SULFOGLYCEROGALACTOLIPID FROM RAT TESTIS: A SUBSTRATE FOR PURE HUMAN ARYLSULFATASE A

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Received September 23,1974

Summary: The major glycolipid from rat testis, 1-O-alkyl-2-O-acyl-3-O-(β-D-galactopyranoside-3'-sulfate)-glycerol, labeled with [^{35}S]sulfate was biosynthesized and isolated. It was hydrolyzed by pure human arylsulfatase A. By each parameter examined, including obligatory bile salt requirement, $MnCl_2$ stimulation, pH optimum and K_m, the conditions were identical to those for the hydrolysis of cerebroside sulfate, the putative physiological substrate for arylsulfatase A. Cerebroside sulfate inhibited the hydrolysis of the testicular sulfolipid in a competitive manner with a K_i of 0.2 mM, which is similar to the K_m when cerebroside sulfate is the substrate. The testicular sulfolipid, like cerebroside sulfate, is not hydrolyzed by arylsulfatase B. Thus, sulfoglycerogalactolipid appears to be a physiological substrate for arylsulfatase A.

It has been established that cerebroside sulfates are the physiological substrates of arylsulfatase A (cerebroside-3-sulfate 3-sulfohydrolase, EC 3.1.6.8) [1], but the ubiquitous occurrence of arylsulfatase A [2] and the relatively restricted occurrence of cerebroside sulfates [3] has seemed paradoxical. The major glycolipid of testis and spermatozoa, a sulfated glycerogalactolipid, has been characterized as 1-O-alkyl-2-O-acyl-3-O-(β-D-galactopyranoside-3'-sulfate)-glycerol [4,5] in which the alkyl group is predominantly cetyl alcohol, while the acyl group is predominantly palmitic acid. Structurally, the testicular sulfolipid is quite similar to brain cerebroside sulfate (Fig. 1), so it prompted the examination of this material as a potential substrate for a highly purified preparation of human arylsulfatase A. The testicular sulfolipid was found to be an equally effective substrate as cerebroside sulfate. A preliminary communication of these findings has been presented [6]. Very recently, Yamato et al. [7] reported that a partially purified arylsulfatase A preparation from boar testis hydrolyzed testicular sulfolipid and they have also suggested that it may be a physiological substrate of arylsulfatase A.

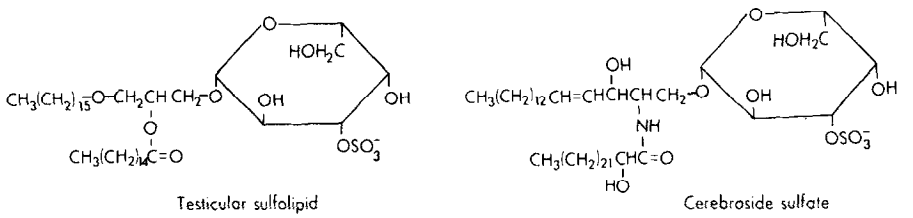

Fig. 1. The structure of testicular sulfoglycerogalactolipid and cerebroside sulfate.

MATERIALS AND METHODS

Preparation of [^{35}S]sulfoglycerogalactolipid. A mature male rat was injected in the testis with 10 mCi (2.5 μmole) of [^{35}S]sulfuric acid (New England Nuclear). On the following day the testes from this animal and 2 non-injected animals were used for the isolation. The tunicae were stripped off and the procedure developed for the isolation of cerebroside [^{35}S]sulfate from brain [8] was applied. Briefly, the lipids were extracted with chloroform-methanol, the lower phase lipids fractionated by TEAE-cellulose chromatography, the glycolipid fraction desalted on Sephadex G-25 and purified by silicic acid thin layer chromatography (chloroform-methanol-acetone-acetic acid-water, 10: 2:4:2:1 [9]). The sulfolipid yield was estimated by the procedure of Kean [10].

Preparation of arylsulfatase A. Human urinary arylsulfatase A was purified essentially to homogeniety as described in a separate report [11]. It had a specific activity of 3520 μmoles of 4-nitrocatechol sulfate hydrolyzed/hr/mg protein when assayed by the arylsulfatase A specific procedure of Baum et al. [12] with the incubation reduced to 30 min.

Enzyme assay. The procedure developed for the determination of cerebroside sulfate sulfohydrolase activity by measuring the release of inorganic [^{35}S]sulfate was used [13] except that the total reaction volume was reduced to 50 μliters.

RESULTS

The yield of testicular sulfoglycerogalactolipid was 24.8 mg from 13 g of testicular tissue at a specific activity of 57 μCi/nmole. The product gave one

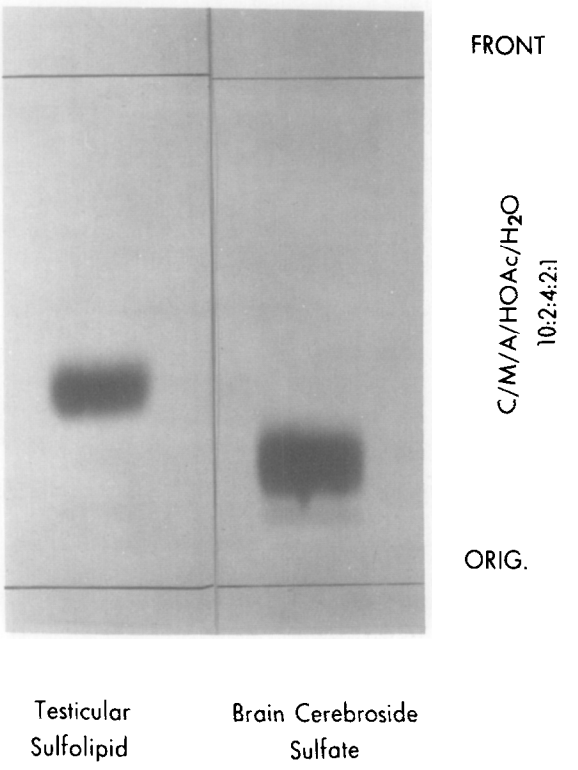

Fig. 2. Radioautograph of a thin layer chromatogram of testicular [^{35}S]sulfolipid. Cerebroside [^{35}S]sulfate is shown for comparative purposes. The sulfolipids (40 μg, $\sim$5000 cpm) were streaked on a E. Merck AG precoated silicic acid plate, developed, and exposed to X-ray film for 14 days.

discrete band on thin layer chromatography which was clearly separated from rat brain cerebroside sulfate (Fig. 2).

The testicular sulfatide was hydrolyzed by arylsulfatase A under conditions established for the hydrolysis of cerebroside sulfate (Table I). No activity was observed in the absence of bile salts; either taurodeoxycholate or cholate fulfilled this requirement. $MnCl_2$, while not an obligatory requirement, was stimulatory. The optimum concentration of sodium taurodeoxycholate showed the same unique sharp maximum identical to that for the hydrolysis of cerebroside sulfate (Fig. 3). The pH optimum of 4.6 was also identical to that for cerebroside sulfate. The reaction was linear for several hours and upon extended incubation essentially complete hydrolysis was achieved at low sub-

TABLE I

REQUIREMENTS FOR HYDROLYSIS OF TESTICULAR SULFOLIPID BY ARYLSULFATASE A

Incubation Mixture	Enzyme Activity	
	(μmole$\cdot$hr$^{-1}\cdot$mg protein^{-1})	
Complete[a]	198	(321)[b]
Minus taurodeoxycholate	0	(1)
Minus $MnCl_2$	70	(132)
Minus enzyme	0	(0)
Cholate[c] instead of taurodeoxycholate	605	(603)

[a]The complete reaction mixture contained 1 mM sulfolipid; 100 mM sodium acetate, pH 4.5; 20 mM $MnCl_2$; 1 mg/ml sodium taurodeoxycholate; 0.4 mg/ml bovine serum albumin; and 0.4 μg/ml of enzyme. The reaction was incubated for 2 hr.
[b]The values in parentheses were derived from parallel experiments with cerebroside sulfate as the substrate.
[c]Sodium cholate was used at a level of 10 mg/ml.

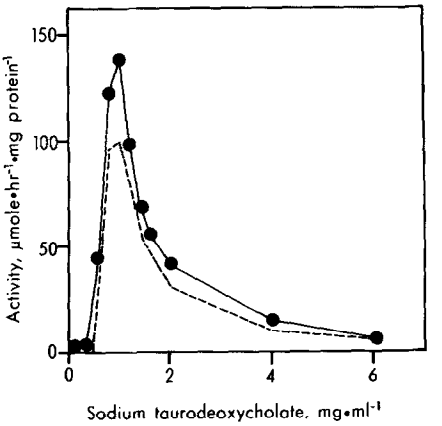

Fig. 3. Effect of sodium taurodeoxycholate on the hydrolysis of testicular sulfolipid by arylsulfatase A. The hydrolysis of cerebroside sulfate is shown for comparative purposes. Standard assay conditions were used with 0.38 mM of substrate. Each 50 μliter of assay mixture contained 23 ng of enzyme and 20 μg of bovine serum albumin. Testicular sulfolipid, ●—●; cerebroside sulfate, – – –.

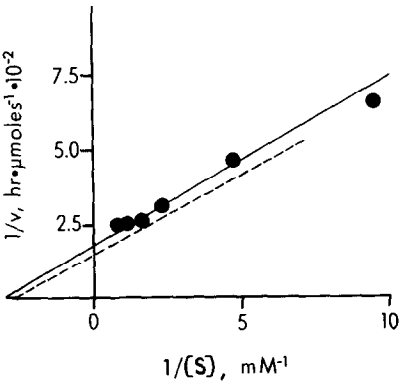

Fig. 4. Lineweaver-Burk plot (v^{-1} vs $[S]^{-1}$) of testicular sulfolipid sulfohydrolase activity of arylsulfatase A. The plot for cerebroside sulfate is shown for comparative purposes. Conditions as in Fig. 3. Testicular sulfolipid, ●—●; cerebroside sulfate,– – –.

strate concentration. At high substrate concentration, inhibition, presumably by accumulated sulfate, prevented complete hydrolysis. The apparent K_m for both sulfolipids was in the 0.2 to 0.5 mM range with similar maximal velocities (Fig. 4). Of greater significance was the competetive inhibition by cerebroside sulfate with a K_i of 0.2 mM which is similar to its K_m when it is the substrate (Fig. 5). The testicular sulfatide, like cerebroside sulfate, was not hydrolyzed by arylsulfatase B.

DISCUSSION

Rat testicular sulfoglycerogalactolipid was hydrolyzed by pure human arylsulfatase A. The optimal reaction conditions and kinetic parameters were identical to those for the hydrolysis of brain cerebroside sulfate. Similar results were obtained by Yamato et al. [7] with partially purified arylsulfatase A from boar testis. The hydrolysis of testicular sulfolipid was inhibited by cerebroside sulfate in a competitive manner with a K_i similar to the K_m when cerebroside sulfate is the substrate. This implies that both substrates occupy the same site on the enzyme and that the precise chemical nature of the hydrophobic moiety is not critical for substrate recognition. This is in accord with the finding that sulfogalactosylsphingosine, i.e., deacylated cerebroside sulfate, is also hydrolyzed by arylsulfatase A [14]. The testicular sulfolipid,

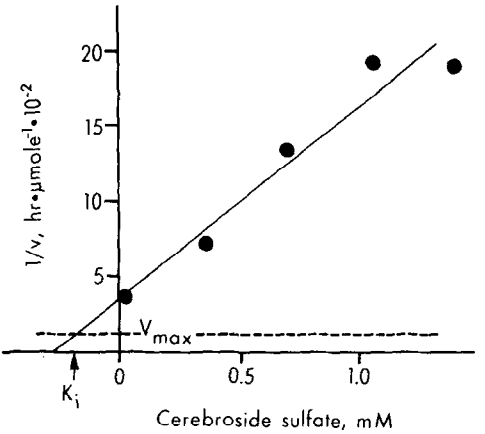

Fig. 5. Dixon plot (v^{-1} vs [I]) of cerebroside sulfate inhibition of testicular sulfolipid hydrolysis. The testicular sulfolipid concentration was 0.36 mM. Conditions as in Fig. 3. V_{max} was determined simultaneously.

cerebroside sulfate, and sulfogalactosylsphingosine are all sulfated in the 3-position of galactose. Slight activity of arylsulfatase A toward galactose-3-sulfate had been observed, but none toward galactose-6-sulfate [1]. It thus appears that while there is high specificity for the position of the sulfate moiety, there is lower specificity for the hydrophobic moiety. Nevertheless, the latter is important for maximal activity.

The significance of arylsulfatase A or of sulfoglycerogalactolipid in testicular tissue is not understood at present. However, the occurrence of sulfolipid as the major glycolipid in testis and spermatozoa has been noted in a number of species including rat, mouse, rabbit, guinea pig, hog, and human [4,5,15,16]. It has been reported that there is a dramatic increase in the amount of the sulfolipid in rat testis at a time coinciding approximately with the appearance of primary spermatocytes [17]. The presence of arylsulfatase A in testis of rats had been established [18], but no physiological significance was implied, presumably because cerebroside sulfate has never been found in testis. Arylsulfatase A had also been found in rabbit sperm acrosomes [19] and it was suggested that it may be involved in the penetration of spermatozoa through the investments of ovum. The presence of the polar sulfoglycerogalacto

lipid, which is probably a membrane component, and its sulfohydrolase in testis and spermatozoa is highly suggestive of a role in reproduction and merits further investigation.

Acknowledgements: This investigation was supported in part by Grants NS-8839, NS-9479 and HD-4612 from the National Institutes of Health.

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