J. Biochem. **134**, 51–55 (2003)
DOI: 10.1093/jb/mvg112

Conversion of Pyridylamino Sugar Chains to Corresponding Reducing Sugar Chains

Chiaki Takahashi, Shin-ichi Nakakita and Sumihiro Hase*
Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043

Received February 20, 2003; accepted April 18, 2003

Pyridylamino sugar chains were converted to the corresponding reducing sugar chains by first converting them to 1-amino-1-deoxy derivatives using the method previously reported [S. Hase, *J. Biochem.* 112, 266–268 (1992)] and then converting the products to the corresponding reducing sugar chains using the Sommlet reaction. The reaction conditions were optimized so as to obtain the maximal product yield using 1-amino-1-deoxylactose and 1-amino-1-deoxy-*N*-acetylglucosamine. When the established procedure was successively applied to pyridylamino high-mannose and complex-type sugar chains, the corresponding reducing sugar chains were obtained in yields of 30%.

Key words: pyridylamino, conversion, reducing sugar, *N*-linked sugar chains.

Abbreviations: BA2-NH_2, 1-amino-1-deoxy derivative of BA2; ESI, electron spray ionization; Fuc, L-fucose; Gal, D-galactose; GalNAc, *N*-acetyl-D-galactosamine; Glc, D-glucose; GlcNAc, *N*-acetyl-D-glucosamine; GlcNAc-NH_2, 1-amino-1-deoxy-*N*-acetyl-D-glucosamine; G1M9-NH_2, 1-amino-1-deoxy derivative of G1M9; HPAEC, high pH anion-exchange chromatography; HPLC, high-performance liquid chromatography; Lac-NH_2, 1-amino-1-deoxylactose; Man, D-mannose; ManN, D-mannosamine; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; PA, pyridylamino; PAD, pulsed amperometric detector; Sia, sialic acid; TFA, trifluoroacetic acid; TLC, thin layer chromatography; TOF, time of flight. The structures and abbreviations of the sugar chains are listed in Fig. 1.

Pyridylamination is widely used for the analysis of sugar chains from glycoconjugates owing to the high sensitivity and excellent separation of pyridylaminated saccharides by reversed-phase HPLC (*1*, *2*). The procedure involves the formation of a covalent linkage by reduction of a Schiff adduct between the acyclic aldehyde form of the sugar chain and 2-aminopyridine, leading to the destruction of the cyclic structure of the reducing-terminal residue. This may affect biological activity or immunogenecity, recognition by lectins, or enzymes of the chitobiosyl core, especially in the case of sugar chains with α1-6– or α1-3–linked fucose residues commonly found on the reducing-terminal residue of *N*-linked sugar chains (*3*). In view of this drawback, conversion of PA-sugar chains to reducing sugar chains was tried using the Sommlet reaction, which converts primary amine to aldehyde (*4–6*), coupled with the procedure reported for the conversion of PA-sugar chains to 1-amino-1-deoxy derivatives (*7*).

MATERIALS AND METHODS

Materials—A Cosmosil 5C18-P column (4.6 × 150 mm) was purchased from Nacalai Tesque (Kyoto), a Mono Q HR 5/5 column (5.0 × 55 mm) from Amersham Biosciences (Upscale), Dowex 50W-X2 (200–400 mesh) from Dow Chemicals (Richmond, VA), Toyopearl HW-40F and TOYOPAK ODS cartridges from Tosoh (Tokyo), and Bio-Gel P-2 from Bio-Red (Hercules, CA). A CarboPac PA1 column (0.2 × 25 cm) was obtained from Dionex (Sunnyvale, CA), and palladium and hexamethylenetetramine were from Wako (Osaka). Insulin chain B (oxidized, bovine) and 2,5-dihydroxybenzoic acid were supplied by Sigma (St. Louis, MO), and neurotensin by Peptide Institute (Osaka). Endo-β-*N*-acetylglucosaminidase H (*Streptomyces griseus*) was purchased from Seikagaku Kogyo (Tokyo). PA-*N*-acetylglucosamine and PA-lactose were prepared as reported (*8*).

1-Amino-1-deoxylactose (Lac-NH_2) and 1-amino-1-deoxy-*N*-acetyl-D-glucosamine (GlcNAc-NH_2) were selected as standard sugars for optimizing the reaction conditions. 1-Amino-1-deoxy derivatives were prepared from the corresponding PA-derivatives as reported previously (*7*). The structures of the compounds were confirmed by mass spectrometry; the peaks of the molecule-related ions, $[M+H]^+$ (m/z 344.22, Calcd. 344.16) for Lac-NH_2 and $[M+H]^+$ (m/z 223.20, Calcd. 223.13) for GlcNAc-NH_2, were observed. PA-G1M9 and PA-BA2 were prepared as reported previously (*9*). G1M9 was prepared from hen egg IgY by digestion with glycopeptidase F (*10*). Bi-PA was prepared as reported (*11*).

High-Performance Liquid Chromatography (HPLC)—Reversed-phase HPLC was performed on a Cosmosil 5C18-P column at a flow rate of 1.5 ml/min at 25°C. The column was equilibrated with 100 mM ammonium acetate buffer, pH 4.0, containing 0.025% 1-butanol. After injecting a sample, the concentration of 1-butanol was raised linearly to 0.5% in 55 min. Elutions were monitored by measuring the fluorescence (excitation wavelength, 315 nm; emission wavelength, 400 nm).

High pH Anion-Exchange Chromatography (HPAEC)—Sugar chains were separated on a CarboPac PA-1 column and the elution was monitored with a pulse-amperometric detector (PAD). Elution condition A: for analysis of mono- and disaccharides, the eluent used was 15 mM

*To whom correspondence should be addressed. Tel: +81-6-6850-5380, Fax: +81-6-6850-5383.

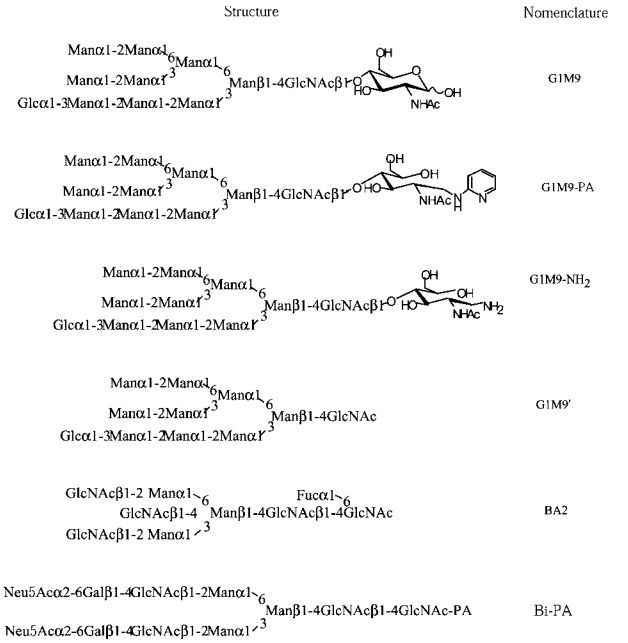

Fig. 1. **Structures and nomenclature of sugar chains used in the present study.**

NaOH at a flow rate of 1.0 ml/min. Elution condition B: for analysis of oligosaccharides two eluents, A and B, were used; Eluent A was 100 mM NaOH aqueous solution and Eluent B 100 mM sodium acetate–100 mM NaOH aqueous solution. The column was equilibrated with Eluent A. Ten minutes after injecting a sample, the percentage of Eluent B was increased linearly to 70% in 35 min. The flow rate was 1.0 ml/min. For purification of sugar chains by HPAEC, peak fractions were collected and immediately added to 0.6 ml of Dowex 50W-X2 (H^+). The suspension was poured onto a glass column (0.5 × 3 cm) and the column was washed with 3 column volumes of water. The washings and the eluate were collected and combined.

Conversion of PA-sugar Chains to 1-Amino-1-Deoxy Sugar Chains—PA-sugars were converted to 1-amino-1-deoxy sugar chains by the method reported previously (*7*). Briefly, a PA-sugar chain was dissolved in 1 ml of water, and the pH of the solution was adjusted to 3 with acetic acid. Palladium black (4 mg) was added to the solution, and the sample was then reduced with hydrogen gas at atmospheric pressure and room temperature for 3 h. After removal of palladium black by membrane filtration, the solution was freeze-dried. To this was added 0.2 ml of anhydrous hydrazine, and the solution was then heated at 70°C for 3 min. Excess hydrazine was removed by repeated evaporation with toluene *in vacuo*. The residues were dissolved in 1 ml of 10 mM acetic acid, then applied onto a Bio-Gel P-2 column (1.2 × 90 cm) equilibrated with 10 mM acetic acid. The elution was monitored by TLC with a ninhydrin reagent. For small sugars, the peak fraction was further concentrated to dryness and dissolved in 1 ml of 10 mM ammonium acetate buffer, pH 6.0, and the solution was then applied onto a HW-40F column (1.2 × 90 cm) equilibrated with 10 mM ammonium acetate buffer, pH 6.0. The elution was monitored by TLC with methanol–sulfuric acid. Structures were confirmed by ESI-MS for Lac-NH_2 and GlcNAc-NH_2 and by MALDI-TOF-MS for G1M9-NH_2 and BA2-NH_2. GlcNAc-NH_2 was quantified by NMR using fucose as an internal standard.

Established Procedure for Conversion of 1-Amino-1-Deoxy Sugar Chains to Reducing Sugar Chains—A 1-amino-1-deoxy derivative (about 1 nmol) was dissolved in 5 μl of water, and to this was added 8 μl of a saturated hexamethylenetetramine aqueous solution (*6*). To the solution was added 1.5 μl a 50% acetic acid aqueous solution (pH of the solution, 4.5), and the reaction mixture was heated at 100°C for 45 min in a sealed tube. After addition of 100 μl of water, the solution was concentrated to about 20 μl with a Speed Vac concentrator to remove the volatile reagents. Then, 200 μl of water was added to the reaction mixture and the solution was applied onto a TOYOPAK ODS cartridge, which was washed thrice with 300 μl of water. The eluate and washings were combined, the solution was added to 0.6 ml of Dowex 50W-X2 (H^+), and the suspension was poured into a small glass column. The product was washed with 3 column volumes of water. The eluate and the washings were combined, concentrated, and freeze-dried.

Mass Spectrometry—For MALDI-TOF-MS, sugar chains were co-crystallized in a matrix of 2,5-dihydroxybenzoic acid and analyzed with a Voyager-DE-RP BioSpectrometry workstation (Perseptive Biosystems, Framingham, MA) equipped with delayed extraction and operated in the reflector mode. Peptide standards (neurotensin and insulin chain B) were used to achieve a two-point external calibration for mass assignment of ions. For ESI-MS, a Mariner mass spectrometer (Perseptive Biosystems, Framingham, MA) was used in the reflector mode.

Acid Hydrolysis of Sugar Chains—Acid hydrolysis of sugar chains and analysis of the hydrolyzates by HPAEC PAD were carried out as reported previously (*12*). Briefly, a sugar chain was hydrolyzed with 2 M TFA at 100°C for 4 h in a sealed tube. The hydrolysate was evaporated to dryness *in vacuo*. Monosaccharides were quantified by HPAEC PAD by comparing the peak areas with those of standard monosaccharides.

Quantitation of Sugar Chains—Reducing sugars were quantified by Park and Johnson's method (*13*). Lac-NH_2, G1M9-NH_2, and BA2-NH_2 were quantified by the phenol–sulfuric acid method using mannose as a standard (*9*). G1M9 and BA2 were quantified in a similar manner using corresponding PA-derivatives as standards.

Other Methods—Sugars were pyridylaminated by the reported method (*8*). For TLC analysis, samples were separated by TLC (Silica gel 60; Merck, Darmstadt) using methanol:ethanol:acetic acid:water (2:1:0.05:0.4, v/v/v/v) as a solvent, spots were visualized with methanol–sulfuric acid (*14*) and a ninhydrin reagent (*15*). For NMR measurement, a sample was exchanged with ^2H_2O and finally dissolved in ^2H_2O. The spectra were recorded at 30°C on a JEOL LAMBDA 500 NMR spectrometer.

RESULTS

Identification of the Reaction Products Obtained from 1-Amino-1-Deoxy Sugars with Sommlet Reagents—Lac-NH_2 was treated with the hexamethylenetetramine Sommlet reagents (Fig. 2) and desalted as described in "MATE-

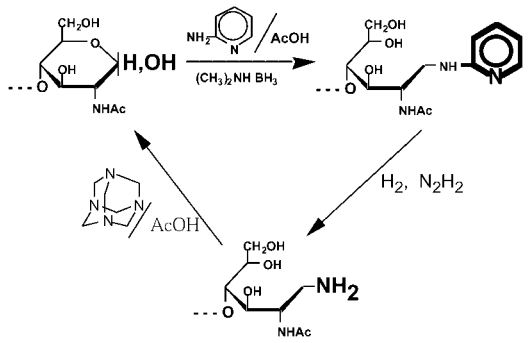

Fig. 2. **Scheme for the conversion of a PA-sugar chain into a free sugar chain.**

RIALS AND METHODS." In TLC, the residue gave a spot eluted at the same position as that of standard lactose. The spot was visualized with methanol–sulfuric acid but not with a ninhydrin reagent (Fig. 3). The residue was also analyzed by HPAEC, and a peak was observed at the same elution position as that of standard lactose (Fig. 4a). The reaction product from GlcNAc-NH_2 was analyzed in the same way, and a peak was detected at the elution position of standard GlcNAc (Fig. 4b). The peak that appeared at ManNAc (17 min) was probably due to epimerization; its amount was less than 5% of that for GlcNAc.

The reducing ability of the products was quantified by the method of Park and Johnson and compared with the values obtained by HPAEC PAD using standard lactose and GlcNAc. The amount of the product quantified by Park and Johnson's method was 89% on a molar basis of the value obtained by HPAEC PAD for Lac-NH_2, and 83% for GlcNAc-NH_2.

Establishment of Optimized Reaction Conditions—Lac-NH_2 and GlcNAc-NH_2 were treated with the Sommlet reagents under various reaction conditions. The reaction mixtures were desalted with a TOYOPAK ODS cartridge and Dowex 50W-X2 (H^+) and the product was quantified by HPAEC. The best results were obtained when the mixtures were treated with 8 μl of saturated hexamethylenetetramine solution (final concetration, 3 M) and 1.5 μl of 50% AcOH (pH 4.5) at 100°C for 45 min

Fig. 3. **TLC analysis of the Sommlet reaction product from Lac-NH_2.** Samples were visualized with methanol–sulfuric acid. Lane 1, reaction mixture; lane 2, lactose; lane 3, Lac-NH_2.

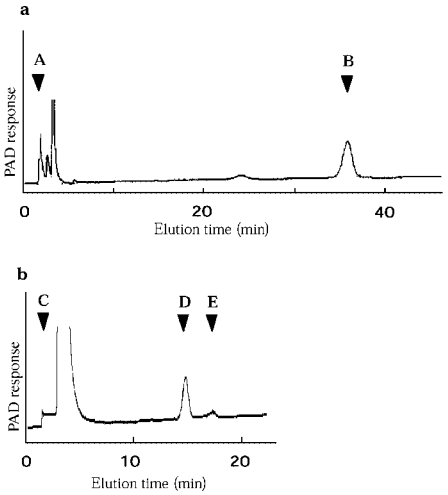

Fig. 4. **HPAEC analysis of the Sommlet reaction products from Lac-NH_2 and GlcNAc-NH_2.** Lac-NH_2 and GlcNAc-NH_2 were separately treated with the Sommlet reagents and the desalted reaction mixtures were analyzed by HPAEC. a: Reaction mixture from Lac-NH_2. b: GlcNAc-NH_2. Arrowheads indicate the elution positions of standard compounds: A, Lac-NH_2; B, lactose; C, GlcNAc-NH_2; D, GlcNAc, and E, ManNAc. Peaks appearing between 1 and 5 min are due to contaminating materials.

(Fig. 5). Under these reaction conditions, the yield was 78% for Lac-NH_2 and 18% for GlcNAc-NH_2.

Stability of Sialyl Linkage under the Established Reaction Conditions—Bi-PA was subjected to the established Sommlet reaction conditions and the reaction mixture was then analyzed by HPLC. Over 90% of Bi-PA was recovered intact, and less than 10% was hydrolyzed to two PA-monosialo sugar chains as determined by anion-exchange chromatography (data not shown) and reversed-phase HPLC (Fig. 6).

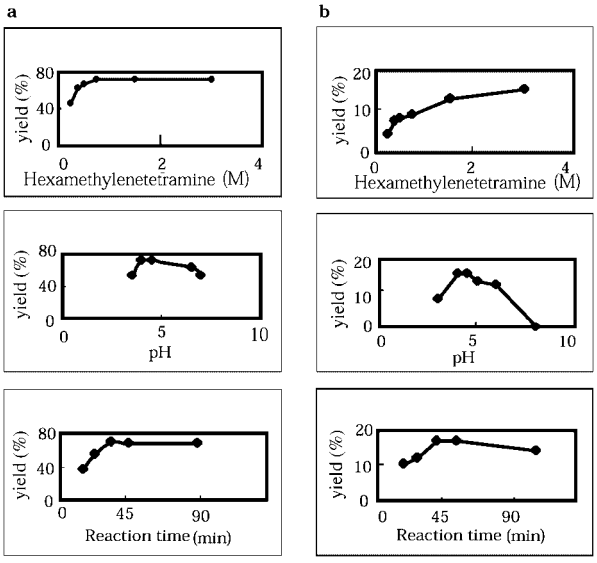

Fig. 5. **Effects of pH, time, and reagent concentration on product yields.** The concentration of hexamethylenetetramine solution, pH, and reaction time were varied and the yields were analyzed by HPAEC. a, Yields of lactose from Lac-NH_2; b, yields of GlcNAc from GlcNAc-NH_2.

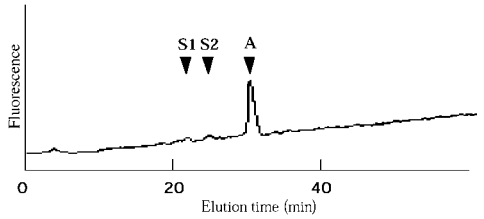

Fig. 6. **Stability of sialyl linkages under the Sommlet reaction conditions.** Bi-PA was heated with the Sommlet reaction reagents at 100°C for 45 min. After removal of the reagents, the reaction mixture was analyzed by reversed-phase HPLC. Arrowheads S1 and S2 indicate the elution positions of authentic monosialo derivatives. Arrowhead A indicates the elution position of Bi-PA

Application of Established Procedure to N-Linked Sugar Chains—The procedure established above was applied to *N*-linked sugar chains. PA-G1M9 was converted to G1M9-NH_2 as described in "MATERIALS AND METHODS" with a yield of 80%. The product was identified by MS, and a molecule-related ion, $[M+Na]^+$ (m/z 2,068.15, Calcd. 2,068.72 for G1M9-NH_2), was observed. G1M9-NH_2 (240 pmol) was then treated with the Sommlet reagents as described in "MATERIALS AND METHODS". The reaction mixture was then desalted and a part of the residue was analyzed by HPAEC (Fig. 7). The reaction mixture was fractionated by HPAEC and the fraction at the elution position of standard G1M9 was collected (Fraction z). The sugar composition of Fraction z was Man:GlcNAc = 9.2:2.0 when the loss due to the reaction during acid hydrolysis was corrected using standard G1M9. To confirm the structure of the reducing-terminal residue, Fraction z was digested with endo-β-*N*-acetylglucosaminidase H. GlcNAc and G1M9′ were detected by HPAEC PAD (Fig. 8), indicating that Fraction z was G1M9 and that it retained the cyclic structure of the reducing end GlcNAc. For further confirmation of the product, Fraction z was pyridylaminated and the PA-derivative was analyzed by reversed-phase HPLC. A peak was eluted at the elution position of G1M9-PA (data not shown). The yield of the product, G1M9, was 30% from G1M9-NH_2. The small peak that appeared at 39 min (Fig. 8) was due to $Glc_1Man_9GlcNAc_1ManNAc_1$, whose structure was confirmed by detecting ManN and ManNAc in the acid hydrolyzates and in the digestion products respectively with endo-β-*N*-acetylglucosaminidase H.

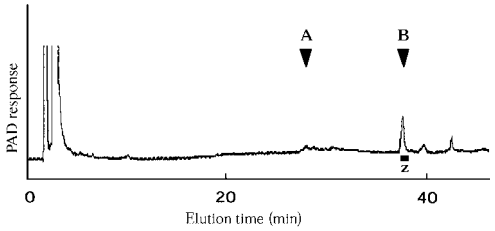

Fig. 7. **HPAEC analysis of the Sommlet reaction products from G1M9-NH_2.** G1M9-NH_2 was treated with the Sommlet reagents. The reaction mixture was desalted and purified by HPAEC. The fraction indicated by Bar z was pooled as the main Sommlet reaction product. Arrowheads A and B indicate the elution positions of G1M9-NH_2 and G1M9, respectively. The peak eluted at 39 min was $Glc_1Man_9GlcNAc_1ManNAc_1$. Peaks appearing between 1 and 3 min are due to contaminating materials.

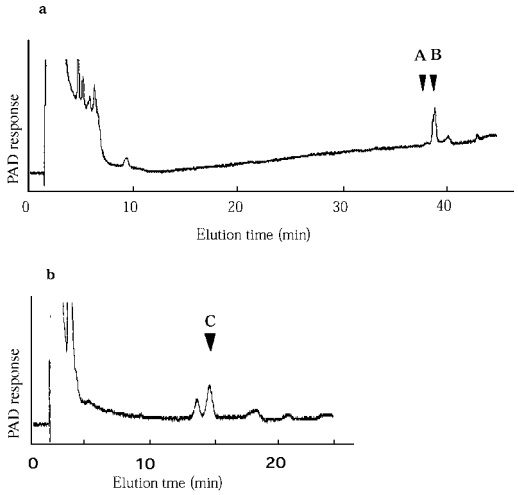

Fig. 8. **Detection of the reducing-end sugar residue of Fraction z.** Fraction z was digested with endo-β-*N*-acetylglucosaminidase H and the products were analyzed by HPEAC. a, Analysis of the non-reducing part of Fraction z; b, the reducing terminal of Fraction z. Arrowheads A, B, and C indicate the elution positions of Fraction z, G1M9′, and GlcNAc, respectively. In a, peaks appearing between 0 and 10 min are due to contaminating materials. In b, peaks appearing at 14 min, 2–5 min, and 16–25 min are due to contaminating materials that appeared in a control experiment. In b, G1M9′ was not eluted within 25 min under the conditions used.

Our method was also applied to complex-type sugar chains. PA-BA2 was converted to BA2-NH_2 as described in "MATERIALS AND METHODS". The product was identified by MS, and a molecule-related ion, $[M+Na]^+$ (m/z 1,689.73, Calcd. 1,689.65), was observed. BA2-NH_2 (200 nmol) was treated with the Sommlet reagents as described for G1M9-NH_2. A peak eluted at the same elution position as BA2 by HPAEC was collected. A molecular-related ion, $[M+Na]^+$ (m/z 1,688.34, Calcd.; 1,688.61), was observed. The product was pyridylaminated and the pyridylamination product was analyzed by reversed-phase HPLC. A peak eluted at the position of BA2-PA was detected (data not shown). The yield of BA2 from BA2-NH_2 was about 30%.

DISCUSSION

Our procurement of products with reducing power shows that 1-amino-1-deoxy sugars can be converted to the corresponding sugar chains by the Sommlet reaction. By using the procedure described, it is now possible to synthesize reducing sugars from the corresponding PA-sugar chains. That is, distinctly structured free sugar chains can be prepared from glycoproteins with heterogeneous structures owing to the excellent separation of PA-sugar chains by reversed-phase HPLC. Although a pure PA-sugar chain can be prepared rather easily from complex mixtures of sugar chains prepared from natural glycoproteins due to the effectiveness of separation by reversed-phase HPLC, pyridylamination involves an acyclic event that leads to the destruction of the reducing-terminal residue of sugar chains. Therefore, PA-sugar chains are in some cases inadequate for investigating proteins that recognize the reducing-terminal residue. For instance,

the affinity of lentil lectin (*Lens culinaris*), which recognizes α-mannose, α-glucose, and 6-*O*–linked fucose on the reducing-terminal GlcNAc, is affected by the reduction of sugar chains.

1-Amino-1-deoxy derivatives were converted into free sugar chains from Lac-NH_2, GlcNAc-NH_2, and oligosaccharides-NH_2 with yields of 78, 18, and 30%, respectively by the method established in this work. As 6-*O*–linked fucose on the reducing-terminal GlcNAc (BA2-NH_2) was not hydrolyzed and did not influence the conversion, sugar chains with 3-*O*–linked fucose seemed to be converted. Most sialyl linkages were not hydrolyzed to monosialo sugar chains, and epimerization was less than 20% for G1M9 and BA2. This epimerization was considered to occur mostly during purification by HPAEC, since epimerization of up to 15% arising from the alkaline conditions of HPAEC has been reported (*16*, *17*).

We speculate that *O*-linked sugar chains with GalNAc and those with neutral sugars at reducing ends were converted to reducing sugars as 1-amino derivatives of neutral sugars, and that *N*-acetylamino sugars were converted to the corresponding reducing sugars. Our procedure was successfully applied to *N*-linked and *O*-linked sugar chains to give yields of around 30% from 1-amino-1-deoxy sugar chains. As sialyl linkages are stable under the reaction conditions used to convert PA-sugar chains to reducing sugar chains, the procedure is applicable to sialylated sugar chains.

Reducing sugars can be modified to 1-*N*-glycyl β-oligosaccharides (*18*), which opens a pathway to synthesizing neoglycoproteins and neoglycolipids that retain the cyclic structure of reducing-end sugars (*19*, *20*). Also, reducing sugars can be used with the reductive amination reaction, including immobilization onto the Surface Plasmon Reasonance Sensor chip (*21*, *22*).

This work was supported in part by the 21st Century COE (Creation of Integrated EcoChemistry), Protein 3000 programs, and the Japan Health Science Foundation.

REFERENCES

1. Hase, S. (1994) High-performance liquid chromatography of pyridylaminated saccharides. *Methods Enzymol.* **230**, 225–237
2. Natsuka, S. and Hase, S. (1998) Analysis of *N*- and *O*-glycans by pyridylamination. *Methods Mol. Biol.* **76**, 101–113
3. Yamamoto, K., Tsuji, T., and Osawa, T. (1982) Requirement of the core structure of a complex-type glycopeptide for the binding to immobilized lentil- and pea-lectins. *Carbohydr. Res.* **110**, 283–289
4. Sommlet, M. (1913) Sur un mode de decomposition des halogenoalcoylates d' hexamethylene-tetramine. *Compt. rend.* **157**, 852–854
5. Angyal, S.J. and Rassack, R.C. (1949) The Sommlet Reaction. Part I. The course of the reaction. *J. Chem. Soc.* 2700–2704
6. Angyal, S.J., Penman, D.R., and Warwick, G.P. (1953) The Sommlet Reaction. Part IV. The preparation of aliphatic aldehydes. *J. Chem. Soc.* 1737–1739
7. Hase, S. (1992) Conversion of pyridylamino sugar chains to 1-amino-1-deoxy derivatives, intermediates for tagging with fluorescein and biotin.*J. Biochem.* **112**, 266–268
8. Yanagida, K., Natsuka, S., and Hase, S. (1999) A pyridylamination method aimed at automatic oligosaccharide analysis of *N*-linked sugar chains. *Anal. Biochem.* **274**, 229–234
9. Ozaki, Y. Omichi, K., and Hase, S. (1996) Preparation of neoglycolipids with a definite sugar chain moiety from glycoproteins. *Anal. Biochem.* **241**, 151–155
10. Tarentino, A.L. and Plummer, Jr.T.H. (1994) Enzymatic deglycosylation of asparagines-linked glycans: Purification, properties, and specificity *Fravobacterium meningosepticum*. *Methods Enzymol.* **230**, 44–57
11. Nakakita, S., Menon, K.K., Natsuka, S., Ikenaka, K., and Hase, S. (1999) β1–4Galactosyltransferase activity of mouse brain as revealed by analysis of brain-specific complex-type *N*-linked sugar chains. *J. Biochem.* **126**, 1161–1169
12. Fan, J.Q., Namiki, Y., Matsuoka, K., and Lee, Y.C. (1994) Comparison of acid hydrolytic conditions for Asn-linked oligosaccharides. *Anal. Biochem.* **219**, 375–378
13. Park, J.T. and Johnson, M.J. (1949) A submicrodetermination of glucose. *J. Biol. Chem.* **181**, 149–151
14. Michel, D., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Fred, S. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**, 350–356
15. Samejima, K., Dairman, W., Stone, J., and Udenfriend, S. (1971) Condition of ninhydrin with aldehydes and primary amines to yield highly fluorescent ternary products II. Application to the detection and assay of peptides, amino acid, amines, and amino sugars. *Anal. Biochem.* **42**, 237–247
16. Speck, Jr.J.C. (1958) Formation and cleavage of the oxygen ring in sugar. *Adv. Carbohydr. Chem.* **13**, 63–103
17. Cataldi, T.R.I., Campa, C., and De Benedetto, G.E. (2000) Carbohydrate analysis by high-performance anion-exchange chromatography with pulsed amperometric detection: The potential is still growing. *Fresenius J. Anal. Chem.* **368**, 739–758
18. Manger, I.D., Rademacher, T.W., and Dwek, R.A. (1992) 1-*N*-glycyl β-oligosaccharide derivatives as stable intermediates for the formation of glycoconjugate probes. *Biochemistry* **31**, 10724–10732
19. Meinjohanns, E., Meldal, M., Paulsen, H., Dwek, R.A., and Bock. K. (1998) Novel sequential solid-phase synthesis of N-linked glycopeptides from natural sources. *J. Chem. Soc. Perkin Trans.* **1**, 549–560
20. Feizi, T., Stoll, M.S., Yuen, C.T., Chai, W., and Lawson, A.M. (1994) Neoglycolipids: probes of oligosaccharide structure, antigenicity, and function. *Methods Enzymol.* **230**, 484–519
21. Shinohara, Y., Sota, H., Gotoh, M., Hasebe, M., Tosu, M., Nakao, J., Hasegawa, Y., and Shiga, M. (1996) Bifunctional labeling reagent for oligosaccharides to incorporate both chromophore and biotin groups. *Anal. Chem.* **68**, 2573–2579
22. Green, R.J., Frazier, R.A., Shakesheff, K.M., Davies, M.C., Roberts, C.J., and Tendler, S.J. (2000) Surface plasmon resonance analysis of dynamic biological interaction with biomaterials. *Biomaterials* **21**, 1823–1835