New Method for Determining the Sugar Composition of Glycoproteins, Glycolipids, and Oligosaccharides by High-performance Liquid Chromatography

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A new method is reported that can be performed within a single vessel to analyze the composition of aldose, hexosamine, and sialic acid residues of glycoproteins, glycolipids, and oligosaccharides. Glycoconjugates are treated with sialidase or subjected to mild acid hydrolysis, before being treated with N-acetylneuraminic acid aldolase to convert the free sialic acid residues to their corresponding N-acetylmannosamines. The reaction mixture is then successively subjected to acid hydrolysis (in order to produce monosaccharides), N-acetylation, and conversion with p-aminobenzoic acid ethyl ester (ABEE). The ABE-converted monosaccharides are simultaneously determined by reverse-phase high-performance liquid chromatography. Determination of the sugar compositions of bovine fetuin, II' NeuGcα-LacCer, and 3'-sialyllactose with this method was found to be highly accurate. Linearity of the peak area vs. the amount of bovine fetuin ranged from 1 to 50 μg in all ABE-converted monosaccharides. With a slight modification to this method, sialic acid residues can be separately determined as NeuAc and NeuGc. This novel method and its modified version are used to demonstrate the sugar compositions of α,β-acid glycoproteins from several sources.

Key words: p-aminobenzoic acid ethyl ester; sialic acid; α,β-acid glycoprotein; monosaccharide analysis

Determination of the sugar composition of glycoconjugates, which contain various monosaccharides, usually employs hydrolysis to produce monosaccharides. The gas-liquid chromatographic analysis of monosaccharides as trimethylsilyl derivatives, involves subjecting glycoproteins to methanolation and subsequent trimethylsilylation. This process can be performed within a single vessel, in which acid-labile sialic acid residues are not destroyed, enabling the amounts of aldose, hexosamine, and sialic acid residues to be simultaneously determined. However N-glycosidic GlcNAc is not quantitatively converted to methyl-GlcNAc by methanolation.

Aldose and hexosamine residues are hydrolyzed to a greater extent than sialic acid residues in a sugar composition analysis by HPLC. There has consequently been no previous report on the simultaneous determination of aldose, hexosamine, and sialic acid residues by HPLC.

A sugar composition analysis, employing ABEE conversion of monosaccharides by reductive amination, is performed prior to applying the sample to the HPLC column. This is a simple and highly sensitive monosaccharide analysis method of the pmol order. ABEE conversion of sialic acids which are not reducing sugars has not previously been reported. On the other hand, it is known that N-acetylneuraminic acid aldolase converts sialic acid residues to N-acetylmannosamines, and there have been reports on the analysis of sialic acid residues by HPLC with this enzyme. We speculated that the simultaneous determination of aldose, hexosamine, and sialic acid residues should be possible by using N-acetylneuraminic acid aldolase. That is, N-acetylmannosamines, to which sialic acid residues were converted with this enzyme, were acid-stable reducing monosaccharides, and thus would not be destroyed by acid hydrolysis, enabling conversion with ABEE. We report in this paper a new method that can be performed within a single vessel that involves five processes: (1) release of sialic acid residues, (2) conversion of sialic acid residues to N-acetylmannosamines, (3) acid hydrolysis, (4) N-acetylation, and (5) conversion with ABEE. The ABEE-converted monosaccharides obtained by these processes were simultaneously determined by reverse-phase HPLC. On the other hand, when the acid hydrolysis and N-acetylation processes were omitted, NeuAc and NeuGc were determined as ABE-NeuNAC and ABE-NeuNGc, respectively, by HPLC under similar conditions.

Materials and Methods

Chemicals. An ABE labeling kit, II' NeuGcα-LacCer, and Honenpak C18 (75 mm × 4.6 mm i.d.) were

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Abbreviations: ABE, p-aminobenzoic acid ethyl ester; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; GLC, gas-liquid chromatography; NeuAc, N-acetylneuraminic acid; NeuGc, N-glycolylneuraminic acid; Gal, d-glucose; Gal, d-galactose; Man, d-mannose; Ara, L-arabinose; Rib, d-ribose; Xyl, d-xylene; GlcNAc, N-acetyl-d-glucosamine; GaINAC, N-acetyl-d-galactosamine; ManNAc, N-acetyl-d-mannosamine; Fuc, L-fucose; Rha, L-rhamnose; ManNGc, N-glycolyl-d-mannosamine; Sia, sialic acid; II' NeuGcα-LacCer, NeuGc2-3Galβ1-4Glcβ1-1'ceramide; 3'-sialyllactose, NeuAcα2-3Galβ1-4Glc; AGP, α,β-acid glycoprotein; AQC, 6-aminquinolinyl-N-hydroxysuccinimidyl carbamate; DMB, 1,2-diamino-4,5-methyleneedioxybenzene; RSD, relative standard deviation.
purchased from Seikagaku Corp. (Tokyo, Japan). HPLC-grade acetonitrile was purchased from Wako Pure Chemical Industries (Osaka, Japan). Bovine fetuin (from fetal calf serum), α1-acid glycoproteins, 3′-sialyllactose, NeuAc, and NeuGc (Lot 027H0485; 88.5% purity, containing 10.9% NeuAc) were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). All other chemicals and standard monosaccharides were of the highest grade commercially available.

Enzymes. N-Acetyleneuraminic acid aldolase (EC 4.1.3.3; from Escherichia coli) and sialidase (EC 3.2.1.18; from Arthrobacter ureafaciens) were purchased from Nacalai Tesque (Kyoto, Japan). N-Acetyleneuraminic acid aldolase was dissolved in a 20 mM sodium phosphate buffer at pH 7.3 (5 munits/μl). Sialidase was dissolved in a 10 mM sodium phosphate buffer at pH 7.3 (1 munit/μl).

Treatment for the simultaneous determination of aldose, hexosamine, and sialic acid residues (Method 1). A sample solution (5 μg of glycoprotein, and approximately 5 μg of 3′-sialyllactose and 10 μg of NeuGcα-LacCer) and an Rha solution (4 nmol) as an internal standard were placed in a tapered glass tube (45 mm × 9.5 mm i.d.) fitted with a Teflon-lined screw cap, which is an attachment for the ABEE labeling kit. The mixture was evaporated to dryness under reduced pressure.

In order to release the sialic acid residues by mild acid hydrolysis, 20 μl of a 0.1 M TFA solution was added, the tube was sealed, and the solution incubated at 80°C for 1 hr. After being cooled to room temperature, the reaction mixture was evaporated to dryness under reduced pressure. Five microliters of an N-acetyleneuraminic acid aldolase solution, 2 μl of distilled water, and 3 μl of an 83 mM sodium acetate buffer at pH 5.0 were added to the resulting residue, and the mixture incubated at 37°C for 17 hr. In the case of releasing the sialic acid residues by sialidase, 5 μl of an N-acetyleneuraminic acid aldolase solution, 2 μl of a sialidase solution, and 3 μl of the 83 mM sodium acetate buffer at pH 5.0 were added to the dried sample, and the mixture incubated at 37°C for 17 hr.

Ten microliters of an 8 M TFA solution was added to the sample solution (10 μl) that had been previously treated with N-acetyleneuraminic acid aldolase, and the solution incubated at 121°C for 2 hr. After being cooled to room temperature, the reaction mixture was evaporated to dryness under reduced pressure. The resulting residue was dissolved in 100 μl of 2-propanol and again evaporated to dryness to remove residual TFA. The residue was treated with a mixture of 40 μl of pyridine/methanol (5/95, v/v) and 10 μl of acetic anhydride at room temperature for 30 min. The mixture was again evaporated to dryness under reduced pressure.

The monosaccharides were converted at their reducing end with ABEE according to the method of Wang et al. Ten microliters of distilled water and 40 μl of the ABEE reagent solution were added to the residue. The mixture was vortexed and then incubated at 80°C for 1 hr. The mixture was cooled to room temperature, before distilled water (0.2 ml) and an equal volume of chloroform were added. After vigorous vortexing, the mixture was centrifuged for 1 min, and the upper aqueous layer was subjected to an HPLC analysis.

Treatment for the determination of NeuAc and NeuGc (Method 2). This treatment was carried out in a manner similar to that for Method 1, except that the acid hydrolysis and N-acetylation processes were omitted. A sample solution (5 μg of glycoprotein, and approximately 5 μg of 3′-sialyllactose and 10 μg of NeuGcα-LacCer) and an Rha solution (4 nmol) as the internal standard were placed in a tapered glass tube (45 mm × 9.5 mm i.d.) fitted with a Teflon-lined screw cap. The mixture was evaporated to dryness under reduced pressure.

In order to release the sialic acid residues by mild acid hydrolysis, 20 μl of a 0.1 M TFA solution was added, the tube was sealed, and the solution incubated at 80°C for 1 hr. After being cooled to room temperature, the reaction mixture was evaporated to dryness under reduced pressure. Five microliters of an N-acetyleneuraminic acid aldolase solution, 2 μl of distilled water, and 3 μl of an 83 mM sodium acetate buffer at pH 5.0 were added to the resulting residue, and the mixture incubated at 37°C for 17 hr. In the case of releasing the sialic acid residues by sialidase, 5 μl of an N-acetyleneuraminic acid aldolase solution, 2 μl of a sialidase solution, and 3 μl of the 83 mM sodium acetate buffer at pH 5.0 were added to the dried sample, and the mixture incubated at 37°C for 17 hr.

Forty microliters of the ABEE reagent solution was added to 10 μl of the solution that had been previously treated with N-acetyleneuraminic acid aldolase, before the mixture was vortexed and incubated at 80°C for 1 hr. The mixture was cooled to room temperature, before distilled water (0.2 ml) and an equal volume of chloroform were added. After vigorous vortexing, the mixture was centrifuged for 1 min, and the upper aqueous layer was subjected to an HPLC analysis.

HPLC analysis and determination of the sugar composition. The ABEE-converted monosaccharides were analyzed according to the HPLC conditions of Yasuno et al. with slight modifications. A Honenpak C18 column (75 mm × 4.6 mm i.d.), which is stable to alkaline solvents, was used. An ABEE-converted monosaccharide was injected into the column at a flow rate of 1.0 ml/min at 30°C and detected by fluorescence monitoring (305 nm excitation wavelength; 360 nm emission wavelength) with an RF-10A instrument (Shimadzu Corp., Kyoto, Japan). Solvent A was a 0.2 M potassium borate buffer at pH 8.9 containing 7% acetonitrile, and solvent B was a 0.02% TFA solution containing 50% acetonitrile. Solvent A was used for 50 min for separation, and solvent B was then passed through the column for 5 min for washing. After solvent A had been passed through the column for 20 min, the next sample was injected into the column.

In order to determine the monosaccharide content of a sample for Method 1, the set of monosaccharides,
Gal, Glc, Man, Fuc, GlcNAc, GalNAc, NeuAc, and Rha (4 nmol each), was used as standards, and for Method 2, NeuAc and Rha (4 nmol each), and NeuGc and Rha (4 nmol each) were used as standards. The standard monosaccharides were treated in an identical manner to that used for the samples. The monosaccharide content of a sample was determined by dividing the peak area of each monosaccharide by that of the Rha internal standard and the response factor for each monosaccharide, which was determined by dividing the peak area of each standard monosaccharide by that of Rha, and then multiplying the result by the amount of Rha added.

**Analysis of the sugar composition by previously proposed methods.** The sugar composition of bovine fetuin was determined by the previously proposed methods. Aldose residues were analyzed by GLC (2% OV-17 column) after methanolysis (1.4 M HCl-methanol at 90°C for 2 hr) and subsequent trimethylsilylation.\(^1\)

Hexosamine residues were analyzed by HPLC after acid hydrolysis (4 M HCl at 100°C for 6 hr) and subsequent conversion with AQC, using an AccQ-Fluor reagent kit (Waters components, Millipore, Milford, MA, U.S.A.).\(^1\)

Sialic acid residues were analyzed by HPLC after mild acid hydrolysis (0.1 M TFA at 80°C for 1 hr) and subsequent conversion with DMB, using a DMB labeling kit (Takara Shuzo Co., Shiga, Japan).\(^1\)

**Results**

**Linearity of the treatment with N-acetyleneuraminic acid aldolase and conversion with ABEE**

N-Acetyleneuraminic acid aldolase quantitatively converts NeuAc and NeuGc to ManNAc and ManNGc, respectively.\(^6,7\) We confirmed the linearity of the ABEE conversion of ManNAc and ManNGc by N-acetyleneuraminic acid aldolase. Various amounts of NeuAc and NeuGc were converted with N-acetyleneuraminic acid aldolase, directly converted with ABEE and analyzed by HPLC. Linearity of the peak area vs. the amount of NeuAc and NeuGc in the range of 1–40 nmol was apparent (Fig. 1). Therefore, NeuAc and NeuGc were quantitatively transformed to ABEE-ManNAc and ABEE-ManNGc, respectively, by the combined conversion with N-acetyleneuraminic acid aldolase and ABEE.

**Relationship between the peak area and amount of glycoprotein**

Bovine fetuin containing N-glycosidic and O-glycosidic sugar chains was used to examine the relationship between the peak area and amount of glycoprotein. Various amounts of bovine fetuin were treated by Method 1 (the sialic acid residues were released with sialidase) and then the resulting solution was analyzed by HPLC. The peak area of the ABEE-converted monosaccharides was plotted against the amount of bovine fetuin treated by Method 1 (Fig. 2). Linearity of the peak area vs. the amount of bovine fetuin in the range of 1–50 μg was apparent in all ABEE-converted monosaccharides.

**Simultaneous determination of aldose, hexosamine, and sialic acid residues in glycoprotein by Method 1**

The simultaneous determination of aldose, hexosamine, and sialic acid residues in bovine fetuin was demonstrated by using Method 1. The chromatograms are shown in Fig. 3, and the sugar composition of bovine fetuin is summarized in Table 1. These results are in good agreement with the values obtained by previously proposed methods based on different principles. RSDs for the results in Table 1 are less than 6% for all monosaccharides (n=10). There was no difference in the amount of sialic acid residues released by mild acid hydrolysis and the sialidase treatment for bovine fetuin. The contents of Gal, Man, Fuc, GlcNAc, and GalNAc in bovine fetuin obtained by Method 1 were in good agreement with the values ("No release" in Table 1) obtained by the method that omits the release of sialic acid and conversion with the N-acetyleneuraminic acid aldolase treatment of Method 1.
Simultaneous determination of aldose, hexosamine, and sialic acid residues in glycolipid and oligosaccharide by Method 1

Method 1 was used to simultaneously determine the compositions of aldose, hexosamine, and sialic acid residues in II\textsuperscript{3}NeuGc\textsubscript{o}-LacCer and 3'-sialyllactose. The chromatograms are shown in Fig. 4, and the sugar compositions of II\textsuperscript{3}NeuGc\textsubscript{o}-LacCer and 3'-sialyllactose are summarized in Table 2. RSDs of the results are less than 6% for all monosaccharides (based on four and ten analyses of II\textsuperscript{3}NeuGc\textsubscript{o}-LacCer and 3'-sialyllactose, respectively). The sugar compositions of II\textsuperscript{3}NeuGc\textsubscript{o}-LacCer and 3'-sialyllactose determined by Method 1 were highly accurate.

Determination of NeuAc and NeuGc by Method 2

Method 2 was used to determine the compositions of NeuAc and NeuGc in bovine fetuin, II\textsuperscript{1}NeuGc\textsubscript{o}-LacCer, and 3'-sialyllactose. The chromatograms are shown in Fig. 5, and the sugar compositions of bovine fetuin, II\textsuperscript{1}NeuGc\textsubscript{o}-LacCer, and 3'-sialyllactose are summarized in Tables 1 and 2. The results for bovine fetuin are in good agreement with the values obtained by the DMB method. The results for 3'-sialyllactose are also in good agreement with the calculated values. On the other hand, NeuAc was detected in II\textsuperscript{1}NeuGc\textsubscript{o}-LacCer, but it was proved by the DMB method that this NeuAc was a contaminant in the sample. The results for II\textsuperscript{1}NeuGc\textsubscript{o}-LacCer are in good agreement with the values obtained by the DMB method. RSDs of the results for bovine fetuin, II\textsuperscript{1}NeuGc\textsubscript{o}-LacCer, and 3'-sialyllactose are less than 6% for all monosaccharides based on ten, four, and ten analyses, respectively. The sums of the NeuAc and NeuGc contents of glycoconjugates obtained by Method 2 are in good agreement with the sialic acid residue contents obtained by Method 1. Therefore, it was not a problem that only NeuAc was used as a stan-
Table 1. Analysis of the Total Sugar Composition of Bovine Fetuin

<table>
<thead>
<tr>
<th>Method</th>
<th>Gal</th>
<th>Man</th>
<th>Fuc</th>
<th>GlcNAc</th>
<th>GalNAc</th>
<th>Sia</th>
<th>NeuGe</th>
<th>NeuAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present one</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sialidase</td>
<td>9.5</td>
<td>7.7</td>
<td>n.d.*</td>
<td>11.2</td>
<td>2.0</td>
<td>10.5</td>
<td>0.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Mild acid hydrolysis</td>
<td>9.5</td>
<td>8.3</td>
<td>n.d.</td>
<td>10.9</td>
<td>2.0</td>
<td>10.3</td>
<td>0.4</td>
<td>9.6</td>
</tr>
<tr>
<td>No release**</td>
<td>9.2</td>
<td>7.4</td>
<td>n.d.</td>
<td>11.2</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLC***</td>
<td>9.6</td>
<td>7.8</td>
<td>n.d.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAA**</td>
<td></td>
<td></td>
<td></td>
<td>10.8</td>
<td>1.9</td>
<td>9.7</td>
<td>0.3</td>
<td>9.4</td>
</tr>
<tr>
<td>DMB**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Mean values of monosaccharide contents are given, and RSDs were less than 6% for all values (n=10) determined by the present methods. Gal, Man, Fuc, GlcNAc, GalNAc, and Sia were determined by Method 1, and NeuGe and NeuAc by Method 2 as described in the Materials and Methods section.
* n.d.: not detectable.
** No release: the sialic acid residue release and conversion processes of Method 1 were omitted.
*** GLC: determined by GLC.
** AAA: determined by an amino acid analysis with AQC-conversion.
*** DMB: determined by the DMB method.

Table 2. Analysis of the Total Sugar Composition of II'-NeuGa0-LacCer and 3'-Sialyllactose

<table>
<thead>
<tr>
<th>Glycoconjugate</th>
<th>Gal</th>
<th>Glc</th>
<th>Sia</th>
<th>NeuGe</th>
<th>NeuAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>II'-NeuGa0-LacCer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present method</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>0.9</td>
<td>0.1</td>
</tr>
<tr>
<td>DMB***</td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Known ratio</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>3'-Sialyllactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present method</td>
<td>1.0</td>
<td>0.9</td>
<td>1.0</td>
<td>n.d.*</td>
<td>0.9</td>
</tr>
<tr>
<td>Known ratio</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

* Mean values of the monosaccharide contents of II'-NeuGa0-LacCer and 3'-sialyllactose are given, and RSD was less than 6% for all values (n=4 and 10, respectively) determined by the present methods. Gal, Glc, and Sia were determined by Method 1, and NeuGe and NeuAc by Method 2 as described in the Materials and Methods section.
*** Molar ratio: the amount of Gal is taken as unity.
* n.d.: not detectable.
*** DMB: determined by the DMB method.

Standard for the quantification of sialic acid residues with a sample containing NeuGe in Method 1.

Sugar composition of α1-acid glycoproteins from several sources

Methods 1 and 2 were used to determine the sugar compositions of AGPs from ovine, canine, bovine, and human sources. The chromatograms are shown in Figs. 6 and 7, and the sugar compositions of AGPs are summarized in Table 3. RSDs of the results are less than 6% for all monosaccharides (n=10). GalNAc was present in ovine and canine AGPs; therefore, O-glycosidic oligosaccharide chains were presumably present in these AGPs. Fuc was present in human AGP, and NeuGe in ovine and bovine AGPs. In this manner, the kind of monosaccharides and the sugar composition of AGP were distinct in different animals, so Methods 1 and 2 can be applied to compare the sugar composition of glycoproteins from different sources.

Discussion

A sugar composition analysis by the present methods is simple and highly sensitive, and the results for bovine

Fig. 5. HPLC Separation of ABEE-converted N-acetylmannosamines from Bovine Fetuin, II'-NeuGa0-LacCer and 3'-Sialyllactose.

Bovine fetuin, II'-NeuGa0-LacCer and 3'-sialyllactose (5 μg, and approximately 10 μg and 5 μg, respectively) were treated by Method 2 and analyzed by HPLC as described in the Materials and Methods section. (A) ABEE-converted monosaccharide standard, (B) ABEE-converted N-acetylmannosamine standard, (C) bovine fetuin treated with sialidase, (D) bovine fetuin subjected to mild acid hydrolysis, (E) II'-NeuGa0-LacCer subjected to mild acid hydrolysis, and (F) 3'-sialyllactose subjected to mild acid hydrolysis. The numbered peaks represent the same sugars as those shown in Fig. 3, and 12 and 13 are ManNeuGc and lactose, respectively.
fetuin, II′NeuGcα–LacCer, and 3′-sialyllactose are in good agreement with the values obtained by previously proposed methods and with the calculated values. The reproducibility of the complete analysis of bovine fetuin, II′NeuGcα–LacCer, and 3′-sialyllactose is within 6% RSD. Therefore, the present methods are proved very useful for the analysis of sugar composition.

The fluorometric response factor for each ABEE-converted monosaccharide was different. We assume that the amount recovered by extraction was different for each ABEE-converted monosaccharide. However, this is not a problem because the plot of the response vs. the amount of monosaccharide was linear (Fig. 1). The degradation of each monosaccharide by acid hydrolysis was different; therefore, standard monosaccharides would be necessary for the analysis of sugar composition by the present methods.

Method 1 resulted in both NeuAc and NeuGc being detected as ABEE-ManNAc. Therefore, we determined the appropriate standards for a sample containing NeuAc and NeuGc. The same amounts of NeuAc and NeuGc were treated with N-acetylenuraminic acid aldolase, and subsequently subjected to acid hydrolysis, N-acetylation and conversion with ABEE. The amounts of ABEE-ManNAc obtained were calculated from the peak areas by HPLC. The relative molar response of ABEE-ManNAc obtained from NeuAc to that from NeuGc was 1.02. Therefore, NeuAc was used as the standard for determining the sugar composition of a sample containing NeuGc. In practice, in the case of using NeuAc as the standard (i.e., by Method 1), the results of the analysis of the sialic acid residue content of samples containing both NeuAc and NeuGc were in good agreement with the values obtained by Method 2 and by a previously proposed method (Tables 1 and 2).

The contents of Gal, Man, Fuc, GlcNAc, and GalNAc in bovine fetuin obtained by Method 1 agree well with the values obtained by the method that omits the release of sialic acid and conversion with the N-acetylenuraminic acid aldolase treatment in Method 1. That is, salts and enzymes used in the release of sialic acid and conversion with the N-acetylenuraminic acid aldolase treatment had no influence on the acid hydrolysis, N-acetylation, conversion with ABEE, and sugar composition results.

Sialic acid residues are acid-labile, and ABEE conver-
sion of sialic acid residues has not previously been demonstrated. Sialic acid residues were converted with N-acetylenuramic acid aldolase to N-acylmannosamines, which are acid-stable reducing monosaccharides and capable of being converted with ABEE. In Method 1, the five processes consisting of (1) release of sialic acid residues, (2) conversion of sialic acid residues to N-acylmannosamines, (3) acid hydrolysis, (4) N-acetylation, and (5) conversion with ABEE were performed within a single vessel. Aldose, hexosamine, and sialic acid residues, which have only been determined separately in previous studies, were determined simultaneously by HPLC as ABEE-converted monosaccharides. Therefore, this method is simple, highly sensitive, and very useful for determining the sugar composition of glycoproteins, glycolipids, and oligosaccharides. On the other hand, with Method 2, in which the acid hydrolysis and N-acetylation processes of Method 1 are omitted, NeuAc and NeuGc were determined as ABEE-ManNac and ABEE-ManNgc, respectively, under similar HPLC conditions. We suggest that Method 2 would be capable of determining sialic acid residues of different linkages by using sialidases of different specificity, as reported by Fu and O’Neill, and that Methods 1 and 2 could be applied to compare the sugar composition of glycoconjugates from different sources, including human cancer cells containing NeuGc.

References