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STRUCTURE OF KIDNEY CERAMIDE DIHEXOSIDE SULFATE

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SUMMARY

1. It has been established by methylation that kidney ceramide dihexoside sulfate is a lactosyl ceramide with the sulfate group at C-3 of the galactose moiety. Thus, it is structurally related on the one hand to kidney neutral glycolipids which possess a lactosyl unit and on the other hand to the simpler galactose-containing cerebroside sulfate esters which have the sulfate group at C-3 of galactose.

2. These results have been obtained by microtechniques using a few mg only of material.

INTRODUCTION

Recently, a sulfatide containing equimolecular amounts of ceramide, glucose, galactose, and sulfate, has been isolated from human kidney. It constitutes 25–30% of the total sulfatide fraction in this organ[1]. This ceramide dihexoside sulfate (CDS) is also present in increased amounts in kidneys of patients with metachromatic leucodystrophy, a hereditary sphingolipidosis in which sulfatides accumulate abnormally in nervous tissue, kidneys, liver, gall bladder, and other organs throughout the body[2]. However, CDS appears to accumulate in kidneys at a slower rate than the more abundant ceramide galactose sulfate[3].

In order to provide support to studies of the metabolic relationships that may exist between this ceramide dihexoside sulfate and the neutral glycolipids of kidney, and also to help define the specificity of the enzymatic systems which are involved in the biosynthesis and the catabolism of sulfatides in general, it was necessary to establish firmly the chemical structure of CDS. For that purpose, periodate oxidation studies have already been carried out[1]. The intact sulfatide was found to be resistant to the action of the oxidant, whereas in the desulfated glycolipid, the galactose moiety was oxidized but the glucose unit was not attacked. These data suggested that no vicinal glycol susceptible to oxidation is present in CDS, which would thus consist of the sequence: ceramide–glucose–galactose, with a sulfate group at C-3 of

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Abbreviation: CDS, ceramide dihexoside sulfate.

galactose and a 1 to 3 linkage between galactose and glucose. Since a 1 to 3 linkage between galactose and glucose has not been found previously either in kidney neutral glycolipids or in other glycolipids, the structure of CDS has been investigated by methylation. This paper presents the results of this study which demonstrates that, contrary to what was indicated by periodate oxidation studies, CDS contains a lactosyl unit.

A preliminary communication has been published[4].

EXPERIMENTAL

Isolation of CDS

The starting material for this investigation was isolated from normal human kidney obtained at autopsy at the Massachusetts General Hospital through the courtesy of Dr. HUGO MOSER. The tissue was kept up to 6 weeks at −70° until CDS was extracted as previously described[1].

Methylation of CDS

Methylation was performed on a very small scale in dimethyl sulfoxide in the presence of dimethylsulfinyl carbanion[5]. Under a stream of nitrogen and with stirring, 20 mg of NaH in oil (Metal Hydrides, Beverly, Mass.) was dissolved in 2 ml of dimethylsulfoxide maintained at 70°. To this solution, cooled to 20°, was added 5 mg of CDS dissolved in 2 ml of dimethylsulfoxide and, after 5 min, 1 ml of methyl iodide was added dropwise. The mixture was kept 0.5 h at room temperature and then diluted with 30 ml of water. The resulting solution was transferred to a column (3 cm × 1.2 cm) prepared in water with a mixture of Norit A–Celite 535 (2:1, w/w). (The mixture had been previously washed with 6 M HCl, ethanol, chloroform–methanol (2:1, v/v) and water.) Elution was carried out successively and exhaustively with 50 ml each of water, water–methanol (1:1, v/v), methanol, methanol–chloroform (1:2, v/v) and finally with methanol–chloroform (1:2, v/v) saturated with 5 M NH_4OH. The fractions were evaporated to dryness under vacuum and the residues were examined by thin-layer chromatography on silica gel G using *n*-propanol–5 M NH_4OH (160:40, v/v) as solvent, and anthrone–sulfuric acid as spraying reagent. Carbohydrate containing material was revealed as one green spot in the fraction eluted with methanol–chloroform–ammonia. This fraction was transferred with 2 ml of methanol into a tube, 1 ml of methanolic hydrogen chloride (15%, w/w) was added, and the tube was sealed and heated for 18 h in a boiling-water bath. The solution was evaporated repeatedly under vacuum with toluene–ethanol (1:1) to eliminate HCl. The residue was dissolved in 5 ml of methanol–water (85:15, v/v) and transferred onto a new Norit A–Celite 535 column (3 cm × 1.2 cm) prepared in the same methanol–water mixture. Further elution with this mixture gave fractions containing methylated methyl hexosides which were detected by thin-layer chromatography on silica gel G with an anthrone–sulfuric acid reagent. The methylated hexosides, thus separated from the sphingosine bases and fatty acid methyl esters, were then hydrolyzed with 2 ml of 1 M H_2SO_4 in a sealed tube at 100° for 15 h. The solution was deionized by passage through a column containing 10 ml of Amberlite CG 45 in the acetate form, methanol–water (2:1, v/v) being used as eluant. The effluent was carefully evaporated under vacuum at a temperature not exceeding 35°.

Identification of the methylated hexoses

The methylated hexoses resulting from the hydrolysis were compared, and thus characterized, by ascending chromatography on thin layer of silica gel G with authentic samples of the 4 isomeric trimethylgalactopyranoses, 4 isomeric trimethylglucopyranoses, tetramethylgalactopyranose, and tetramethylglucopyranose. The plates were irrigated with acetone–water–conc. NH_4OH (250:3:1.5, v/v/v), and sprayed with aniline phthalate (Fig. 1).

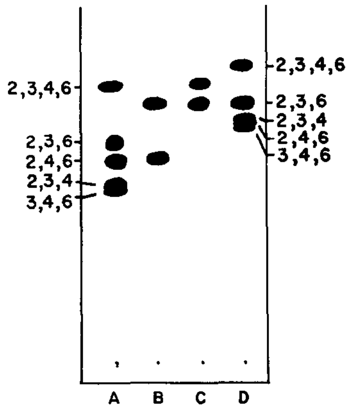

Fig. 1. A. Tetramethyl and isomeric trimethyl-D-galactose standards. B. Methylated hexoses from hydrolyzate of intact permethylated CDS. C. Methylated hexoses from desulfated and then permethylated CDS. D. Tetramethyl and isomeric trimethyl-D-glucose standards.

Desulfation of CDS and methylation of the resulting ceramide dihexoside

CDS, 5 mg, was desulfated with methanolic HCl as previously described[1]. The resulting neutral glycolipid was methylated as described above and the methylated hexoses obtained after hydrolysis were also compared with authentic methylated derivatives of glucose and galactose. In a trial run for this experiment, ceramide lactose (galactosyl 1 → 4 glucosyl ceramide) was prepared by partial hydrolysis of gangliosides[6] and purified by column chromatography on silica gel G with chloroform–methanol (4:1, v/v) as solvent. It was methylated and the corresponding methylated hexoses were isolated and characterized as described above.

RESULTS

It is apparent in Fig. 1 (Sample B) that spots corresponding in R_F value to 2,4,6-trimethylgalactose and 2,3,6-trimethylglucose are obtained by chromatography of the methylated hexoses resulting from the methylation and hydrolysis of intact ceramide dihexoside sulfate.

On the thin-layer chromatogram of the methylated hexoses obtained after methylation and hydrolysis of the desulfated glycolipid (Sample C), no spot corresponding to 2,4,6-trimethylgalactose is present, but a new spot corresponding to 2,3,4,6-tetramethylgalactose is revealed. No 2,3,4,6-tetramethylglucose is detected. However, 2,3,6-trimethylglucose is present here as it is in the mixture resulting from the methylation of the intact glycolipid. Thin-layer chromatography of the methylated

hexoses resulting from the methylation of ceramide lactose obtained from gangliosides gives identical results.

These data lead to the conclusion that the sulfate group in CDS is located at C-3 of the external galactose unit which, in turn, is linked at C-4 of the internal glucosyl residue itself linked to the ceramide (Fig. 2).

KIDNEY CERAMIDE DIHEXOSIDE SULFATE

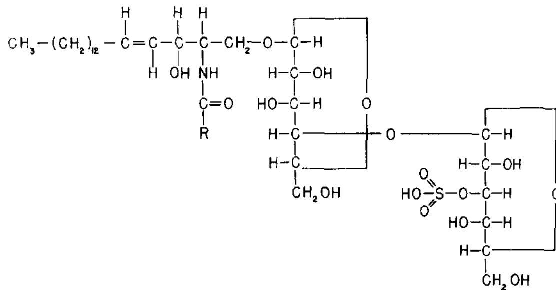

Fig. 2. Structure of kidney ceramide dihexoside sulfate.

DISCUSSION

The results of this investigation show unequivocally that the sulfate group in CDS is located solely at C-3 of the galactose, as it is in the more abundant sulfatide containing only galactose[7,8]. This indicates that the enzymatic system responsible for the synthesis of these sulfated glycolipids introduces the sulfate group specifically at C-3 of galactose. In this connection, it is interesting to recall that the sulfating systems of brain and kidney described by McKHANN, LEVY AND HO[9] are unable to sulfate ceramide glucose. Ceramide lactose, however, acts as a sulfate acceptor[10]. The presence of a lactosyl residue in kidney CDS shows the structural relationship of this sulfatide with the neutral glycolipids of kidney and further emphasizes the common occurrence of this type of structure in glycolipids. The resistance to periodate oxidation of the glucosyl unit in CDS and desulfated CDS is not unique for these compounds. It has been observed in gangliosides[11] also, as well as in ceramide dihexoside from spleen[1], and may represent a special case of steric hindrance. The method of methylation devised by HAKOMORI[5] and which gives complete methylation in a very short operation, combined with the procedures of isolation of the methylated products on charcoal columns, and the thin-layer chromatographic system giving a neat separation of highly methylated hexoses, make the procedure of methylation here described valuable for the study of the structure of small amounts of naturally occurring glycolipids.

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