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The retarded rate of acid-catalyzed solvolysis of glycoside bonds between reducing-end glucose residue and ceramide in glycosphingolipids compared with that of glycoside bonds between hexopyranosides

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Rates of acid-catalyzed solvolysis of glycoside bonds in glycosphingolipids were compared to establish a basis for conducting saccharide analysis. Permethylated globotetraosylceramide and asialogangliotriaosylceramide as model compounds for methylation and sugar composition analysis, respectively, were solvolyzed under acidic conditions and the sugar components thus obtained were determined at specified times by gas liquid chromatography, after they had been derivatized. Reducing-end glucose residues in both compounds were liberated more slowly than other sugar residues. Glycoside bonds between reducing-end glucose and ceramide in glycosphingolipids would thus appear to be more resistant towards acid-catalysed solvolysis than other glycoside bonds between hexopyranosides.

Glycosphingolipid; Glycoside bond; Solvolysis; Saccharide analysis

1. INTRODUCTION

The solvolysis of glycoside bonds is generally conducted under acid-catalysed conditions. This is fundamental for the saccharide analysis of glycoconjugates. The rate of solvolysis of each glycoside bond in glycoconjugates depends on the particular conditions under which it is carried out. Consequently, special attention should be directed to providing conditions which permit maximum release of each saccharide. This still remains a problem in the saccharide analysis of glycoconjugates though great progress has been made in analyzing unit saccharides, such as by gas liquid chromatography (GLC) [1] and high performance liquid chromatography (HPLC) [2]. Previously, in conducting methylation analysis of a glycosphingolipid, the generation of reducing-end glucose residue was found to be less than predicted. The present study was conducted to clarify the reason for this. Glycoside bonds between reducing-end glucose and ceramide in glycosphingolipids were shown to be more resistant toward acid-catalyzed solvolysis than ordinary hexose–hexose glycoside bonds.

Abbreviations: GLC, gas liquid chromatography; HPLC, high performance liquid chromatography; Gal-Gb_3, III^3 Gal-α-globotriaosylceramide; Gb_4, globotetraosylceramide; $AsGM_2$, asialogangliotriaosylceramide; HPTLC, high performance thin layer chromatography; GLC-MS, gal liquid chromatography-mass spectrometry; TFA, trifluoroacetic acid.

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2. MATERIALS AND METHODS

2.1. *Glycolipids*

III^3 Gal-α-globotriaosylceramide (Gal-GB_3) was prepared as described previously [3]. Globotetraosylceramide (Gb_4) was purified from porcine erythrocytes. Asialogangliotriaosylceramide ($AsGM_2$) was prepared from gangliotriaosylceramide (Hunakoshi, Japan) by enzymatic hydrolysis with sialidase (from arthrobacter ureafaciens, Nakarai, Japan).

2.2. *Methylation analysis*

Methylation of glycolipids was conducted by the method of Hakomori [4], and permethylated glycolipids were purified by high performance thin layer chromatography (HPTLC) (Merck, Germany). The products were hydrolyzed in 2 N trifluoroacetic acid (TFA) at 120°C, reduced, acetylated and analyzed by gas liquid chromatography-mass spectrometry (GLC-MS) according to Waeghe et al. [5]. For GLC-MS analysis, a JEOL JMS HX-100 mass spectrometer and MS-GC06 gas chromatograph (JEOL, Japan) with fused silica capillary column (DB-5; J&W Scientific Co., USA) were used.

2.3. *Saccharide composition analysis*

Saccharide compositions of glycolipids were determined by GLC analysis [1], after methanolysis (in 0.5 N methanolic HCl, at 65°C), re-*N*-acetylation and trimethylsilylation had been conducted. GLC was carried out using a gas chromatograph (Shimadzu GC-8A, Japan) equipped with a fused silica capillary column (DB-5; J&W Scientific Co., USA).

3. RESULTS

Methylation analysis of Gal-Gb_3 was performed according to the method of Waeghe et al. [5]. Permethylated glycolipid was hydrolyzed in 2 N TFA at 120°C for 1 h, and the liberated saccharides were derivatized to partially methylated alditol acetates which were analyzed by GLC-MS. A total ion chromatogram of

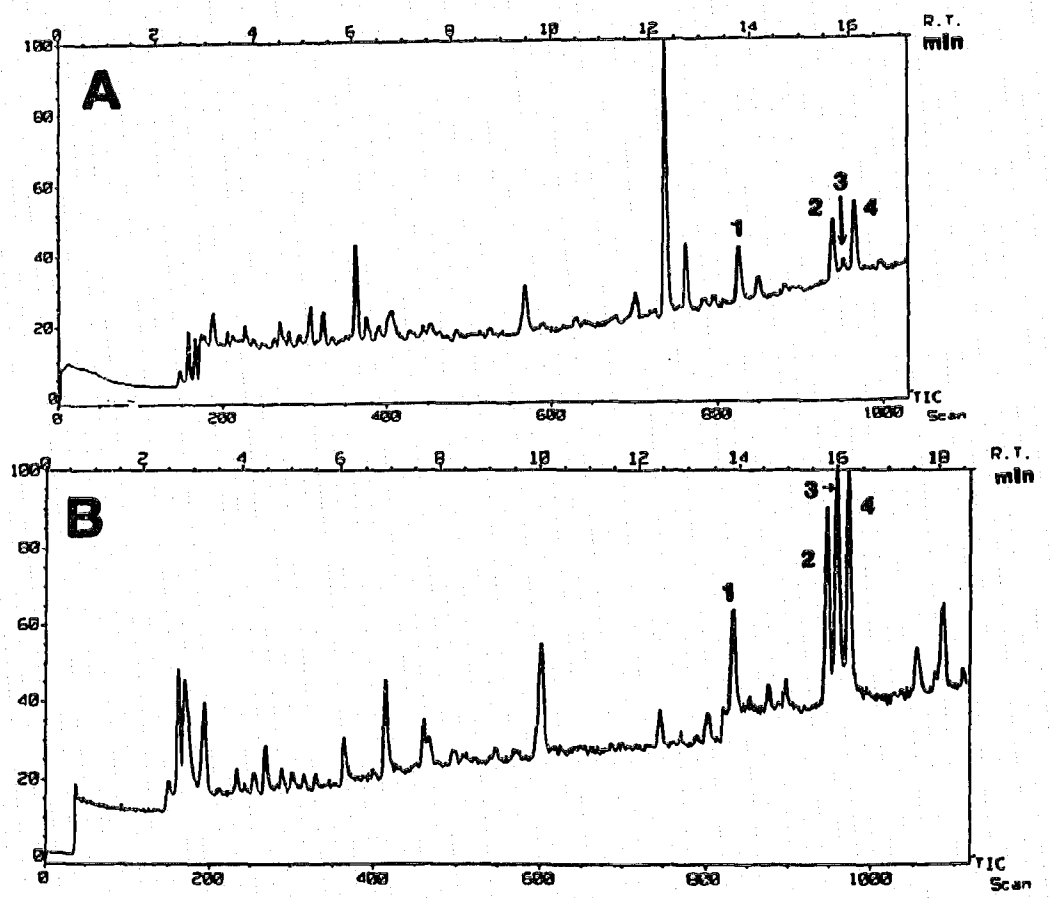

Fig. 1. Total ion mass chromatogram of partially methylated alditol acetates derived from Gal-Gb_3 in PC-12 cells [3]. Permethylated Gal-Gb_3 was hydrolyzed in 2 N TFA at 120°C for 1 h (A) or for 5 h (B), then derivatized to alditol acetates. The designations of the peaks 1–4 refer to the following compounds: (peak 1), 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol; (peak 2), 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactitol; (peak 3), 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol; (peak 4), 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol.

analysis is shown in Fig. 1A. Assignment of each peak appearing in the chromatogram was based on analysis of each mass spectrum [5]. The intensity of the peak for the reducing-end glucose residue (4-substituted glucose) was somewhat weaker than those for other saccharides, possibly due to the peculiar features of glucose-ceramide glycoside bonds in relation to acid-catalyzed hydrolysis. The fact that the intensity of the peak for the reducing-end glucose residue became stronger and comparable to others on prolonging the reaction time of the hydrolysis to 5 h appears to provide evidence for this notion (Fig. 1B).

Thus, using model compounds, attempts were made to demonstrate that the unique features of the glycoside bond between reducing-end glucose residue and ceramide are also to be generally observed in other glycosphingolipids. First, permethylated Gb_4 was subjected to acid-catalysed hydrolysis as a model for methylation analysis. Relative peak intensities for the saccharide components were analyzed by GLC and plotted against time (Fig. 2). The delayed appearance of the reducing-end glucose was clearly evident for this model compound. The weaker intensity of GalNAc residue was due to its lability to acidic conditions and low response to the flame ionization detector. On non-derivatized $AsGM_2$, acid-catalysed methanolysis was subsequently conducted under the same conditions for saccharide composition analysis [1]. The liberated sugar components were determined at specified times by GLC after they had been converted to trimethylsilyl ethers (Fig. 3). The delayed liberation of reducing-end glucose was also noted in the acidcatalyzed methanolysis of this glycosphingolipid. Based on the above findings, the delayed liberation of reducing-end glucose in glycosphingolipids is concluded to occur generally in acid-catalyzed solvolysis.

4. DISCUSSION

The acid-catalyzed solvolysis of glycoside bonds is initiated by protonation on glycoside oxygen, followed by cleavage of glycoside bonds (C_1–O) with subsequent generation of carbonium ions at the C_1 position. These ions are attacked by bases such as $H_2\ddot{O}$ and $CH_3\ddot{O}H$ to give more stable products [6]. Factors that promote the transient formation of carbonium ions and stabilize

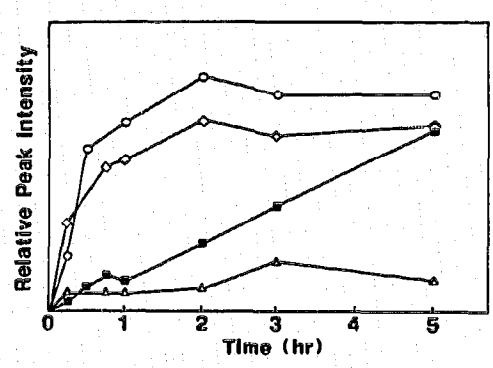

Fig. 2. Time course of the liberation of composing sugar units from permethylated Gb_4 by acid-catalyzed hydrolysis (in 2 N TFA at 120°C). Partially methylated sugars released from permethylated Gb_4 were derivatized to alditol acetates and analyzed by GLC. Relative peak intensity of each saccharide at each time to that of myo-inositol added as internal standard is shown. (○), Values of 1,4,5-tri-O-acetyl-2,3,6-tri-*O*-methyl-D-galactitol; (◇), values of 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol; (■), values of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol; (△), values of 1,5-di-*O*-acetyl-3,4,6-tri-*O*-methyl-*N*-acetyl-*N*-methyl-D-galactitol.

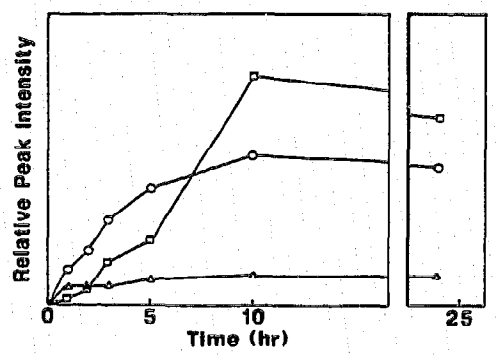

Fig. 3. Time course of the liberation of composing sugar units from $AsGM_2$ by acid-catalyzed methanolysis (in 0.5 N methanolic-HCl at 65°C). Released sugars were derivatized to trimethylsilyl ether and analyzed by GLC. (□), Values of 2,3,4,6-tetra-*O*-trimethylsilyl methyl glucoside; (○) values of 2,3,4,6-tetra-*O*-trimethylsilyl methyl galactoside; (△), values of 3,4,6-tri-*O*-trimethylsilyl-*N*-acetyl methyl galactoside.

them may possibly accelerate the acid-catalyzed solvolysis of glycoside bonds. Galactose or mannose residues in saccharide chains, possessing 1,3-diaxial substituents in the chair conformation, are usually solvolyzed more quickly than glucose residues not having 1,3-diaxial substituents. Explanations for this is as follows [7]: the transition of hexopyranoside residues to C_1-carbonium ions, stabilized by resonance between ring oxygen, accompanies change in their conformation from the chair to half-chair form to release repulsive energy arising from 1,3-diaxial interactions that occur in galactose or mannose residues, in the chair conformation. This transition to the carbonium ions is thus facilitated in galactose or mannose residues to a greater extent than in glucose residues. This may possibly be one reason why the reducing end glucose residue in glycosphingolipids behave differently from other residues in acid-catalyzed solvolysis.

Stereochemical conditions that may affect anomeric carbons in saccharide residues should be also taken into consideration. Transition to C_1-carbonium ions following the cleavage of glycoside bonds dissipates steric hindrance of bulky aglycons. Aglycons of glycoside bonds between hexopyranosides, with linkage by 1–3 or 1–4, are relatively bulky secondary alcohols, while those of reducing-end glucose residues in glycosphingolipids are ceramides, which are primary alcohols. The extent of steric acceleration due to release of aglycons may affect the rate of the solvolysis. This, too, may possibly explain the slower rate of solvolysis of the bonds between reducing-end glucose residues and ceramides. Presence of certain intra- or inter-molecular interactions, which prevent access of the reactants to the reducing-end glucose residues, may also be an explanation for the retarded rate of the solvolysis.

If glycolipids are solvolyzed via its lyso form, the ammonium ion formation in a sphingosin base should suppress protonation at the glycoside oxygen of reducing-end glucose residues. This may also suffice as explanation for the slow rate of solvolysis between glucose residues and ceramides. However, this would not account appreciably for the reduction in rate since no glucopsycosin was detected during the acid-catalysed hydrolysis of glucosylceramide (data not shown).

In this study, TFA was used as the catalyst for the solvolysis of glycoconjugates since methylated saccharides are more easily solubilized in TFA than in mineral acids and TFA can thereafter be quickly removed by evaporation. The present study shows a longer reaction time for the acid-catalyzed solvolysis of glycosphingolipids than for other saccharides to be better when using a mild acid such as TFA as catalyst.

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