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Developmental regulation of galactoglycerolipid and galactosphingolipid sulphation during mammalian spermatogenesis

Evidence for a substrate-selective inhibitor of testicular sulphotransferase activity in the rat

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The synthesis of sulphatoxygalactosylacylalkylglycerol (SGG) is a differentiation marker of mammalian spermatogenesis. Maximal sulphation is observed in rat testis at about 20 days after birth and rapidly declines to low levels as the testis matures. The present data show that this decline in SGG synthesis is due to the appearance of an inhibitor of galactolipid sulphation. The inhibitor is a soluble testicular factor which is first detected at about 25 days after birth. Testicular homogenate can sulphate exogenous galactosylacylalkylglycerol (GG), galactosylceramide (GC) and lactosylceramide (LC) *in vitro*. The testicular inhibitor is most effective in preventing GG sulphation and inhibits GC and LC sulphation to a lesser extent; this correlates with the finding that glycolipid sulphation shifts from SGG production in 20-day-old testis to GC and LC sulphation at later stages of testicular development. The effect of the inhibitor on sulphotransferase activity from brain and kidney was also determined. The inhibitor decreased the sulphation of GG *in vitro* by both testis and kidney, inhibited testicular sulphation of GC less effectively and had no effect on GC sulphation by kidney and brain homogenates. A 9500-fold purification of the inhibitory activity has been obtained in a fraction isolated by h.p.l.c.

INTRODUCTION

Sulphatoxygalactolipids are present in the testes of all animal species so far tested (Murray *et al.*, 1980). SGG is the major glycolipid in the testis of several mammalian species (Murray *et al.*, 1980; Kornblatt *et al.*, 1972; Ishizuka *et al.*, 1973) and has not been detected in lower animal phyla (Murray *et al.*, 1980). The testes of lower phyla contain sulphatoxygalactosylceramide (SGC) and sulphatoxylactosylceramide (SLC) or both (Murray *et al.*, 1980).

SGG appears to be located primarily in the plasma membranes of testicular germ cells (Klugerman & Kornblatt, 1980; Shirley & Schachter, 1980). GG sulphation to form SGG occurs at a specific stage in mammalian spermatogenesis, probably just before the appearance of pachytene spermatocytes (Kornblatt *et al.*, 1974; Letts *et al.*, 1978; Lingwood, 1985); there is no further synthesis or degradation of SGG at later stages of spermatogenesis (Kornblatt, 1979). Sterile mutant male mice lacking early germ-cell stages are deficient in SGG (Kornblatt *et al.*, 1974).

Testicular homogenates contain a sulphotransferase that converts GG into SGG. The specific activity of this enzyme reaches a maximum in the testes from 20-day-old rats and rapidly declines as the testis matures (Kornblatt *et al.*, 1974). The present report shows that this decline in activity can be attributed to the appearance in the testis of a developmentally regulated soluble factor that selectively inhibits sulphation of GG.

EXPERIMENTAL PROCEDURES

Materials

[^{35}S]PAPS (0.9–5.1 Ci/mmol) and $H_2{}^{35}SO_4$ (43 Ci/mg, theoretical maximum) were purchased from New England Nuclear.

Enzyme preparation

Testes were removed from Sprague–Dawley rats immediately after asphyxiation with CO_2. The tunicae were removed and the tubules homogenized by twelve strokes in a Dounce homogenizer in sufficient 0.32 M-sucrose/1 mM-EDTA to give 40–60 mg of protein/ml. Enzyme preparations were stored in liquid N_2 without loss of activity.

In some experiments solubilized enzyme from 20-day-old rats was used. The homogenate was centrifuged at 100000 *g* for 30 min. The pellet was resuspended in 10 mM-Tris/HCl (pH 7.4)/25% (v/v) glycerol and adjusted to a final concentration of 0.5% Triton X-100. The suspension was incubated at 4 °C for 1 h with occasional shaking and centrifuged at 100000 *g* for 1 h to a final supernatant protein concentration of about 20 mg/ml. About 90–95% of the sulphotransferase activity was

Abbreviations used: SGG, sulphatoxygalactosylacylalkylglycerol; GG, galactosylacylalkylglycerol; GC, galactosylceramide; LC, lactosylceramide; SGC, sulphatoxygalactosylceramide; SLC, sulphatoxylactosylceramide; MGDA, monogalactosyldiacylglycerol; PAPS, 3'-phosphoadenosine-5'-phosphosulphate.

recovered in this supernatant. The enzyme was stable for a week at 4 °C but routinely stored at −70 °C, when enzyme activity was stable for more than 2 months.

Preparation of sulphation inhibitor

Testes from adult (250–300 g) rats were homogenized as described above. The homogenate was centrifuged at 8000 *g* for 2 min, the supernatant was passed through a Millipore filter (0.45 μm pore size) and portions were freeze-dried and stored at −20 °C until required.

Glycolipid substrates

SGG was prepared from bovine testes as previously described (Lingwood *et al.*, 1980). GG was prepared from SGG by anchinmeric-assisted solvolysis in acetone/HCl (Lingwood *et al.*, 1983). LC was prepared from human erythrocytes as described by Laine *et al.* (1974). GC and monogalactosyldiacylglycerol (MGDA) were commercial preparations from Supelco. Galactolipid concentrations were determined by subjecting portions to acid hydrolysis (Lingwood, 1979) and assaying free galactose with galactose dehydrogenase (Schachter, 1980).

Sulphotransferase assays

Glycolipid substrate (25 nmol unless otherwise stated) was dissolved in 25 μl of chloroform/methanol (2:1, v/v) containing 4% Triton X-100, the solution was evaporated to dryness under N_2 and resuspended in 0.1 ml of 0.1 Tris/HCl, pH 8.6, containing 10 mM-ATP, 2.5 mM-$MgCl_2$, 80 mM-K_2SO_4, either 2 or 30 μM-[^{35}S]-PAPS and 25 μl of sulphotransferase preparation. Under these conditions the assay is linear with time (up to 2 h) and protein concentration (< 0.5 mg/assay). After 2 h at 37 °C, the incubation mixture was partitioned into 5 vol. of chloroform/methanol, 2:1 (v/v), and aq. 0.88% KCl, the lower phase was subjected to t.l.c. on silica-gel G plates (chloroform/methanol/water, 65:25:4, by vol.) and sulphated products were detected by autoradiography. No radiolabelled SGG was found in the upper phase. Non-radioactive glycolipid standards were detected by an orcinol/H_2SO_4 acid spray for carbohydrate. Radioactive products were scraped from the plate, dissolved in 4 ml of ACS (Amersham) scintillation fluid and counted for radioactivity in an LKB Racbeta II liquid-scintillation counter.

Sulphation of endogenous glycolipids

Tubules from 20-day-old or adult rat testes were finely chopped with a sharp razor blade and suspended in 50 ml of phosphate-buffered saline (0.1 M-phosphate buffer, containing 0.15 M-NaCl, pH 7.2. The suspension was allowed to settle in a measuring cylinder for 5 min at room temperature and the supernatant was then centrifuged at 800 *g* for 10 min. The pellet was resuspended in phosphate-buffered saline containing 1% fructose at a concentration of about 10^6 cells/ml. A portion (100 μCi) of $H_2{}^{35}SO_4$ was then added to a 1 ml aliquot and the cells were incubated at room temperature for 5 h with occasional gentle agitation. Cell viability at the end of the incubation was about 60% as judged by Trypan Blue exclusion. About 10 vol. of chloroform/methanol (2:1, v/v) was added, lower-phase glycolipids were separated by t.l.c. (chloroform/methanol/water, 40:10:1, by vol.), and radioactive products were detected by autoradiography.

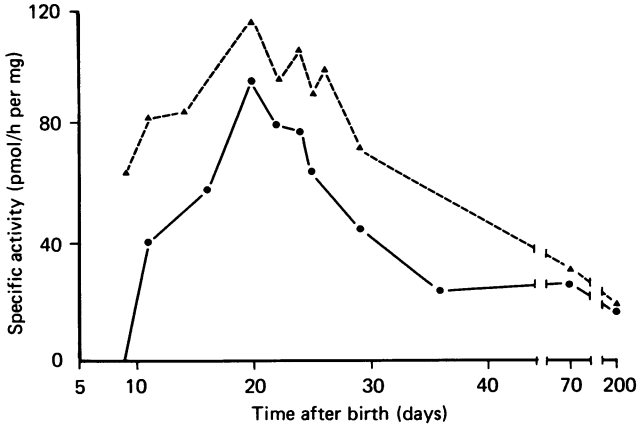

Fig. 1. Regulation of galactolipid sulphotransferase during testicular development

The specific activity of the testicular sulphotransferase (pmol of SGG synthesized/h per mg of protein) was measured with GG and 30 μM-PAPS as described in the Experimental procedures section (●). The average result for duplicate assays is shown. The effect of the 8000 *g* supernate of each homogenate on the sulphotransferase activity present in the testicular homogenate of 22-day-old rats (enzyme/supernatant, 1:1, v/v) was also measured (▲). The protein contents of the additional supernatant were not included in the calculation of specific activities. Results of one of three similar experiments are shown.

Purification of inhibitor

The Millipore filtrate of the adult testicular homogenate supernatant was prepared as described above. The filtrate was extracted with 10 vol. chloroform/methanol (2:1, v/v) and filtered through a sintered-glass funnel without separation of the phases. The filtrate was rotary evaporated to dryness, resuspended in chloroform/methanol (2:1, v/v) and partitioned against 6 vol. of water. The upper phase was removed and freeze-dried. The upper-phase extract was redissolved in the original volume of water, filtered through a Millipore 0.45 μm-pore-size filter and a portion was separated by h.p.l.c. on a C_{18} reverse-phase column (25 cm × 0.4 cm), with water containing 0.1% acetic acid as the mobile phase (1 ml/min). The column effluent was monitored at 240 nm and fractions corresponding to peak readings were collected. These fractions were freeze-dried and reconstituted in the orginal sample volume for assay of sulphotransferase inhibitory activity. Sulphotransferase from 20-day-old testicular homogenate, solubilized in 0.5% Triton as described above, was used to monitor inhibitory activity during purification. Protein concentrations were measured by the Bradford (1976) method.

RESULTS

Inhibition of sulphotransferase activity during testis development

The specific galactolipid sulphotransferase activity of rat testicular homogenates at various days after birth is shown in Fig. 1. As previously described (Kornblatt *et al.*, 1974), activity reached a maximum between 18 and 21 days after birth and was followed by a marked decrease to values characteristic of the adult testis by 35 days.

Table 1. Acceptor specificity of adult testicular sulphotransferase inhibitor

		Specific activity (pmol of SO_4^{2-}/h per mg)*					
	[[^{35}S]PAPS]...	2 μM			30 μM		
(*a*) Alone†	Inhibitor...	−	+	% of Control	−	+	% of Control
GG		5.3	2.8	53±7 (8)	101.7	56.7	63±8 (20)
GC		13.1	5.3	69±21 (11)	136.5	122.7	88±16 (11)
LC		4.4	5.0		70.8	63.3	83±10 (9)
MGDA		1.6	1.2		22.2	11.4	

		Specific activity (pmol of SO_4^{2-}/h per mg)		
(*b*) Mixed‡	[PAPS] (μM)	SGG	SGC	SLC
GG+GC+LC−inhibitor	2	1.7	5.1	1.53
+inhibitor	2	1.1 (64%§)	5.5 (107%§)	1.45 (95%§)
−inhibitor	30	36.7	141.4	38.0
+inhibitor	30	17.5 (47%)	92.9 (70%§)	41.9 (110§)

* Values in duplicate from a single representative experiment are shown. The degree of inhibition of sulphotransferase activity (expressed as a percentage of a control-incubation value in the absence of inhibitor ± S.D.) is calculated from the numbers of separate determinations indicated in parentheses. The inhibitor preparation had no effect on unrelated enzyme activities such as alkaline phosphatase, creatine kinase or uridine diposphatidylglucosamine glycopeptide transferase (results not shown).

† Saturating substrate concentrations (25 nmol of GG, 2 nmol of GC, 25 nmol of MGDA and 10 nmol of LC) were incubated with homogenate from 20-day-old rats ± Millipore filtrate of adult testicular homogenate supernatant as described in Fig. 1.

‡ One-third substrate concentrations (8 nmol of GG, 0.67 nmol of GC and 3.2 nmol of LC) were incubated as above in the same assay tube. Radiolabelled products were detected by autoradiography and the corresponding bands were scraped off and counted for radioactivity.

§ Of control.

Fig. 1 also shows the sulphotransferase specific activity of homogenate from 22-day-old rat testes assayed in the presence of the 8000 *g* supernatants from testes of various ages. These supernatants were essentially free of sulphotransferase activity, since the enzyme is membrane-bound (Knapp *et al.*, 1973) and therefore sedimented. The data suggested the appearance, at about 25–30 days, of a testicular factor capable of inhibiting sulphotransferase activity. At early times (18–22 days) an apparently stimulatory activity can be detected (C. A. Lingwood, unpublished work). The activity of the inhibitor with time parallels the decrease in the sulphotransferase activity observed for testicular homogenates during development. This suggests that decreased GG sulphation during testis development may be due to the appearance of a soluble developmental regulated sulphotransferase inhibitor.

Inhibitor activity was assayed in subsequent studies by including, in the standard sulphotransferase assay (see the Experimental procedures section), 25 μl of the Millipore filtrate of the supernatant from adult testicular homogenates (about 0.4 mg of protein). Such an incubation results in about 50% inhibition of sulphotransferase activity when 20-day-old-rat testis homogenates serve as enzyme source. More than 90% inhibition can be achieved at higher inhibitor concentrations (results not shown). Under these conditions new radiolabelled products are observed corresponding in t.l.c. migration to the sulphation of endogenous GC and LC. The inhibition therefore appears to reduce GG sulphation preferentially. Further evidence for this substrate selectivity is presented below.

Substrate selectivity of the sulphation inhibitor

SGG is the only radioactive glycolipid detected in testis after the injection of sodium [^{35}S]sulphate into rats (Lingwood *et al.*, 1981). Testicular homogenates can, however, sulphate a variety of exogenously added galactolipids *in vitro*, e.g., GG, GC, LC and MGDA. The K_m values for PAPS were calculated as follows: GG, 38.3 μM; GC, 37.5 μM; LC, 40.5 μM. The physiological substrate, GG, is not the most active *in vitro* (reaction rate: GC > LC ≥ GG). Competition studies have shown that a single testicular sulphotransferase may be responsible for the sulphation of GG, GC and LC *in vitro* (Handa *et al.*, 1974; C. A. Lingwood, unpublished work). Table 1 provides data supporting the preferential action of the testicular inhibitor on GG sulphation *in vitro*. The inhibitor was most effective in decreasing the sulphation of GG as compared with GC. Little effect on the sulphation of LC was observed. This selectivity of inhibition was most striking when the galactolipid substrates were assayed in combination.

Fig. 2 shows that this selective action of the inhibitor *in vitro* may be expressed during spermatogenesis. Testicular homogenates from rats of various ages were tested for sulphation of endogenous GG and of low concentrations of exogenous GC and LC. The synthesis of both SGC and SLC relative to SGG synthesis increased with age. Similar results were obtained by metabolic labelling of testicular cell suspensions with [^{35}S]sulphate (results not shown). This approach depends entirely on endogenous glycolipid acceptors. The ratio

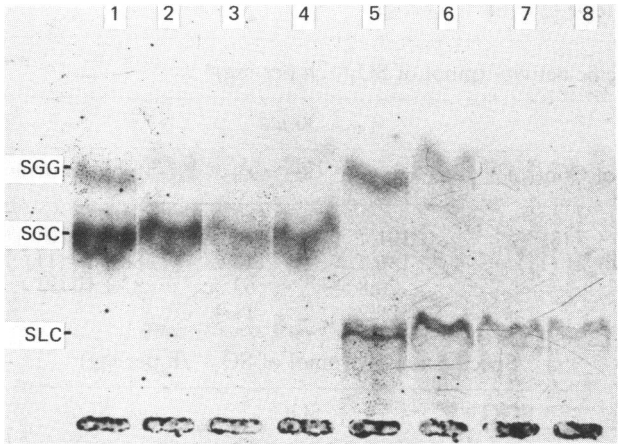

Fig. 2. Change in acceptor specificity *in vitro* of testicular sulphotransferase during spermatogenesis

Lanes 1–4, sulphation of 10%-saturation GC (0.2 nmol); lanes 5–8, sulphation of LC (1.0 nmol) in the presence of 30 μM-PAPS. Testicular homogenates from 20-day (lanes 1 and 5), 36-day (lanes 2 and 6), 80-day (lanes 3 and 7) and adult rats (lanes 4 and 8) are shown. Ratios of biosyntheses (SGC/SGG) were 3.4, 6.3, 7.8 and 6.0, and for SLC/SGG they were 1.3, 3.7, 5.5 and 3.2 respectively. Substrate K_m values calculated from reciprocal plots at 30 μM-PAPS in 20-day- and adult-rat homogenates were respectively 3.7 and 6.3 μM for GC and 33.3 and 4.6 μM for LC (owing to high endogenous incorporation, the K_m for GG could not be accurately measured).

of SGC to SGG production was 0.2 for 20-day-old testis and 2.0 for adult testis. The respective ratios for SLC production relative to SGG production were 0.16 and 2.8. Thus both testis homogenates and intact cells show a shift from SGG synthesis to SGC and SLC production as the testis matures.

It should be pointed out that the sulphated products formed from endogenous lipid acceptors have only been tentatively identified as SGC and SLC. These products co-migrate with SGC and SLC standards on t.l.c., are resistant to mild alkaline hydrolysis [unlike glycerol-based sulphoglycolipids (Lingwood *et al.*, 1981)], and this assignment is consistent with the substrate selectivity of the inhibitor (Tables 1 and 2).

Comparative study of testicular-sulphation-inhibitor action on sulphotransferases from rat testis, kidney and brain

Kidney, brain and testis are major sites of sulphoglycolipid biosynthesis in mammals. Sulphotransferases capable of converting GC into SGC have in fact been isolated from both rat kidney and brain (Tennekoon & McKhann, 1978; Tennekoon *et al.*, 1981). Kidney homogenate was prepared from adult rat kidney as described for testis homogenate. A partially purified GC sulphotransferase from 20-day-old rat brain was kindly provided by Dr. G. Tennekoon (Johns Hopkins University, Baltimore, MD, U.S.A.). The conditions used for assay of the testicular sulphotransferase were also used for the brain and kidney enzymes. Both of these preparations, as well as 20-day-old testis homogenate, converted GC into SGC (Table 2) and LC to SLC (results not shown); the ratio of SGG formation to SGC formation was respectively 0.22, 0.07 and 0.20 for the enzyme preparation from 20-day-old testis, brain and adult kidney. Thus both testis and kidney can form SGG, although kidney does not appear to synthesize SGG *in vivo* (Lingwood *et al.*, 1981); presumably kidney lacks the precursor GG. The brain enzyme, however, is essentially inactive with GG as an acceptor.

Table 2 shows the effect of sulphation inhibitor from adult rat testis on GC sulphation by these three enzyme sources. Only the testis GC sulphotransferase activity was decreased by the testis inhibitor. However, the substrate selectivity of the inhibitor (Table 1) was again demonstrated in that the inhibitor decreased the sulphation of GG by kidney (Table 2). Thus, the testis inhibitory activity shows selectivity for both the tissue source of sulphotransferase and for the glycolipid substrate.

Table 2. Enzyme selectivity of testicular sulphotransferase inhibitor

Experimental conditions were as described in Fig. 1. Testis homogenate was from 20-day-old, and kidney was from adult, rats.

[^{35}S]PAPS concn. (μM)	Enzyme source	Substrate	Inhibitor	Sulphate product (pmol/h per mg) Expt. 1	Expt. 2
2	Testis	GC	−	31.4	24.0
		GC	+	22.5 (72%*)	17.9 (74%*)
	Brain	GC	−	797	122.5
		GC	+	829 (104%*)	126.1 (103%*)
	Kidney	GC	−	7.8	11.5
		GC	+	8.6 (110%*)	10.1 (88%*)
30	Kidney	GG	−	16.1	18.9
		GG	+	6.5 (40%*)	7.0 (37%*)
		GC	−	31.5	34.5
		GC	+	28.2 (90%*)	31.7 (92%*)
		LC	−	35.9	11.4
		LC	+	37.2 (104%*)	15.6 (137%*)

* Expressed as a percentage of control incubation in the absence of inhibitor.

Table 3. Purification of testicular inhibitor of sulphotransferase activity*

Fraction	[Protein] (mg/ml)	Protein required for 50% inhibition (mg)	Purification factor	Inhibitory activity recovered (%)†
Adult testicular homogenate	64.3	1.6	1	100
Millipore filtrate of homogenate supernatant	48.2	1.2	1.3	100
Upper phase of chloroform/methanol extract	2.33	0.23	7.0	25
H.p.l.c. peak I	0.0023	1.7×10^{-4}	9411.8	30

* Measured at 2 μM-PAPS.
† Accumulative recovery is given.

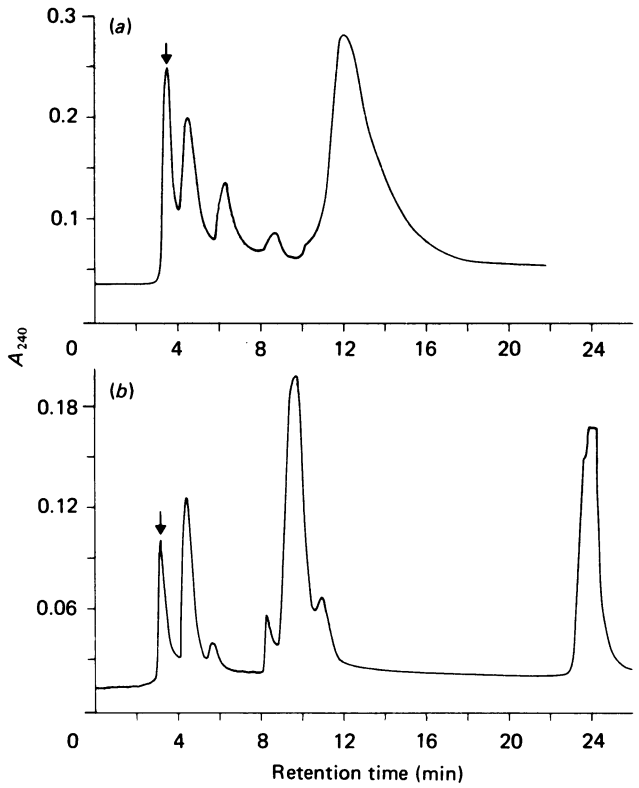

Fig. 3. Separation of testicular inhibitor of sulphotransferase activity by reverse-phase h.p.l.c.

A portion of the Millipore filtrate of the testicular supernatant (*a*) or the upper phase of the Folch extract thereof (*b*) was applied on a C_{18} μ-Bondapak column previously equilibrated with (*a*) water or (*b*) water containing 0.1% acetic acid. The column was eluted at a flow rate of 1 ml/min and peak fractions were collected. Sulphotransferase inhibitory activity was found in the peak indicated (retention time 3.5 min).

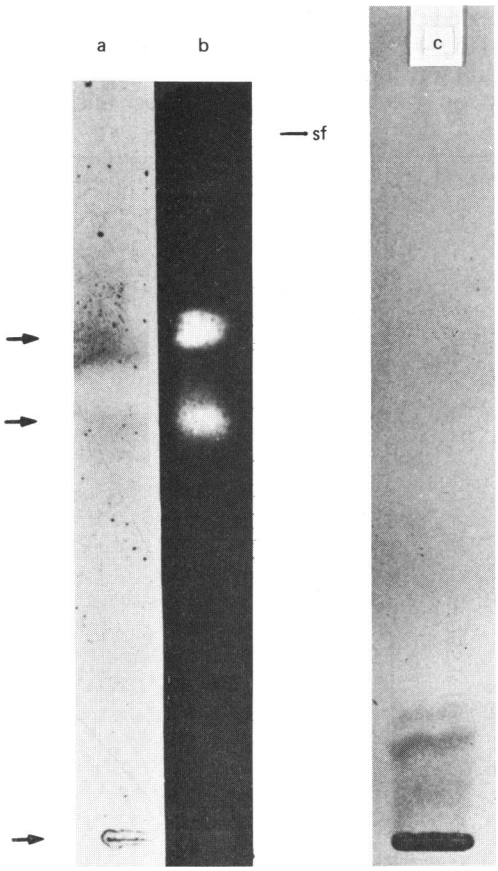

Fig. 4. T.l.c. analysis of h.p.l.c.-purified inhibitor

A portion of peak I from the h.p.l.c. purification was separated by t.l.c. in chloroform/methanol/water (2:10:4, by vol.) (lanes a and b) or chloroform/methanol/water (55:25:4) (lane c). Individual species were revealed by orcinol spray for carbohydrate (lanes a and c) or fluorescamine spray for protein under u.v. illumination (lane b). Abbreviation used: sf, solvent front.

Purification of inhibitor

The sulphotransferase inhibitory activity was found to be present in the upper phase of a Folch extract of the high-speed testicular supernatant. Approx. 25% of the activity present in the Millipore filtrate was recovered in this fraction (Table 3). No inhibitory activity was found in the lower phase. The upper-phase fraction was resolved into several peaks by reverse-phase h.p.l.c. (Fig. 3). Of these subfractions, only the first (most hydrophilic) contained inhibitory activity. A purification of approx. 9500-fold was obtained relative to the adult testicular homogenate (Table 3). The h.p.l.c.-purified inhibitor was

Table 4. Substrate selectivity of purified (h.p.l.c. peak I) inhibitor

Triton X-100-solubilized enzyme was incubated in quadruplicate with GG or GC as described in the Experimental procedures section, in the presence of 30 μM-PAPS ± inhibitor. Inhibitor was reconstituted in 30% of the volume of the original adult testicular homogenate from which it was purified.

Galactolipid substrate	Product formed (pmol/h per mg)	+ Inhibitor (% of control + S.D.)
GG	64.69	52.89 ± 9.1
GC	114.79	75.75 ± 14.4

dialysable and trypsin labile. Analysis of this fraction by t.l.c. revealed three carbohydrate-containing amino components (Fig. 4). I am unable to separate these components and maintain inhibitory activity. Species of similar mobility on t.l.c. were found to be missing in the upper-phase testicular extract of a mutant rat which was deficient in testicular sulphotransferase inhibitory activity [see the following paper (Lingwood *et al.*, 1985)]. Moreover, the purified inhibitor showed a substrate selectivity similar to the original inhibitory activity in the adult testicular homogenate supernatant in that the sulphation of GG *in vitro* was decreased more than that of GC (Table 4).

Some preliminary enzyme-kinetic studies were also performed with the purified inhibitor and solubilized sulphotransferase (Fig. 5). Reciprocal plots at constant nucleotide (Fig. 5*a*) or galactolipid-acceptor concentration (Fig. 5*b*) suggest uncompetitive inhibition against GG and non-competitive inhibition against PAPS.

DISCUSSION

The present results provide evidence for a novel physiological mechanism of regulation of sulphogalactolipid biosynthesis during spermatogenesis in the rat. In the rat the first wave of spermatogenesis occurs during the first 50 days after birth. Tissue autoradiography

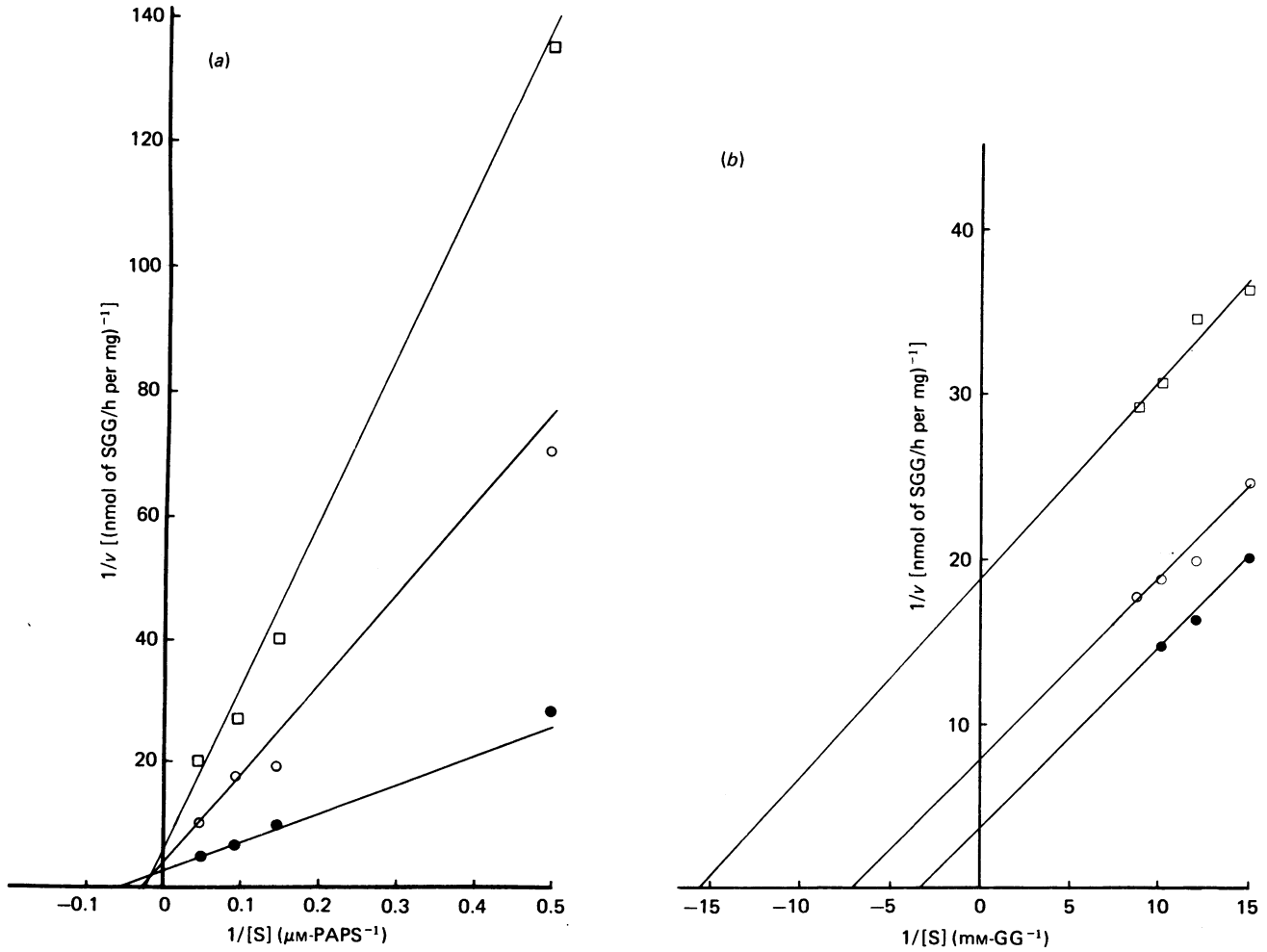

Fig. 5. Lineweaver–Burke analysis of kinetics of inhibition of testicular sulphotransferase

The inhibitory activity of the h.p.l.c.-purified inhibitor on the detergent-solubilized testicular sulphotransferase was determined (*a*) as a function of PAPS concentrations at fixed GG substrate (10 nmol) and (*b*) as a function of GG concentration at fixed PAPS concentration (0.2 nmol). In (*b*) the exogenous concentration of GG is given. Values for endogenous incorporation have not been subtracted. ●, Control; ○, 0.2 μg of inhibitor; □, 0.4 μg of inhibitor. Different preparations were used in (*a*) and (*b*).

(Lingwood, 1985) has shown that the zygotene spermatocyte is responsible for the onset of SGG synthesis. Thus, *in vitro*, SGG biosynthesis is maximal at about 20 days after birth, corresponding (Fig. 1) to the appearance of early primary spermatocytes. SGG synthesis is greatly decreased in the later stages, this decrease corresponding with the appearance of sulphotransferase inhibitory activity in the testicular homogenate supernatant. Measurement of the sulphotransferase activity of testicular homogenate from 22-day-old rats in the presence of the supernatant of the homogenate from other ages modulates the activity to mimic the changes observed during testicular development (Fig. 1). Thus the changes in specific activity of the homogenate during development may reflect the levels of endogenous inhibitor.

Although the testicular homogenate can sulphate a variety of galactolipids *in vitro*, the sulphation of the physiological substrate GG was preferentially decreased (Table 1). Thus the substrate specificity of the sulphotransferase was altered. Since the inhibitory activity is developmentally regulated (Fig. 1), the galactolipid substrate specificity of the homogenate sulphotransferase should also change during development. This was found to be the case (Fig. 2). Although the specific sulphotransferase activity is decreased during testicular development, the ability to sulphate galactosphingolipids is preferentially retained. Similar findings were obtained for metabolic labelling of sulphoglycolipids by intact spermatogenic cells *in vitro*. Although incorporation is greatly decreased for adult cells, radiolabelled species corresponding to SGC and SLC were detected as the major products, whereas SGG is the major product of cells from 20-day-old animals.

Sulphogalactolipids are also synthesized in the brain (Ishizuka *et al.*, 1978) and kidney (Lingwood *et al.*, 1981). Sulphotransferase preparations from rat testis, kidney and brain all sulphate exogenous GC and LC to SGC and SLC respectively. GG is sulphated by testis and kidney, but very poorly by brain. This suggests that the brain enzyme differs from the testis and kidney enzymes or that testis and kidney have at least two enzymes. Competition studies (Handa *et al.*, 1974; C. A. Lingwood, unpublished work) indicate that a single testis enzyme acts on GG, GC and LC. Since all three tissues sulphate GC *in vitro*, it was of interest to determine the effect of the testicular inhibitor on the sulphotransferase activity of these tissues. The inhibitor was effective at decreasing GC sulphation only for the testicular enzyme (Table 2), suggesting that testicular and kidney enzymes are also distinct.

The inhibitor shows tissue specificity [GC sulphation is inhibited for the testis enzyme but not for the kidney or brain enzyme (Table 2)] and substrate specificity [testicular sulphation of GG is inhibited preferentially (Table 1), and only GG sulphation is inhibited for the kidney enzyme (Table 2)]. It is therefore not clear whether the inhibitor binds to the enzyme protein, galactolipid substrate or both. To answer such questions, it is necessary to purify the inhibitor and sulphotransferase. A significant purification of the inhibitor has been achieved (Table 3). The purified preparation comprises carbohydrate containing primary amino species. Several species are found in this preparation (Fig. 4). The relationship between these components requires further investigations. Since the inhibitory activity was trypsin-sensitive, it is possible that the active species is a glycopeptide. Carbohydrate-containing species of similar mobility on t.l.c. were found to be deleted or decreased in a mutant rat that showed a deficiency in sulphotransferase-inhibitory activity [see Fig. 4 and the following paper (Lingwood *et al.*, 1985)]. The purified inhibitory preparation shows substrate selectivity (Table 4) similar to that of the Millipore filtrate of the adult testicular homogenate supernatant (Table 1), in that the sulphation of GG is preferentially decreased compared to that of GC. The dose response for the purified inhibitor is linear with the exception of a small stimulatory activity observed at very low concentrations. This may relate to the slight stimulation of sulphotransferase activity found for the testicular homogenate supernatant from young animals (Fig. 1). Thus stimulation-inhibition of galactolipid sulphotransferase activity may be dose-related phenomena. Initial kinetic studies indicate that the purified species is an uncompetitive inhibitor for GG (Fig. 5) and non-competitive (hyperbolic) for PAPS, suggesting a complex, perhaps allosteric, mechanism of action. Thus inhibition is independent of galactosylglycerolipid substrate concentration and cannot be a result of substrate breakdown during the assay. Inhibition does vary with PAPS concentration, being more marked at lower nucleotide concentrations (see also Table 1). At present the PAPS pool size within the Golgi *in vivo* is not known. Further studies to purify the sulphotransferase are needed. When this is done it will be possible to analyse the kinetics of interaction in detail. The data presented cannot as yet be used to infer the mechanism of sulphotransferase activity *in vivo*. They do, however, offer a plausible explanation for the changes in sulphotransferase activity *in vitro* observed during spermatogenesis. It is possible that the developmental regulation of sulphogalactolipid synthesis in the mammalian testis may be due to the appearance of a unique soluble factor capable of inhibiting galactolipid sulphation in a preferential manner.

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REFERENCES

Bradford, M. (1976) Anal. Biochem. **72**, 248–254
Handa, S., Yamato, K., Ishizuka, I., Suzuki, A. & Yamakawa, T. (1974) J. Biochem. (Tokyo) **75**, 77–83
Ishizuka, I., Suzuki, M. & Yamakawa, T. (1973) J. Biochem. (Tokyo) **73**, 77–86
Ishizuka, I., Inomata, M., Ueno, K. & Yamakawa, T. (1978) J. Biol. Chem. **253**, 898–907
Klugerman, A. & Kornblatt, M. J. (1980) Can. J. Biochem. **58**, 225–229
Knapp, A., Kornblatt, M. J., Schachter, H. & Murray, R. K. (1973) Biochem. Biophys. Res. Commun. **55**, 179–186
Kornblatt, M. J. (1979) Can. J. Biochem. **57**, 225–258
Kornblatt, M. J., Schachter, H. & Murray, R. K. (1972) Biochem. Biophys. Res. Commun. **48**, 1489–1494
Kornblatt, M. J., Knapp, A., Levine, M., Schachter, H. & Murray, R. K. (1974) Can. J. Biochem. **52**, 689–697

Laine, R. A., Stellner, K. & Hakomori, S. (1974) Methods Membr. Biol. **2**, 205–244
Letts, R. J., Hunt, R. C., Shirley, M. A., Pinteric, L. & Schachter, H. (1978) Biochim. Biophys. Acta **541**, 59–75
Lingwood, C. A. (1979) Can. J. Biochem. **57**, 1138–1143
Lingwood, C. A. (1985) J. Cell Sci. **75**, 1–10
Lingwood, C. A., Hay, G. & Schachter, H. (1981) Can. J. Biochem. **59**, 556–563
Lingwood, C. A., Murray, R. K. & Schachter, H. (1980) J. Immunol. **124**, 769–774
Lingwood, C. A., Sakac, D. & Vella, G. (1983) Carbohydr. Res. **122**, 1–9
Lingwood, C. A., Kunz, H. W. & Gill, T. J., III (1985) Biochem. J. **231**, 401–405
Murray, R. K., Narassimhan, R., Levine, M., Pinteric, L., Shirley, M. A., Lingwood, C. A. & Schachter, H. (1980) in Cell Surface Glycolipids (Sweeley, C. C., ed.), pp. 105–125, American Chemical Society Press, Washington
Schachter, H. (1980) Methods Enzymol. **41B**, 3–10
Shirley, M. A. & Schachter, H. (1980) Can. J. Biochem. **58**, 1230–1239
Tennekoon, G. I. & McKhann, G. M. (1978) J. Neurochem. **31**, 329–340
Tennekoon, G. I., Fragia, J., Aitchison, S. & Price, D. L. (1981) J. Cell Biol. **91**, 332–339

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