ANALYSIS OF THE ANOMERIC CONFIGURATION OF A GALACTOFURANOSE CONTAINING GLYCOLIPID FROM AN EXTREME THERMOPHILE

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1. Introduction

The anomeric configuration of the galactofuranose containing glycolipid of the extremely thermophile, *Thermus thermophilus,* strain HB8 was determined by comparison of its oxidation by chromium trioxide (CrO_3) with those of authentic methyl-α- and methyl-β-galactofuranoside.

Laine and Renkonen [1] determined the anomeric configurations of the sugar residues of glycolipids by acetylation and oxidation with CrO_3 in acetic acid. Acetylated hexapyranosides in which the aglycone occupies an equatorial position were oxidized easily to acetylated 5-ketohexulosonates. On the other hand, anomers in which the aglycone occupies an axial position were oxidized only very slowly. Namely, pyranosic glucose, galactose, mannose, *N*-acetyl-glucosamine and *N*-acetyl-galactosamine bound to the lipids through β-glycosidic linkages reacted to the extent of 80–97%, whereas anomers with α-glycosidic linkages reacted only 0–6%. The oxidations of hexafuranosides with CrO_3 has not yet been studied.

A novel glycolipid isolated from *T. thermophilus* was reported to have the structure: Gal(f)(1-2)Gal(p)(1-6)GlcN(15-methylhexadecanoyl)(1-2)Glc(p) diglyceride(2,3). The non-reducing terminal galactose residue is in the furanose form. We studied the configuration of the galactofuranose containing glycolipid by oxidation with chromium trioxide.

Abbreviations: Gal (f): galactofuranose, Glc (p): glucopyranose. Note: This organism was originally named *Flavobacterium thermophilum.* Later it was transferred to the new genus *Thermus* [6] and named *Thermus thermophilus.* See ref. [7] for details.

2. Materials and methods

The novel glycolipid of *T. thermophilus,* strain HB8 was purified as reported elsewhere [2,3].

The purified methyl-α-galactofuranoside was kindly given by Dr Yamakawa of Tokyo University. Methyl-β-galactofuranoside was prepared by treatment with 3% HCl in dry methanol at 22°C for 24 h. and Dowex-1 × 1 (OH^- form, 100–200 mesh) column chromatography, following the method of Matsushima and Miyazaki [4].

Oxidation of the acetylated novel glycolipid and methyl galactofuranosides with CrO_3 was carried out essentially as described by Laine and Renkonen [1]. Samples of 1 μmole were acetylated by treatment with 0.1 ml of pyridine–acetic anhydride (1:1 v/v) at 100°C for 15 min. Then the solvent was dried by evaporation and 0.2 ml of saturated solution of CrO_3 (100 mg/ml) in acetic acid was added with stirring for 15 min. at 40°C. Equal amounts of water and chloroform were added to the reaction mixture and the chloroform extracts were washed with water and evaporated to dryness. It was subjected to methanolysis (3% HCl in dry methanol, 100°C, 3h). D-Mannitol (1.0 μmol) was added as an internal standard. After methanolysis the sample was dried, trimethylsilylated and analyzed by gas–liquid chromatography. The fatty acid methyl esters of glycolipid obtained by methanolysis were extracted before trimethylsilylation. The recoveries of the monosaccharides were calculated for each experiment.

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The gas chromatography was carried out using a glass column of 4% SE-30 or 6% tripalmitin on Celite 545 (silanized with hexamethyldisilasane) at 135–140°C.

3. Results and discussion

On treatment of methyl-α-galactofuranoside with CrO_3, 8% ± 0.2 remained unoxidized, so that 92% must have been oxidized. On the other hand, on treatment of methyl-β-galactofuranoside with CrO_3, 73% ± 0.4 remained unoxidized, so that less than 27% was oxidized. Thus galactofuranoside with the α-configuration was more easily oxidized than that with the β-configuration. This is the reverse of findings on hexapyranosides in which the α-configuration is more resistant to oxidation than the β-configuration [1]. The acetylated methyl-β-galactofuranoside molecule is probably more stable stereochemically and so more resistant to CrO_3 oxidation than the methyl-α-galactofuranoside [5], because all the substituted residues in the molecule have the *trans* configuration.

Next the novel glycolipid was subjected to oxidation and the results, expressed as molar ratios of monosaccharides are shown in table 1. The findings that one mole of glucopyranose was retained in the oxidation while one mole of glucosamine was completely degraded mean that the glucopyranoside has the α-configuration and the glucosamine has the β-configuration. One mole of galactopyranose has been shown to have the α-configuration by treatment with α-galactosidase [3]. During the oxidation two moles of galactose were retained. Thus the other galactose residue which is in the furanose form

Table 1

Sugar	Molar ratio Intact Glycolipid	After CrO_3 oxidation
Galactose	2.02	2.08
Glucose	1.00	0.95
Glucosamine	0.85	0.00

Calculated from the peak areas on gas chromatography using D-mannitol as an internal standard. Average of three different experiments.

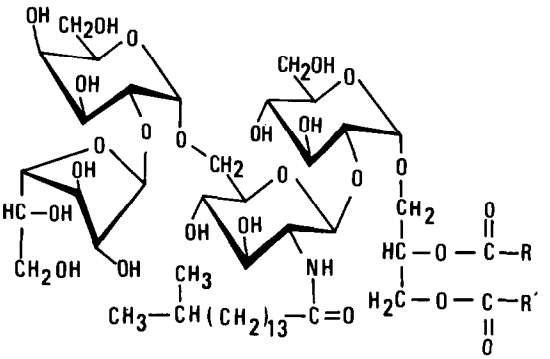

Fig. 1

probably has the β-configuration, because the β-configuration of galactofuranoside is more resistant to CrO_3 oxidation.

These results show that the anomeric configuration of the sugar residues of the novel glycolipid is: Gal(f)$^{\beta}$Gal(p)$^{\alpha}$GlcN(15-methylhexadecanoyl)$^{\beta}$Glc(p)$^{\alpha}$ diglyceride. The structure of the glycolipid of *T. thermophilus* is shown in fig. 1.

Hudson's isorotation rule also suggests that the galactofuranose in the glycolipid molecule has the β-configuration. The molecular rotation ($[M]_D = [\alpha]_D^{24} \times$ (mol.wt./100) of this glycolipid and the alkaline deacylated glycolipid are + 446 (in $CHCl_3$) and + 70.4 (in water–ethanol, 1:1, v/v), respectively. The $[M]_D$ of the known methylglycoside and glycoside are; α-D-Glc(p)(1-1)glycerol: + 325, α-D-Gal(p): + 252, β-D-GlcN(p): – 94, α-D-Gal(f): + 189 and β-D-Gal(f): – 100 (in water). The configuration of the glycerol residue of the glycolipid is still unknown. It is still unknown how many of the three fatty acyl residues in the glycolipid molecule are involved in the conformational change. However, it seems reasonable that the novel glycolipid has a galactofuranose residue of the β-configuration, because a galactofuranoside with the α-configuration would make the molecule more dextro-rotatory than it actually is.

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