SULFATIDES AND SODIUM ION TRANSPORT, SPHINGOLIPID COMPOSITION OF THE RECTAL GLAND OF SPINY DOGFISH

Karl-Anders KARLSSON, Bo E.SAMUELSSON and Göran O.STEEN
Institute of Medical Biochemistry, University of Göteborg, Fack, 400 33 Göteborg 33, Sweden

Received 7 October 1968

1. Introduction

It was recently found, that sulfatides are present in relatively high concentrations in the outer part of kidney medulla [1] and in the avian salt gland [2], tissues known to have a corticosteroid dependent sodium ion transport [3,4]. Based on these findings, it was proposed [2] that sulfatides are involved as carriers or receptors in this process. To evaluate this hypothesis further, the rectal gland of spiny dogfish [5] was analyzed for the presence of sulfatides. The only known function of this organ, as well as of the salt gland, is excretion of sodium chloride in a concentration higher than that of sea water.

2. Materials and methods

Rectal glands of spiny dogfishes from the Swedish westcoast were taken out within 8 hours after animal capture. The tissue was cut in small pieces and lyophilized. Lipids were extracted with hot chloroform–methanol (2:1, v/v), twice with 20 ml/g dry tissue and eight times with 10 ml/g. After mild alkaline hydrolysis with 0.1 M KOH in methanol–water 9:1, v/v, at room temperature for 10 hours, the hydrolysate was acidified to pH 2 with hydrochloric acid. After partition (chloroform–methanol–water 8:4:3, v/v/v), the two phases were worked up separately. The upper phase was dialyzed against tap water and lyophilized. Essentially only sulfatides were eluted from silicic acid columns (table 1). The lower phase was subjected to repeated chromatography on silicic acid and magnesium silicate (Florisil) until pure fractions were obtained. Fractions were examined by thin layer chromatography, using the anisaldehyde reagent [6] for detection of glycolipids. Sphingomyelins were finally purified as ceramides after phospholipase hydrolysis [7]. The figures in table 1 were gravimetrically determined, that for sphingomyelins after multiplication of ceramide weight by 1.43. Identification was made by comparison with human brain sphingolipids, using thin layer chromatography and analysis of hydrolysis products (table 1, 2, and 3). Long-chain bases were analyzed as described elsewhere [8]. Fatty acids were separated by gas chromatography on glass columns packed with 3% OV 1 on 100/120 mesh Gas-Chrom Q (Applied Science Laboratories), using a flame ionization detector. Methyl esters were first separated into normal and α-hydroxy esters on thin layer plates. Isolated α-hydroxy esters were analyzed as their trimethylsilyl ethers [9]. For the ratio of normal to α-hydroxy esters, an internal standard procedure was used [10]. Fatty acids of bovine brain cerebrosides [11] were used as reference material.

Table 1
Sphingolipid composition of the rectal gland of spiny dogfish.

Cerebrosides	0.2
Diglycosylceramides	0.2
Sphingomyelins (crude)	6.1
Sulfatides:	
Upper phase	0.7
Lower phase	2.2

Figures are given as mg/g dry tissue. Sulfatides contained galactose as the only carbohydrate, analyzed by gas chromatography [15].

North-Holland Publishing Company – Amsterdam

Table 2
Relative amounts of individual α-hydroxy fatty acids to total fatty acids of rectal gland sulfatides (lower phase).

Acid	<20	20 : 0	21 : 0	22 : 1	22 : 0	23 : 1
%	tr	4	4	3	24	2
Acid		23 : 0	24 : 1	24 : 0	25 : 1	26 : 1
%		15	32	6	1	tr

The figure before the colon means carbon chain length, the figure after the colon number of double bonds, and tr means trace amount.

3. Results and discussion

As shown in table 1, sulfatides make up an appreciable part of the sphingolipids, and the composition is thus similar to that of the avian salt gland (8.6 mg sulfatides/g dry tissue) [2]. Other glycolipids than those presented in table 1 were not detected. Sulfatides of the rectal gland appear as a double spot on thin layer chromatograms, whereas salt gland sulfatides show a single spot. This is probably due to the presence of trihydroxy bases (phytosphingosine and related bases) in the rectal gland (table . , bases which are absent from the salt gland. More than 90% of rectal sulfatide fatty acids are α-hydroxy fatty acids (table 2). These are more unsaturated but otherwise similar to those of salt gland sulfatides [2]. In sphingomyelins the dominating acid is nervonic acid. Very small (a few per cent) but definite amounts of α-hydroxy acids (the major acid is hydroxynervonic acid) are present. This has not been reported before for sphingomyelins in general and will be discussed in detail elsewhere [10].

The sodium-potassium ion activated adenosine triphosphatase (NaATPase), thought to be an essential component of the sodium ion transport system [12], is present in high activities in the rectal gland [5] and in the salt gland [13], and the enzyme-sulfatide ratios of these tissues are approximately the same. The possibility that sulfatides and NaATPase are components of the same sodium ion transporting unit [2] will be studied in different ways, mainly using the salt gland. A subcellular fractionation may reveal a similar distribution of enzyme and sulfatides, and functional adaptations [14] of the salt gland may change enzyme activity and sulfatide concentrations in similar ways.

Acknowledgements

The present paper is number 5 of the series "Structure and Function of Sphingolipids", which is part of

Table 3
Long-chain base composition of rectal gland sphingolipids.

Relative amounts of aldehydes			Probable parent bases	
Aldehyde	Sulfatides	Sphingomyelins	Sulfatides	Sphingomyelins
14:0	1	1	t17:0	d16:0
iso 15:0	1	–	iso t18:0	–
15:0	30	1	t18:0	d17:0
14:1	–	1	–	d16:1
iso 16:0	24	–	iso t19:0	–
16:0	1	2	d18:0 + t19:0	d18:0
?	5	–	t	–
15:1	–	1	–	d17:1
iso 16:1	1	4	iso d18:1	iso d18:1
16:1	33	82	d18:1	d18:1
16:2	1	1	d18:2	d18:2
iso 17:1	5	7	iso d19:1	iso d19:1

The method of analysis and identification has been described elsewhere [8]. Isolated dinitrophenyl derivatives of trihydroxy (t) and dihydroxy (d) bases were oxidized by lead tetra-acetate and the derived aldehydes analyzed by gas chromatography. The figure before the colon means carbon chain length and the figure after the colon number of double bonds. Iso means an iso branched carbon chain.

a project supported by a grant from the Tri-Centennial Fund of the Bank of Sweden. The authors wish to thank Ragnar Fänge, Institute of Zoophysiology, for valuable advice concerning collection of material, Karin Nilsson for important assistance, and Jan Olsson for the capture of spiny dogfishes.

References

[1] K.–A.Karlsson, B.E.Samuelsson and G.O.Steen, Acta Chem. Scand., in press.
[2] K.–A.Karlsson, B.E.Samuelsson and G.O.Steen, submitted for publication.
[3] J.Crabbé, Nature 191 (1961) 817.
[4] D.Bellamy and J.G.Phillips, J. Endocrinol. 36 (1966) 97.
[5] S.L.Bonting, Comp. Biochem. Physiol. 17 (1966) 953.
[6] E.Stahl, Dünnschicht-Chromatographie (Springer-Verlag, Berlin, 1962) p. 498.
[7] K.–A.Karlsson, in preparation.
[8] K.–A.Karlsson, B.E.Samuelsson and G.O.Steen, Acta Chem. Scand. 22 (1968) 1361.
[9] K.–A.Karlsson, Acta Chem. Scand. 19 (1965) 2425.
[10] K.–A.Karlsson, B.E.Samuelsson and G.O.Steen, in preparation.
[11] J.S.O'Brien and G.Rouser, J. Lipid Res. 5 (1964) 339.
[12] R.W.Albers, Ann. Rev. Biochem. 36 (1967) 727.
[13] S.L.Bonting, L.L.Caravaggio, M.R.Canady and N.M. Hawkins, Arch. Biochem. Biophys. 106 (1964) 49.
[14] G.L.Fletcher, I.M.Stainer and W.N.Holmes, J. Exp. Biol. 47 (1967) 375.
[15] C.C.Sweeley and D.E.Vance, in: Lipid Chromatographic Analysis, ed. G.V.Marinetti (Marcel Dekker, New York, 1967) p. 465.