STUDIES ON THE BIOSYNTHESIS OF TESTICULAR SULFOGLYCEROGALACTOLIPID: DEMONSTRATION OF A GOLGI-ASSOCIATED SULFOTRANSFERASE ACTIVITY

A. Knapp, M.J. Kornblatt, H. Schachter and R.K. Murray

The Dept. of Biochemistry, University of Toronto, Toronto, Canada.

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SUMMARY

Preparations of rat testis have been found to contain a sulfotransferase activity that catalyses the transfer of sulfate from 3'-phosphoadenosine-5'-phosphosulfate to monoalkyl-monoacyl-glyceryl monogalactoside. These preparations also sulfate galactosylceramide and monogalactosyl diglyceride. Cell fractionation studies reveal the enzyme activity to be enriched in a Golgi apparatus fraction.

INTRODUCTION

A monoalkyl-monoacyl-glyceryl monogalactoside sulfate has been isolated from rat testis (1); this sulfoglycerogalactolipid has been found to be the major glycolipid in the testis of rat, mouse, guinea pig, rabbit, and human (2). Independently, Ishizuka et al (3,4) have shown this compound to be the principal glycolipid of boar sperm and have proposed its structure to be 1-O-hexadecyl-2-O-hexadecanoyl-3-β[3'-sulfogalactosyl]-glycerol. As an initial step in elucidating the pathway of biosynthesis of this novel compound, we present evidence that rat testis contains a sulfotransferase activity - highly enriched in a GA[1] fraction (cf. 5) - capable of transferring sulfate from PAPS to the desulfated derivative of the above lipid.

MATERIALS AND METHODS

Preparation of Acceptor. Purified rat testis sulfoglycerogalactolipid was desulfated by refluxing approximately 5 mg in 10 ml of anhydrous dioxane for 1 hour (6). The desulfated lipid was purified by preparative TLC on glass

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[1] Abbreviations: GA, Golgi apparatus; PAPS, 3'-phosphoadenosine-5'-phosphosulfate; TLC, thin layer chromatography; C, chloroform; M, methanol; GLC, gas liquid chromatography; MGDG, monogalactosyl diglyceride; DGDG, digalactosyl diglyceride; gal-cer, monogalactosyl ceramide.

plates coated with silica gel G using a solvent system of C:M (9:1, v:v); the R_F of the desulfated lipid was approximately 0.40 in this system (corresponding to that of the small amount of non-sulfated glycerogalactolipid found in rat testis (1)), whereas the parent compound had an R_F of less than 0.05. The product was eluted from the chromatograms with C:M (2:1, v:v) and stored in this solvent mixture for further use. The concentrations of this lipid and of other galactolipids used as sulfate acceptors were measured by determination of the galactose content of acid hydrolysates with galactose dehydrogenase (7).

Assay of Sulfotransferase Activity. The procedure used was based on systems previously described (8,9) for the assay of PAPS: cerebroside sulfotransferase activity. The assay system is described in Table 1. Product identification was based on co-chromatography of [^{35}S]-lipid product with carrier testis sulfolipid in solvent systems of C:M:H_2O (65:25:4, v:v) and C:M:H_2O: ammonia (65:23:3:1, v:v); also, radioactive product was deacylated with mild alkali and the resulting product was shown to co-chromatograph with carrier deacylated testis sulfolipid in the system C:M:H_2O (65:25:4, v:v).

Preparation of Cell Fractions. Rat testes were homogenized in 3 volumes of 0.32 M sucrose containing 1 mM EDTA and 3 mM Na_2HPO_4 at pH 7.0. Aliquots of the total homogenate were retained for analysis and a microsome fraction was prepared by centrifugation of the post-mitochondrial supernatant (18,800 x g for 20 minutes) at 105,000 x g for 1 hour. The microsome pellet was suspended in homogenisation medium to a final protein concentration of 12.5 mg per ml; this preparation was used for enzyme assays. GA fractions of rat testis were prepared by the method of Mollenhauer et al. (10). Protein determinations were performed by the method of Lowry et al. (11).

Sources of Chemicals. [^{35}S] PAPS (0.6 Ci per mmole) was obtained from New England Nuclear. MGDG and DGDG were purchased from Applied Science Labs. (State College, Pa.). Synthetic gal-β-cer was obtained from Miles Labs. Galactose dehydrogenase and ATP were from Sigma. Triton X-100 was obtained from Mann Research Labs.

TABLE 1. (A) Requirements for testicular PAPS:galactolipid sulfotransferase
(B) Substrate specificity studies.

Assay procedure: 0.015 µmole desulfated testis glycolipid (or other glycolipid) was dissolved in 10 µl of 4% (v:v) Triton X-100 in C:M (2:1, v:v) and dried in a small test-tube under vacuum; to the tube were added 40 µl 0.25 M imidazole-HCl (pH 7.5), 10 µl 0.1 M ATP, 5 µl 0.016 M K_2SO_4, 5 µl 0.1 M KCl, 5 µl [^{35}S] PAPS (3.4×10^5 cpm, 0.6 Ci per mmole) and enzyme (125 µg and 250 µg of microsome fraction) in a final volume of 0.1 ml. The reaction mixtures were incubated for 1 hour at 37° and the reaction was terminated by addition of 0.5 ml of C:M (2:1, v:v). The upper phase was discarded and the lower phase was washed 4 times with 0.2 ml of C:M:0.1 M KCl (3:47:48, v:v). The final lower phase was dried down and taken up in 0.1 ml of C:M (2:1, v:v). Half of this solution was taken for determination of radioactivity. The other half was mixed with about 0.03 µmole of non-radioactive carrier testis sulfolipid (or other appropriate carrier), applied in a 1 cm channel at the origin of a thin layer plate coated with silica gel G and developed in C:M:H_2O (65:25:4, v:v). The position of the testis sulfolipid (or of the sulfated products of the other lipids) was visualized by brief exposure to iodine vapour; this area and other areas in the same channel were scraped separately into scintillation vials and their radioactivity determined. It was found that essentially similar results were obtained if the separation of radioactive product by TLC was omitted; the washing procedure described above removes all the [^{35}S] PAPS from the lower phase and at least 85% of the radioactivity in the washed lower phase can be recovered in the area of the chromatogram corresponding to testis sulfolipid (or to the sulfated derivatives of the other glycolipids used as acceptors).

(A) Incubation mixture	[^{35}S] incorporated (cpm/250 µg enzyme/hour)
Complete	2050
Minus acceptor lipid	190
Minus Triton X-100	8
Minus K_2SO_4	1690
Minus ATP	1690
Minus ATP and K_2SO_4	1140
Minus ATP, K_2SO_4, and KCl	640
Minus K_2SO_4 and KCl	640
Plus dithiothreitol (0.21 µmole)	1050
Plus dithiothreitol (0.42 µmole)	580
Minus active enzyme, plus heat-inactivated enzyme (5 min at 100°)	10

(B) Acceptor	[^{35}S] incorporated (cpm/250 µg enzyme/hour)
Desulfated testis glycolipid	2000
Gal-cer	1970
MGDG	1250
DGDG	50

RESULTS

We have shown the presence in rat testis of a sulfotransferase activity that catalyses the transfer of [^{35}S] sulfate from [^{35}S] PAPS to monoalkyl-monoacyl-glyceryl monogalactoside (Table 1). The pH optimum of the reaction is about 7.5. Table 1A shows the requirements of the reaction. In the absence of exogenous acceptor, enzyme activity fell to 8-10% of that observed with the complete system; the residual activity is presumably due to endogenous lipid acceptors. Omission of ATP, K_2SO_4 and KCl all resulted in decreased activity. Dithiothreitol was inhibitory. Enzyme activity was lost if the enzyme was boiled or if Triton X-100 was omitted. Under the standard assay conditions, the rate of enzyme activity was proportional to enzyme protein concentration (up to 500 μg) and was constant with time up to 2 hours. The concentration of [^{35}S] PAPS used in the standard assay (Table 1) was not saturating; however, sufficient [^{35}S] PAPS was present to ensure adequate proportionality with respect to protein concentration and time.

The substrate specificity of the testis sulfotransferase is shown in Table 1B. Gal-cer and MGDG were both excellent substrates whereas DGDG did not accept sulfate.

Competition studies were carried out with gal-cer and desulfated testis lipid as substrates at saturating concentrations (Table 2). It is apparent that the incorporation of sulfate when both substrates are present together in a single tube is approximately the average of the incorporation for each substrate alone indicating competition between the substrates. The product resulting from incubations in the presence of both substrates was subjected to analysis by TLC; it was found that 78% of the [^{35}S] sulfate had been incorporated into sulfo-gal-cer and 22% was present in the testis sulfo-glycerogalactolipid.

Initial studies revealed that the sulfotransferase specific activity of the microsome fraction was approximately 1 to 2-fold that of the crude homogenate. To characterize further the subcellular location of the sulfo-

TABLE 2. Competition studies.

Standard enzyme assays were carried out as described in Table 1 using desulfated testis glycolipid and gal-cer as substrates either individually or together in the same tube. The enzyme used was a microsome fraction.

Desulfated testis glycolipid (μmoles)	Gal-cer (μmoles)	[^{35}S] incorporated (cpm/hour) 140 μg enzyme	280 μg enzyme
0.015		970	2540
0.030		900	1740
	0.014	1080	2250
	0.028	1170	1960
0.015	0.014	1020	1970

TABLE 3. Specific activities of PAPS:galactolipid sulfotransferase in subcellular fractions of rat testis.

The conditions of enzyme assay are described in Table 1. Desulfated testis galactolipid was used as acceptor.

Cell fraction	[^{35}S] incorporated (cpm/50 μg enzyme/hour)		Relative specific activity	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Homogenate	580	540	1.0	1.0
Microsome fraction	1400	507	2.4	0.9
GA fraction	10100	17500	17.3	32.4
105000 x g Supernatant	0	--	0	--

transferase, crude homogenate, microsome fraction and GA were assayed for enzyme activity (Table 3). In two experiments, the sulfotransferase was enriched 17.3 and 32.4 times in the GA fraction relative to homogenate. Rat testis GA preparations have been monitored for glycoprotein glycosyltransferases[2] in studies similar to those previously carried out in rat liver (12); it was found[2] that the testis GA fractions were enriched about 50-fold relative to homogenate for a fucosyl-, galactosyl- and N-acetylglucosaminyl-

[2] P.J. Letts, L. Pinteric and H. Schachter, in preparation.

transferase. The testis GA fractions were also examined by electron microscopy and shown to have the structures characteristic of the Golgi apparatus from liver and other organs[2]. These findings indicate that our testis GA fractions were indeed enriched in Golgi apparatus elements.

DISCUSSION

The above results clearly indicate that adult rat testis contains an enzyme activity capable of sulfating several galactolipids. The testis galactolipid (4), MGDG (13) and synthetic gal-cer used in this study all contain a terminal β-galactose residue. DGDG, which showed little or no acceptor capacity, contains a terminal α-galactose residue (14). These observations suggest that the rat testis sulfotransferase will sulfate a variety of galactolipids - with either glycerol or sphingosine backbones - containing a terminal β-galactose residue. Presumably the sulfate is attached at C-3 of the galactose moiety (4,15). Farrell and McKhann (8) have demonstrated that the PAPS: cerebroside sulfotransferase of rat brain will sulfate a variety of galactosphingolipids containing a terminal β-galactose. Recent studies in our laboratory indicate that rat brain contains a small amount of the predominantly testicular sulfoglycerogalactolipid; it thus appears possible that the brain sulfotransferase will also be found to sulfate certain glycerogalactolipids. The substrate competition studies revealed clear competition between gal-cer and the desulfated testis glycolipid, suggesting that the same PAPS: galactolipid sulfotransferase in rat testis acts on both galactosphingolipids and galactoglycerolipids. These studies also indicate that gal-cer is the preferred substrate; further work is in progress to determine various kinetic parameters of the sulfotransferase with respect to these substrates. However, in the light of these findings, it appears probable that the observed absence of the classical sulfatide (SO_4-gal-cer) in rat testis may reflect the negligible amounts of gal-cer found in testis (1,4).

We have recently reported that there is a dramatic increase in the amount of the sulfoglycerogalactolipid in rat testis at a time coinciding

approximately with the appearance of primary spermatocytes (2); assays of the PAPS: galactolipid sulfotransferase activity from testes at various stages in spermatogenesis should assist in elucidating the mechanism of this phenomenon and also of its possible importance in the processes of differentiation occurring in the developing testis.

The subcellular fractionation studies revealed a marked enrichment of the sulfotransferase activity in a GA fraction (Table 3). Of particular significance in this respect is the recent finding by Fleischer and Zambrano (5) that the PAPS: cerebroside sulfotransferase activity of rat kidney is markedly enriched in GA fractions of that organ. As stated by these workers, this was the first direct evidence that the GA functions in mammalian cells not only to transport and modify secretory products, but also to modify a lipid which ultimately becomes a component of the membranes of the cell. The sulfoglycerogalactolipid of testis and sperm is probably also a membrane component; establishment of the membrane system (or systems) in which it is principally located may afford a useful model for analysis of the function of the GA in membrane biogenesis. Evidence is now available that there is a marked enrichment of certain of the glycosyl transferases involved in ganglioside biosynthesis in GA fractions of rat liver (16). The results reported here thus support the view that this organelle plays an important role in at least certain steps in the biosynthesis of the various glycolipids found in mammalian tissues. They also lend support to the generalization of Young (17) that the enzymes required for the transfer of inorganic sulfate to a variety of acceptor molecules may be located in the Golgi complex.

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Footnote - At the 9th International Biochemistry Congress in Stockholm , Handa et al. (18) reported the presence of a galactolipid sulfotransferase in mammalian testis with generally similar properties to that described here; the abstract of their presentation does not, however, refer to this finding.

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