STRUCTURAL STUDIES OF GLYCOASPARAGINES FROM URINE OF A PATIENT WITH ASPARTYLGLYCOSYLAMINURIA (AGU)

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1. Introduction

Patients with aspartylglycosylaminuria (AGU) excrete in their urine two isomeric glycoasparagines composed of two moles each of galactose and *N*-acetylglucosamine and one mole each of *N*-acetylneuraminic acid and asparagine, in addition to glycoasparagines with smaller carbohydrate moieties including 2-acetamido-*N*-(4′-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (GlcNAc-Asn) [1–3]. Isolation and characterization of these two isomeric glycoasparagines have been reported previously [3]. This paper describes their complete structure based on the results of periodate oxidation and methylation studies.

2. Materials and methods

Glycoasparagines from AGU urines were fractionated as described previously [2]. Fraction B was further fractionated by paper chromatography to yield two glycoasparagines, a faster moving B-F and a slower moving B-S. They have the same chemical composition and gave an identical glycoasparagine after removal of *N*-acetylneuraminic acid [3].

Periodate oxidation was carried out according to Eibl and Lands [4]. Methylation of glycoasparagines was carried out according to the method of Stellner et al. [5] and the methylated sugars were analyzed using a LKB-Shimadzu 9000 gas chromatography mass spectrometer (GC-MS). For gas–liquid chromatography of methylated sugars, a column of 2% OV-17/Gas-Chrom Q (3 mm × 2 m) was used, and the column temperature was programmed from 150–250°C at a rate of 2°C/min. Flow rate of helium was 24 ml/min. The mass spectrometry was carried out as follows: ionizing potential 70 eV for total ion analysis and 20 eV for single ion analysis; accelerating voltage, 3.5 kV; ion source temperature, 210°C; and separator temperature, 190°C. Identification of methylated derivatives of galactitol and 2-*N*-methyl-acetamido-2-deoxyglucitol was made mainly according to the data of Stellner et al. [5] and Lindberg [6].

3. Results and discussion

Thirty nmol B-F or B-S was oxidized in 0.1 ml 18.5 mM $NaIO_4$ in 0.05 M boric acid at room temperature in the dark. By following the increase in ultraviolet absorption due to $NaIO_3$, the oxidation was found to be complete after 60 min, as shown in fig.1. From the data it was concluded that B-F consumed 2 mol $NaIO_4$/mol based on the amount of aspartic acid, whereas B-S consumed 4 mol. Sialic acid-free B-F and B-S consumed 2 mol $NaIO_4$ due to newly exposed terminal galactose residue. Since sialic acid at the non-reducing end consumes 2 mol $NaIO_4$, 2 of the 4 mol consumed by B-S can be ascribed to oxidation of the penultimate galactose residue which should have free hydroxyl groups at C-2, C-3 and C-4. On the other hand, B-F should have the penultimate galactose residue substituted at C-3. Further it is anticipated that the non-terminal galactose and two *N*-acetylglucosamine residues in the sialic acid-free glyco-asparagines are substituted at C-3 and at C-3 or C-4, respectively, since no sugars other than the terminal galactose consumed periodate.

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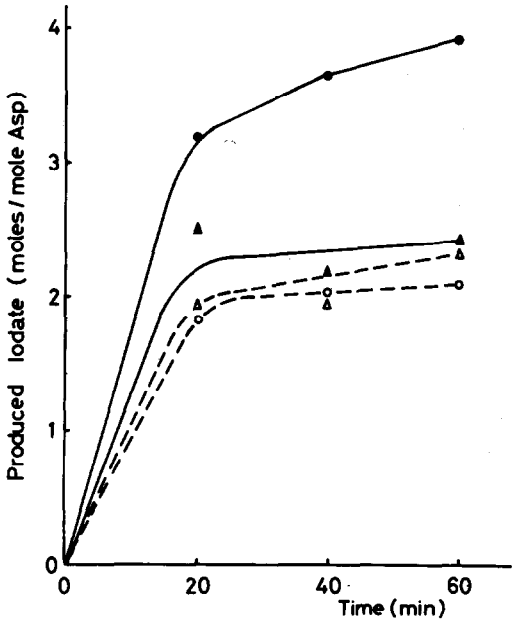

Fig.1. Periodate consumption of glycoasparagines from AGU urine. B-F (▲—▲), B-S (●—●), sialic acid-free B-F (△- - -△), sialic acid-free B-S (○- - -○).

B-F and B-S (100–300 nmol) were methylated, and aliquots of the resulting methylated sugars were analyzed by GC-MS in which single ions of m/e 117 and 161 were monitored for methylated galactitol and those of m/e 158 and 116 for methylated 2-*N*-methylacetamido-2-deoxyglucitol. Identification of each peak was further carried out by total ion analysis.

B-F gave 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol and 1,4.5-tri-*O*-acetyl-3,6-di-*O*-methyl-2-*N*-methylacetamido-2-deoxyglucitol, indicating that both galactose residues are substituted at C-3 and both *N*-acetylglucosamine residues at C-4. B-S gave 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol and 1.5,6-tri-*O*-acetvl-2,3,4-tri-*O*-methylgalactitol in a molar ratio of 1.0 : 0.9 and 1,4,5-tri-*O*-acetyl-3,6-di-*O*-methyl-2-*N*-methyl-acetamido-2-deoxyglucitol, indicating that the penultimate galactose and the inner galactose residues are substituted at C-6 and C-3, respectively, and both *N*-acetylglucosamine residues are substituted at C-4 as in B-F.

Based on the foregoing results, the structures of two isomeric glycoasparagines are deduced as shown in table 1, in which structures of other glycoasparagines with a similar core structure so far isolated from AGU urine are shown for comparison. B-F and B-S are sialyl derivatives of the glycoasparagines with another *N*-acetyllactosamine residue attached to the core glycoasparagine, that is, *N*-acetyllactosaminylasparagine. It is unclear whether or not these glycoasparagines are the catabolic products of glycoproteins, since glycoproteins with this type of oligosaccharide have not been shown to occur. However, it is interesting to note that B-F is very similar to a *N*-acetylglucosamine-containing ganglioside (GlcNAc-ganglioside (NANA)) (see table 1) from human erythrocytes [7] which is supposed to be involved in binding of staphylococcal α-toxin to the cells [8]. In the ganglioside, the innermost *N*-acetylglucosamine in B-F is replaced by glucose that is then linked to ceramide.

Table 1
Glycoasparagines from AGU urine – Structure and concentration in urine

Glycoasparagines from		μmol/1 urine
Fraction D[a]	β-GlcNAc-Asn	1000
	β-Gal-(1→4)-β-GlcNAc-Asn	18
Fraction C[a]	α-NANA-(2→3)-β-Gal-(1→4)-β-GlcNAc-Asn	82
	α-NANA-(2→4)-β-Gal-(1→4)-β-GlcNAc-Asn	14
Fraction B, B-F	α-NANA-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-GlcNAc-Asn	8
B-S	α-NANA-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-GlcNAc-Asn	2
GlcNAc-ganglioside (NANA)[b]	α-NANA-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-Glc-Ceramide	

[a]Fractions D and C are eluted from Sephadex G-25 column after B fraction (see ref. [3])
[b]See ref. [7]

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