MANNOSIDOSIS IN ANGUS CATTLE: PARTIAL CHARACTERIZATION OF TWO MANNOSE CONTAINING OLIGOSACCHARIDES

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Received 18 July 1973

1. Introduction

In 1957 Whittem and Walker described a lethal nervous disease of Angus cattle that microscopically resembled the human lipidoses [1]. Being unable to demonstrate lipid within vacuolated neurones they referred to the condition as neuronopathy and pseudolipidosis. More recently it has been demonstrated that an oligosaccharide containing three mannose and one glucosamine residue could be isolated from lymph nodes of affected calves, but not from normal animals, and that affected calves had a severe deficiency of α-mannosidase in tissues [2]. The disease was inherited as an autosomal recessive and heterozygotes were shown to have less than half the normal level of α-mannosidase in plasma. This bovine condition was thus shown to be similar to human mannosidosis which is also associated with a deficiency of α-mannosidase and accumulation of mannose containing oligosaccharides [3, 4] in tissues. The term 'mannosidosis' of Angus cattle was suggested as being preferable to the name 'pseudolipidoses' [2]. The purpose of the present investigation was to study the stored material in calf brain and the urinary exretion of mannose containing oligosaccharides. Two oligosaccharides were isolated from the calf brains. Chromatographic behavior and sugar analysis suggest that both are tetrasaccharides with two D-mannose and two *N*-acetyl-D-glucosamine residues. Neither of these tetrasaccharides are identical with oligosaccharides isolated from urines of human patients with mannosidosis.

2. Materials and methods

Brain tissues and urine (15–20 ml) from two Angus calves with mannosidosis, two normal Angus calves, and urine from two heterozygotes were used. Diagnosis of mannosidosis or normality was made on the basis of clinical grounds, microscopic examination of the central nervous system and tissue assays for α-mannosidase activity. The heterozygote animals were selected on the basis of plasma α-mannosidase levels from a group of ten calves born to known heterozygote parents. All tissues were stored at −12°C, except during transportation from New Zealand to Sweden when they were packed in dry ice for approximately 72 hr.

The trisaccharide R_L 0.82*, the tetrasaccharide R_L 0.46 and the pentasaccharide R_L 0.21 were obtained from the urines of patients with mannosidosis as

* Abbreviations used are: $R_L = R_{Lactose}$, Man = mannose and GlcNAc = *N*-acetylglucosamine.

described previously [5, 6]. The structures of these oligosaccharides have been established** [5, 6].

Bacterial growth was prevented by the addition of phenyl mercuric nitrate (final dilution 1:50000). Brain tissue was homogenized in water using a Potter–Elvehjelm homogenizer at 0°C. The homogenate was defatted by chloroform extraction and ultrafiltered at 4°C by the method described by Berggård [7] using Visking 23/32 inch dialysis tubing. Solvents were removed from solutions by rotatory evaporation at 40°C. Gel chromatography on Sephadex G-25, preparative zone electrophoresis on Pevikon C 870, and paper chromatography were carried out as described previously [8, 9] using the following systems: a) 2 M acetic acid (pH 1.9); b) ethyl acetate–acetic acid–water (3:1:1; v/v); c) *n*-butyl acetate–acetic acid–water (3:2:1; v/v); d) ethyl acetate–pyridine–water (2:1:2; v/v); e) butanol–pyridine–water (6:4:3; v/v); f) *n*-propanol–ethyl acetate–water (6:1:3; v/v); g) iso-propanol–ethyl acetate–water (42:35:23; v/v). The carbohydrate containing spots on paper chromatograms were located by using a $AgNO_3$ dip reagent [10]. The urines were ultrafiltered and treated as above.

Colorimetric methods for the determination of total hexose, fucose and hexosamine were the same as previously used [8, 9]. The resorcinol method of Svennerholm was used to analyze sialic acid after hydrolysis and isolation on Dowex 2. Peptides were estimated by the Folin method [12]. Sugar analyses by gas chromatography were carried out according to the method of Sawardeker et al. [13]. The optical rotations were measured with a Perkin Elmer 141 polarimeter.

3. Results

Analysis of neutral sugars in defatted brain tissue showed that the mannose content in bovine mannosidosis was 4–6 times higher than in normal brain. No differences were observed for other neutral sugars. Gel chromatography of the concentrated ultrafiltrates

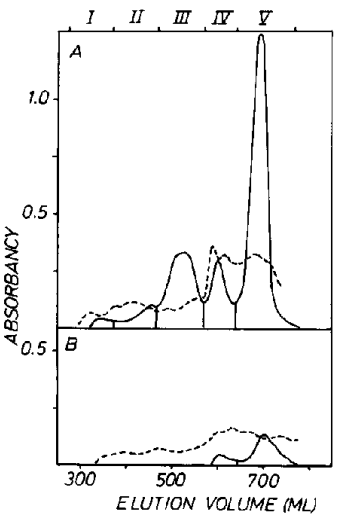

Fig. 1. Ultrafiltered water extracts from 100 g brain tissue (wet weight) fractionated on a column of Sephadex G-25 (128 × 2.9 cm; V_0 = 350 ml). Eluted fractions were assayed for total hexose (——) and peptides (– – –): A) Brain from calf with mannosidosis; B) normal calf brain. The fractions were pooled as indicated by the vertical lines.

(fig. 1) showed that there was a considerable increase of carbohydrate material and a moderate increase in Folin reacting material in bovine mannosidosis. Region III of the affected brains had a broad hexose containing peak with a maximum at K_{av} 0.35. This was absent in normal brains.

Fractions I–V, pooled as indicated were analyzed for the presence of hexosamine, sialic acid and individual neutral sugars. No marked qualitative differences in sugar composition were seen between normal and

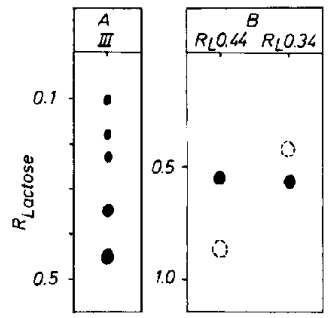

Fig. 2. A) Paper chromatography of fraction III. The paper was developed in ethyl acetate–acetic acid–water (3:1:1; v/v); B) compounds R_L 0.44 and R_L 0.34 purified on papers developed in *n*-butyl acetate–acetic acid–water (3:2:1; v/v).

** R_L 0.82 = α-D-Man–(1 → 3)–β-D-Man–(1 → 4)–D-GlcNAc.
R_L 0.46 = α-D-Man–(1 → 2)–α-D-Man–(1 → 3)–β-D-Man–(1 → 4)–D-GlcNAc.
R_L 0.21 = α-D-Man–(1 → 2)–α-D-Man–(1 → 2)–α-D-Man–(1 → 3)–β-D-Man–(1 → 4)–D-GlcNAc.

Table 1
$R_{Lactose}$-values of compounds R_L 0.44 and R_L 0.34 in four different solvent systems.

Solvent system	R_L 0.44	R_L 0.34
Ethyl acetate–pyridine–water 2:1:2	0.49	0.47
Butanol–pyridine–water 6:4:3	0.47	0.45
N-propanol–ethyl acetate–water 6:1:3	0.63	0.63
Iso-propanol–ethyl acetate–water 42:35:23	0.54	0.54

mannosidosis brain in any other fraction except III, which had higher amounts of mannose and hexosamine.

Pooled fraction III was purified by preparative zone electrophoresis (system a, 3.5 V/cm for 24 hr). The stationary material was fractionated by preparative paper chromatography (system b) into five components (fig. 2A). Components with R_L 0.44 and R_L 0.34 were purified further (system c) (fig. 2B), and shown to be homogeneous in four paper chromatographic systems (d–g inclusive) (table 1). The components R_L 0.44 and R_L 0.34 were finally passed through a column of Biogel P-2 from which they eluted as symmetrical peaks. Table 2 shows the yields and analytical data. Comparison of the two components with oligosaccharides R_L 0.82, R_L 0.46 and R_L 0.21 obtained from the urines of patients with mannosidosis, revealed them to be different from any of the human oligosaccharides.

The urines from normal, mannosidosis and heterozygote calves were fractionated on a column of Sephadex G-25 (140 × 5 cm; V_0 = 1027 ml). Again, the distribution of hexose containing-compounds was different in the diseased animals, with an abnormally high excretion of hexose containing-compounds with K_{av} 0.15–0.42 and a maximum at K_{av} 0.35. The mannose content of this fraction was about 20 times higher than in the corresponding fraction from normal urines.

Table 2
Yields and analytical data of the two isolated oligosaccharides R_L 0.44 and R_L 0.34. The yields are given as mg per 100 g brain tissue (wet weight).

Sugar	Yield	$[\alpha]_D^{20}$	Per cent composition (molar ratio)	
			Man	GlcNAc
R_L 0.44	2.39	+1.9	35 (1.0)	43 (1.0)
R_L 0.34	1.80	+5.0	29 (1.1)	32 (1.0)

No significant abnormality was seen in urines from heterozygotes.

4. Discussion

Sugar analysis, chromatography and electrophoretic studies indicate that the oligosaccharides R_L 0.44 and R_L 0.34 are tetrasaccharides containing two mannose and two *N*-acetylglucosamine residues. In the gas chromatographic analyses only about 60–80% of the dry weight of these oligosaccharides could be accounted for. The most likely explanation for this may be the retarded hydrolysis of the hexosaminidic linkages due to concomitant *N*-deacetylation. Since mannose and *N*-acetylglucosamine are present in equimolar amounts it is suggested that the two *N*-acetylglucosamine residues are not linked to each other. In view of the very much decreased activity of α-mannosidase and accompanying oligosaccharide accumulation, the non-reducing terminus of the oligosaccharides most probably consists of an α-linked mannose residue. The data are consistent with the sequence Man–GlcNAc–Man–GlcNAc for both tetrasaccharides.

Another tetrasaccharide consisting of one *N*-acetylglucosamine and three mannose residues has previously been isolated from the lymph nodes of calves with mannosidosis [2]. This compound might be identical to one of the additional compounds with lower R_L values that was observed in the brain extract. The urines of calves with mannosidosis contain mannose rich substances eluted with the same K_{av} value as R_L 0.44 and R_L 0.44 and R_L 0.34 indicating that these compounds also are excreted in the urine. None of the bovine mannosidosis oligosaccharides are present in the urines of human patients with this disease where the predominant component isolated is a trisaccharide [5]. No information regarding the substrate specificities of

the α-mannosidase in human and bovine mannosidosis is available. Hence it is not possible to state whether the observed differences can be explained in terms of different enzyme specificities or by a different glycoprotein architecture in the two species.

Acknowledgements

This investigation was supported by grants from the Swedish Medical Research Council (13X-2 and 19X-2222). The authors are indebted to Mrs. Gunilla Pettersson, Mrs. Elwy Wallin and Mrs. Birgit Boman for valuable technical assistance.

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