (containing 6-oxo-galactose) were modified with 2-(dansylamido)ethylamine by reductive amination. The N terminus of the peptide portion was then modified with naphthylacetic acid. Alternatively, the peptide portion of the monosialylated glycopeptide was first modified with naphthylacetic acid and the sialic acid located on the Manα(1–3)Man branch was oxidized with periodate under controlled conditions. The oxidized glycopeptides (oxo-sialic acid) were coupled with 2-(dansylamido)ethylamine by reductive amination. These doubly fluorescence-labeled glycopeptides were used for conformational studies of biantennary glycopeptides by energy transfer (see the accompanying article (Wu, P., Lee, K. B., Lee, Y. C., and Brand, L. (1996) J. Biol. Chem. 271, 1470–1474)). Furthermore, the unmodified branch of the fluorescent labeled glycopeptides were digested stepwise with exoglycosidases. Resonance energy transfer experiments were done with each of the resulting derivatives to determine the effects of removing sugars at each stage of peeling on any conformational change on the resulting branch antennae.

Recognition of carbohydrates is a form of biological signal which is rapidly gaining attention in recent years (e.g., Ref. 1). To better understand the interaction between oligosaccharides and proteins, information about the solution conformation of oligosaccharides is indispensable. NMR is the most frequently used technique for studies of solution conformation of oligosaccharide (2–4). However, the NMR technique is most suitable for measurement of distances in the range of 2–5 Å, and for the 10–50 Å distances, resonance energy transfer is more suitable.

Resonance energy transfer techniques have been used to measure intramolecular distances in biological molecules such as proteins, oligonucleotides, and lipids (5–8). Recently, we have demonstrated the usefulness of fluorescence energy transfer measurement in the conformational analysis of some complex-type oligosaccharide (9, 10). In these studies, the solution conformations of triantennary glycopeptides derived from bovine fetuin was examined. The glycopeptide was modified with a naphthyl group at the N terminus of the peptide and with a dansyl group at one of the Gal residues.

Time resolved energy transfer measurement revealed that two of the three antennae of oligosaccharide were flexible. In order to extend such studies to biantennary glycopeptide, we have chosen to use the major monosialylated biantennary glycopeptide derived from readily available bovine fibrinogen of the structure shown in Fig. 1 (11).

Controlled periodate oxidation (12) was used to oxidize the terminal NeuAc7 on the Manα(1–3)Man branch, and galactose oxidase was used to oxidize the terminal Gal6 on the Manα(1–6)Man branch. The oxo-galactose or oxo-sialic acid were easily modified with 2-(dansylamido)ethylamine by reductive amination. These doubly fluorescent-labeled glycopeptides were used for energy transfer measurement to study the conformation of the biantennary glycopeptide (see the accompanying paper, Ref. 27). Moreover, the unlabeled branch of the fluorescent labeled glycopeptide was removed stepwise by successive exoglycosidase digestion followed, and conformational changes of the resulting series of derivatives were measured, by the same technique as described previously (13).

EXPERIMENTAL PROCEDURES

Materials

Bovine fibrinogen (95% clottable) was obtained from Miles, Inc. Kankakee, I1. Pronase (protease, Streptomyces griseus) was purchased from CalBiochem (La Jolla, CA). Neuraminidase from Arthobacter ureafaciens was a gift from Dr. Yoji Tsukada (Kyoto Research Institute, Uji, japan). Glycopeptidase A was purchased from Seikagaku America, chromatography; PA-, pyridyl-2-amino-; SA, sialic acid; 6-oxo-Gal, 6-aldehydo-6-deoxy-α-galactose; AUFS, absorbance units full scale.

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Inc. (Rockville, MD). Sodium cyanoborohydride, borane-pyridine complex, 1-hydroxybenzotriazole, and 2-naphthylacetic acid (Nap) were purchased from Aldrich. 2-Dansylamidoethylamine (Dan) was purchased from Molecular Probes, Inc. Spherisorb HPLC columns were from Phase Separation (Norwalk, CT). Hypersil ODS column was purchased from Alltech Associates, Inc. (San J. ose, CA). A Gilson HPLC System equipped with two Gilson Model 302 pumps, a Rheodyne 7125 injector, a Hitachi TC 50 column oven, and an ISCO V4 UV detector was used for all HPLC operations. Fluorescence detection of HPLC eluate was performed with a Perkin-Elmer LS40 scanning fluorescence detector. Dithiothreitol, iodoacetamide, dicyclohexylcarbodiimide, galactose oxidase (EC 1.1.3.9), and catalase (EC 1.11.1.16) were from Sigma. Dialysis tubing was from Spectrum Medical Industries (Los Angeles, CA). Sodium periodate was from GFS Chemical (Columbus, OH). Sodium cyanoborohydride, borane-pyridine complex, and sodium acetate were from J. T. Baker Chemical Co. Sodium cyanoborohydride was used for all HPLC operations. Fluorescence detection of HPLC eluate was performed with a Bio-LC system Dionex (Sunnyvale, CA) equipped with a CarboPac PA-1 column (0.46 × 25 cm) and a pulsed amperometric detector. The following settings of pulse potentials and durations were used: \( E_1 = 0.05 \), \( E_2 = 0.60 \), \( E_3 = -0.60 \). \( T_1 = 480 \) ms, \( T_2 = 120 \) ms, and \( T_3 = 60 \) ms. The sensitivity was set at 1000 nA full scale.

Eluants used were: A, 200 mM NaOH; B, water; C, 1 mM sodium acetate. The column was eluted at 1 ml/min with a linear gradient of sodium acetate rising from 0 to 400 mM over 30 min. Sodium hydroxide (300 mM) was added to the column effluent prior to the detector cell at a flow rate of 0.5 ml/min.

Monosaccharides were released by the hydrolytic conditions described by Fan et al. (18). Amino acid analysis was performed as described previously (19).

The Structure of the Monosialylated Oligosaccharides—The monosialylated glycopeptide (15 nmol in 20 μl of 50 mM ammonium acetate) was digested with 0.4 milliunit of glycopeptidase A in 10 μl of 10 mM ammonium acetate, pH 6.0, for 16 h at 37°C and purified on a Sephadex G-10 column. The released oligosaccharides were reductively amminated with 2-amino pyridine as described previously (20). To remove the excess reagent, the reaction mixture was applied to a Sephadex G-10 column (1.6 × 30 cm) eluted with 10 mM ammonium acetate. The column was monitored by uv absorption at 254 nm. The void volume was collected, freeze-dried, and purified on a C18 RP-HPLC column (0.46 × 25 cm). The pyridylaminated oligosaccharides were analyzed with a RP-HPLC column (Hypersil ODS 5 micron, 0.46 × 25 cm). The column was eluted 1 ml/min with a gradient formed by 50 mM ammonium acetate (Eluant 1) and 50% acetonitrile in 50 mM ammonium acetate (Eluant 2), varying the Eluant 2 from 5 to 20% over 35 min. The effluent was monitored by fluorescence (excitation, 320 nm; emission, 400 nm). The structural confirmation of the PA-derivatized monosialylated oligosaccharide was performed by Dr. S. Hase (Osaka University), using a Cosmosil 5C18P column by the published method (21).

2-Naphthylacetylation of the Amino Terminus of the Monosialylated Glycopeptides—The monosialylated glycopeptide was 2-naphthylacylated as described previously (9). The 2-naphthylacetic acid (1 mmol) was activated by dissolving dicyclohexylcarbodiimide (1 mmol) and 1-hydroxybenzotriazole (1 mmol) into 5 ml of N,N-dimethylformamide and reacted overnight. The reaction mixture was suction-filtered through a Whatman filter paper (Qualitative 1). The glycopeptide (10 μmol) in 5 ml of 100 mM sodium bicarbonate, pH 8.0, was reacted with 1 mmol of activated 2-naphthylacetic acid in 5 ml of N,N-dimethylformamide. The reaction mixture was evaporated and applied to a Sephadex G-10 column eluted with 10 mM ammonium acetate, effluent being monitored at \( A_{254 \text{ nm}} \). The freeze-dried reaction mixture (20 nmol) was applied to a C8 RP-HPLC column (0.46 × 25 cm) and eluted with a gradient formed by 50 mM ammonium acetate (Eluant 1) and 50% acetonitrile in 50 mM ammonium acetate (Eluant 2), raising the Eluant 2 from 5 to 50% over 40 min at a flow rate of 1 ml/min. The effluent was monitored at \( A_{254 \text{ nm}} \) at 2 AUFS. The mixture of derivatized glycopeptides (10 nmol) was treated with 1 milliunit of neuraminidase in 10 mM ammonium acetate analyzed by RP-HPLC. For preparative separation, the derivatized glycopeptide (200 nmol) was applied to a C8 column (0.46 × 25 cm) and eluted isocratically with 8% of Eluant 2. The collected glycopeptides were freeze-dried and stored at −20°C.

Periodate Oxidation of the Monosialylated N-2-Naphthylacetyl Glycopeptides—To optimize the periodation of sialic acid, N-naphthylacylated monosialylated glycopeptides containing NeuAc (GP2-Nap, 50 nmol) were oxidized in different final periodate concentrations of 0.5, 1, and 1.5 mM in 10 mM phosphate buffer, pH 7.0, at 0°C for 20 min (12). A large scale oxidation was accomplished as follows. A solution of glyco-
peptides (1.5 μmol) in 6 ml of 10 mM phosphate buffer, pH 7.0, was mixed with 6 ml of 2 mM periodate in 10 mM phosphate buffer, pH 7.0 (both previously chilled in an ice bath). The reaction mixture was kept in the dark at 0 °C for 20 min. The oxidation was terminated by adding 200 μl of 1 M sodium metabisulfite in the phosphate buffer and left in the dark for an additional 10 min. The reaction mixture was desalted on a Sephadex G-10 column (1.6 × 30 cm) eluted with water, monitored at A_{220} nm. The monosialylated N{-2-naphthylacetylated glycopeptide containing NeuGc (GP1-Nap) was oxidized under identical conditions.

Reductive Amination of the Periodate-oxidized Monosialylated Glycopeptide with 2-(Dansylamido)ethylamine—The periodate-oxidized monosialylated N-2-naphthylacetylated glycopeptide (1 μmol) was dissolved into 1 ml of sodium phosphate, pH 7.0, and mixed with 10 mg of 2-(dansylamido)ethylamine in 250 μl of ethanol. The reaction mixture was kept at 37 °C for 30 min, and the void volume peak was collected and freeze-dried. The dried sample was dissolved in 1 ml of sodium phosphate, pH 7.0, and 20 mg of 2-(dansylamido)ethylamine in 200 μl of 100 mM phosphate buffer, pH 7.0, containing 50 μg/ml catalase. The reaction was performed at 37 °C for 18 h. The reaction mixture was applied to a Sephadex G-10 column, monitored at A_{210} nm, and the void volume peak was collected and freeze-dried. The dried sample was dissolved in 1 ml of sodium phosphate, pH 7.0, and 20 mg of 2-(dansylamido)ethylamine in 200 μl of ethanol was added. The reaction mixture was kept at 37 °C overnight. The reaction mixture was applied to the G-10 column and eluted with 10 mM ammonium acetate and monitored at A_{254} nm with 0.5 AUFS. The product peak was collected, freeze-dried, and further purified with a C_{18} RP-HPLC column (0.46 × 25 cm, Fig. 8A). The column was eluted 1 ml/min with a linear gradient.

Oxidation of the Monosialylated Glycopeptide with Galactose Oxidase and Coupling with 2-(Dansylamido)ethylamine—The monosialylated N-2-naphthylacetylated glycopeptide containing NeuGc (GP1-Nap) was oxidized under identical conditions.

Reductive Amination of the Periodate-oxidized Monosialylated Glycopeptide with 2-(Dansylamido)ethylamine—The periodate-oxidized monosialylated N-2-naphthylacetylated glycopeptide (1 μmol) was dissolved into 1 ml of sodium phosphate, pH 7.0, and mixed with 10 mg of 2-(dansylamido)ethylamine in 250 μl of ethanol. The reaction mixture was kept at 37 °C for 30 min, and the void volume peak was collected and freeze-dried. The dried sample was dissolved in 1 ml of sodium phosphate, pH 7.0, and 20 mg of 2-(dansylamido)ethylamine in 200 μl of 100 mM phosphate buffer, pH 7.0, containing 50 μg/ml catalase. The reaction was performed at 37 °C for 18 h. The reaction mixture was applied to a Sephadex G-10 column, monitored at A_{210} nm, and the void volume peak was collected and freeze-dried. The dried sample was dissolved in 1 ml of sodium phosphate, pH 7.0, and 20 mg of 2-(dansylamido)ethylamine in 200 μl of ethanol was added. The reaction mixture was kept at 37 °C overnight. The reaction mixture was applied to the G-10 column and eluted with 10 mM ammonium acetate and monitored at A_{254} nm with 0.5 AUFS. The product peak was collected, freeze-dried, and further purified with a C_{18} RP-HPLC column (0.46 × 25 cm, Fig. 8A). The column was eluted 1 ml/min with a linear gradient.
formed by 50 mM ammonium acetate (Eluant 1) and 50% acetonitrile in 50 mM ammonium acetate (Eluant 2, which was varied from 30 to 70% over 55 min). The effluent was monitored at 280 nm and 480 nm.

The collected products were freeze-dried and stored at -20 °C.

Coupling of 2-Naphthylacetic Acid to the Dansyl-labeled Glycopeptide—The mixture of dansyl-labeled glycopeptide (1 μmol) was dissolved in 500 μl of 100 mM sodium bicarbonate, pH 8.0, and 10 μmol of activated 2-naphthylacetic acid in 5 ml of N,N-dimethylformamide was added. After 37 °C for 18 h, the reaction mixture was applied to the G-10 column eluted with 50 mM ammonium acetate and the void volume peak was collected. The column was monitored at A_{254 nm}. The freeze-dried sample was separated using a C_{18} RP-HPLC column (0.46 × 25 cm, Fig. 8B). The elution conditions were the same as described above. The collected product was freeze-dried and stored at -20 °C. The product was characterized by monosaccharide and amino acid analyses.

Stepwise Exoglycosidase Digestion of Fluorescent Labeled Glycopeptides—The dried GP5-DanNap (Scheme 1, 500 nmol) was dissolved in 100 μl of 10 mM ammonium acetate, pH 5.4, and 25 milliunits of neuraminidase in 7 μl of 10 mM ammonium acetate were added. After incubation at 37 °C for 18 h, the product was purified with a C_{18} column (0.46 × 25 cm) eluted at 1 ml/min, using the same two eluents described above. The Eluent 2 was increased linearly from 30% to 70% over 55 min, and the effluent was monitored at A_{254 nm} with 2 AUFS. The freeze-dried glycopeptides (10 μmol) were dissolved in 500 μl of 100 mM sodium acetate buffer, pH 4.3, and 1 unit of α-mannosidase was added. After 18 h at 37 °C, the final product was purified on the C_{18} column as described above. A portion of the collected material (9°, Scheme 1) was freeze-dried and used for energy transfer measurement. The remainder was dissolved in 100 μl of citrate-phosphate buffer, pH 4.3, and incubated with 2.5 units of β-N-acetyl-D-glucosaminidase from bovine kidney in 50 μl of 3.2 mM ammonium sulfate at 37 °C for 18 h.

The resulting product was purified on the C_{18} column as described above. A portion of the collected material (9°, Scheme 1) was freeze-dried and used for energy transfer measurement. Finally, the remainder was dissolved in 50 μl of sodium acetate buffer, pH 4.3, and 1 unit of α-mannosidase was added. After 18 h at 37 °C, the final product was purified on a C_{18} column. The elution conditions were the same as described above. The collected material (9°, Scheme 1) was freeze-dried and used for energy transfer measurement. The other doubly labeled glycopeptide, GP2-DanNap (Scheme 2, 500 nmol) was also sequentially digested with the exoglycosidase without initial neuraminidase treatment and purified on the RP-HPLC C_{18} column as described above.

Liquid Secondary Ionization Mass Spectrometry—Liquid secondary ion mass spectra were obtained on a Kratos Concept double sector mass spectrometer (Kratos Analytical, Manchester, UK) in the positive mode. The accelerating voltage was 4 kV. The samples were diluted in water and imbedded in a matrix consisting of a 1:1 mixture of glycerol/monothioglycerol which contained 1% trifluoroacetic acid.
RESULTS

Preparation of Glycopeptides—For large-scale preparation of biantennary glycopeptides, bovine fibrinogen was the most suitable source, because it contains only biantennary-type oligosaccharides (11). Glycopeptides were generated by exhaustive Pronase digestion of reduced and alkylated bovine fibrinogen. Separation of glycopeptides from low molecular weight peptides was achieved by gel filtration on a Sephadex G-50 column (Fig. 2). The final recovery was 75% (80 mmol of biantennary type glycopeptides) from 10 g of bovine fibrinogen.

Separation and Analysis of Glycopeptides—Glycopeptides were separated into three fractions based on the levels of sialylation on a DEAE-cellulose column eluted with a phosphate gradient (Fig. 3). Fractions A, B, and C represented 20, 43, and 32% of the applied glycopeptides. Fraction A was a very heterogeneous mixture as analyzed by high performance anion-exchange chromatography on a CarboPac PA-1 column (data not shown). Each of fractions B and C contained two major peaks (Fig. 4, A and C). Neuraminidase treatment of fractions B and C caused each fraction to converge into a single peak, while yielding NeuAc and NeuGc, respectively (Fig. 4, B and D). These results indicate that fractions B and C differ only in the degree of sialylation. The monosaccharide compositions of fractions B and C closely match those of the biantennary oligosaccharide structure (Table I). It is also apparent that fractions B and C are the monosialylated and disialylated biantennary glycopeptides, respectively. The amino acid analyses of fractions B and C (Table I) are consistent with the known peptide sequence near the glycosylation site (22, 23).

Assignment of Sialylation Position in Monosialylated Oligosaccharide Using PA Derivatives—The structure of the PA-derivatized monosialylated oligosaccharide was determined by comparing its elution position with those of the authentic standard PA-oligosaccharide derivatives by RP-HPLC (Hypersil ODS). The sample was co-eluted with the authentic PA derivative of Gal[β1–4GlcNAcβ1–2Manα1–3]Manβ1–4GlcNAcβ1–4GlcNAc–PA (Fig. 5). The NeuAc is α(2–6)-linked to the terminal galactose located on the Manα1–3Man branch (11). NeuGc-containing PA-oligosaccharide derivatives were not analyzed by this method because of unavailability of authentic standards.

N-2-Naphthylacetylation of the Monosialylated Glycopeptides—Monosialylated glycopeptides modified with 2-naphthylacetic acid (purified with Sephadex G-10 column) were separated on a C8 RP-HPLC column into four peaks as shown in Fig. 6A. The combined yield was about 75%, but the unre-
acted glycopeptides (about 25% of the total recovered glycopeptides) eluted at the void volume could be recycled to raise the net yield eventually. Peaks 1 (GP1-Nap) and 3 (GP3-Nap) contained NeuAc and peaks 2 (GP2-Nap) and 4 (GP4-Nap) contained NeuGc. After neuraminidase treatment, peaks 1 and 2 became peak 5 and peaks 3 and 4 became peak 6 (Fig. 6).

Amino acid analyses showed that peaks 1 and 2 contained Asn and Glu in a ratio of 1:1. Peaks 3 and 4 contained Asn, Glu, and Gly in a ratio of 1:0.8:0.7.

Periodate Oxidation of Naphthyl-glycopeptides and Coupling with 2-(Dansylamido)ethylamine—

GP2-Nap (N-2-naphthylacetylated monosialylated glycopeptide, peak 2 in Fig. 6A) was oxidized at 0°C using various periodate concentrations, and the periodate-oxidized derivative was coupled with 2-(dansylamido)ethylamine by reductive amination. Oxidation with 0.5 mM NaIO₄ for 20 min (optimal condition) yielded two major products (Fig. 7A). The earlier eluting peak was the product of the C₇-C₈ bond cleavage, and the later eluting peak was the product of the C₈-C₉ bond cleavage. Increasing the NaIO₄ concentration to 1.5 mM resulted in the nearly total disappearance of the C₈-NeuAc derivative and an increase of the C₇-NeuAc derivative (Fig. 7B). Under these conditions, no oxidation of other sugars was observed. The glycopeptides labeled with both dansyl and naphthyl groups were purified on a C₁₈ RP-HPLC column (Fig. 8A). Peaks 1 and 2 represented GP5-Dan and GP6-Dan, respectively. B, product of N-2-naphthylacetylation of dansyl-labeled glycopeptides. Peaks 3 and 4 represent GP5-DanNap and GP6-DanNap, respectively. C, the neuraminidase treated mixture of doubly fluorescence labeled glycopeptides (GP5-DanNap and GP6- DanNap). The C₁₈ RP-HPLC column was eluted with a gradient formed with the same two eluants as described in the legend to Fig. 5. Eluant 2 was varied from 30 to 70% over 55 min at a flow rate of 1 ml/min. The effluent was monitored by A₂₅₄ nm, at 2 AUFs.

Trimming of the Unmodified Branch by Stepwise Exoglycosidase Digestion—

Galactosyl residues modified at C-6 is not released by bovine testicular β-D-galactosidase (13, 24). Therefore, by stepwise exoglycosidase digestion of the unmodified branch in the fluorescent-labeled glycopeptide, we were able to generate a series of biantennary glycopeptides of decreasing sizes, eventually generating a linear glycopeptide still containing the two fluorophores at the terminal positions of molecule. Each of the trimmed derivatives was purified on a C₁₈ reverse phase column (Figs. 10 and 11). Each enzyme behaved in an expected fashion during the trimming operation, suggesting that no side reaction had taken place during the trimming procedure. Monosaccharide analysis of final products agreed with the expected trimming pattern.
with the expected linear glycopeptides derivatives. As expected, removal of a sugar from the nonreducing terminus caused the product to elute later.

The structures of final products were confirmed by mass spectrometry analysis. The molecular ions at m/z 1801.9 (M+H)⁺ and 1824.1 (M+Na)⁺ are consistent with a product 9′′.

**DISCUSSION**

In order to introduce fluorescent probes specifically at each branch in the biantennary glycopeptide, we could have taken the approach of random modification and HPLC separation as we have done for the triantennary glycopeptide (25). Instead, we chose to use biantennary bovine fibrinogen glycopeptide monosialylated only on the Manα(1–3)Man branch, because of its ready availability.

Separation of the sialylated glycopeptides based on the extent of sialylation on the glycopeptides was readily accomplished by anion-exchange chromatography with DEAE-cellulose. Fractions B (monosialyl) and C (disialyl) contained relatively homogeneous glycopeptides. However, the major monosialyl species was that with the sialyl group on the Gal6 of the Manα(1–3)Man branch (see Fig. 1). So our strategy was developed to utilize this specific structure.

We used the well established pyridylamination method (20) to characterize the structure of monosialylated oligosaccharide. It confirmed that the sialic acids of monosialylated glycopeptide are preferentially located on the terminal Gal6 on the Manα(1–3)Man branch. The propensity of the sialic acid on the Gal6 residue is in agreement with a previous report of Galβ(1–4)GlcNAcβ(1–2)Manα(1–3) branch being the preferred site for the bovine colostrum β-galactoside α(2–6)sialyltransferase (26).

N-2-Naphthylacetylation of the monosialylated glycopeptides facilitates their separation and detection by increasing their hydrophobicity and uv absorbance. We were also able to separate the NeuAc-containing glycopeptides from the NeuGc containing glycopeptides by RP-HPLC. The N-2- naphthylacetylated monosialyl glycopeptides were used as substrate for the peridate oxidation. We could selectively oxidize the terminal sialic acid on Gal6 located on the Manα(1–3)Man branch under controlled conditions without affecting the other sugar components. Galactose oxidase was able to oxidase the Gal6 residue without affecting the sialylated Gal6. By this strategy,
biantennary glycopeptides labeled at the N-terminal amino acid and at the terminal sugar of either of the branches were prepared. These were used for energy transfer experiments (see accompanying paper, Ref. 27).

We observed previously that the flexibility of two of the branches of triantennary oligosaccharide derived from bovine fetuin was lost when converted to a monoantennary structure by sequential digestion with exoglycosidases. In order to determine at what stage of trimming such a transition in the conformational structure occurs, we performed stepwise deglycosylation of the unmodified branch in the glycopeptides. After each stage of exoglycosidase trimming, the product was purified and submitted to resonance energy transfer measurements. The branch labeled with the fluorophore survived during the sequential exoglycosidase digestion because β-galactosidase was incapable of releasing the galactose modified at C-6 with 2-(dansylamido)ethylamine.

The introduction of the large fluorophores to oligosaccharides could possibly cause an artificial conformational change. However, previous studies (9) revealed that triantennary glycopeptides fluorophore-tagged at the C-6 positions of Gal residues did not cause notable changes in the binding affinity for asialoglycoprotein receptor on hepatocytes. From this result we can assume that introduction of fluorophores to biantennary glycopeptides could cause very minimal or negligible conformational change.

The results of resonance energy transfer experiments with these fluorescent labeled glycopeptides and their exoglycosidase-trimmed derivatives are described in the accompanying paper (27).

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