Enzymatic sulfation of glycochenodeoxycholic acid by tissue fractions from adult hamsters

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Abstract Using a radiometric assay with glycochenodeoxycholic acid as substrate, bile acid:3′-phosphoadenosine-5′-phosphosulfate sulfotransferase activity was found in 105,000 *g* supernatant fractions of liver, proximal intestine, and adrenal gland homogenates from adult hamsters. Optimum conditions for measurement of the hepatic enzyme were determined. In both male and female animals sulfation only occurred at the 7α-position. Saturation analysis with glycochenodeoxycholic acid revealed that the higher activity observed in fractions from female compared to male hamsters was due to a 4-fold lower apparent K_m (79 μM vs. 317 μM) for this bile acid in the females. The sulfation of glycochenodeoxycholic acid was competitively inhibited by glycolithocholic acid, chenodeoxycholic acid, and ursodeoxycholic acid. The data are consistent with the concept that sulfation of many, if not all, bile acids can occur in vivo.—**Barnes, S., P. G. Burhol, R. Zander, G. Haggstrom, R. L. Settine, and B. I. Hirschowitz.** Enzymatic sulfation of glycochenodeoxycholic acid by tissue fractions from adult hamsters. *J. Lipid Res.* 1979. **20:** 952–959.

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The importance of sulfation of lithocholic acid, as a means of preventing accumulation of this potentially toxic bile acid (1–3), arose from the pioneering work of Palmer (4) and Palmer and Bolt (5). An enzyme capable of catalyzing the transfer of sulfate from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to taurolithocholic acid (TLCA) in vitro has been detected in rat liver and kidney and has been partially purified (6, 7). Bile acid:PAPS:sulfotransferase (BAST) activity in liver soluble supernatant in vitro correlated well with the sulfation rate of TLCA recovered in the bile in rats (8), suggesting that the liver is the site of bile acid sulfate synthesis in normal physiology. Sulfation of bile acids in vitro is not restricted to the monohydroxy bile acids; in a test system with the renal BAST enzyme, dihydroxy bile acids at 100 μM were sulfated at one third of the rate observed with TLCA (7). Because the dihydroxy bile acids constitute a much greater proportion of the total bile acids than the monohydroxy bile acids, it may be postulated that substantial amounts of dihydroxy bile acid sulfates are synthesized in vivo. Sulfation of dihydroxy bile acids occurs in human cholestasis, where 80% of all the bile acid sulfates in urine are those of chenodeoxycholic acid (9–12). By analogy with TLCA, sulfation of dihydroxy bile acids would provide a potential regulatory control for their pool size and synthesis.

In this study BAST activity, using glycochenodeoxycholic acid (GCDC) as substrate, has been investigated in the hamster to determine *a*) its tissue and organ location, *b*) the optimum conditions for its measurement, and *c*) the type and quantitative inhibition between individual bile acids for sulfation. The hamster was chosen because it does not reduce the toxicity of bile acids by alternative mechanisms, i.e., by hydroxylation, as happens in the rat (13), and because after bile duct ligation large amounts (approximately equivalent to the initial pool size over a 3-day period) of bile acid sulfates are excreted in the urine (14).

MATERIALS

Sodium glycochenodeoxycholic acid and cholylglycine hydrolase (glycerol preparation, #C-3763) were

Abbreviations: BAST, bile acid:3′-phosphoadenosine-5′-phosphosulfate sulfotransferase; CDC, chenodeoxycholic acid; GCDC, glycochenodeoxycholic acid; TLCA, taurolithocholic acid; GLCA, glycolithocholic acid; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMS, trimethylsilyl; GLC–MS–DS, gas–liquid chromatography–mass spectrometry–data system. Trivial names: lithocholic acid, 3α-hydroxy-5β-cholanoate; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoate; ursodeoxycholic acid, 3α,7β-dihydroxy-5β-cholanoate.

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obtained from Sigma Chemical Co., St. Louis, MO. Glyco[11,12-$^3H_2$]chenodeoxycholic acid (40 mCi/μmol) was kindly donated by Dr. A. F. Hofmann, Department of Medicine, University of California, San Diego, CA. 3′-Phosphoadenosine-5′-phosphosulfate (PAPS) was purchased from P-L Biochemicals, Milwaukee, WI, and its concentration was determined from its absorbance at 260 nm in water, using the molar extinction coefficient of 14.7×10^3.

METHODS

Isolation of tissue fraction

Adult golden Syrian hamsters (Southern Animal Farms, Prattville, AL) weighing 100–120 g were killed and the organs were quickly excised and placed in ice-cold 0.25 M sucrose containing 5 mM sodium phosphate buffer, pH 7.0. After blotting dry and weighing, the organs were homogenized in 4 ml/g of ice-cold buffer by six strokes in a Potter-Elvehjem homogenizer with a Teflon pestle. Subcellular fractions were isolated by differential centrifugation at 1,000 *g* for 10 min (nuclei), 20,000 *g* for 20 min (mitochondria), and at 105,000 *g* for 60 min to obtain a microsomal pellet and a soluble supernatant fraction. Particulate fractions were resuspended in the homogenization medium and then recentrifuged. Each fraction was studied immediately after preparation. The soluble fraction was stored for further studies at −20°C.

BAST assay

The tissue homogenate fraction, equivalent to 0.5–0.8 mg of protein, was incubated at 37°C for 60 min in a final volume of 200 μl containing 10 μmol of sodium phosphate buffer, pH 7.0, 1 μmol of $MgCl_2$, 36 nmol of PAPS, and from 10 to 100 nmol of GCDC along with 0.15 μCi of glyco-[11,12-$^3H_2$]chenodeoxycholic acid. In experiments to determine whether other bile acids inhibited sulfation of GCDC, the concentration of added bile acid causing 50% inhibition of sulfation of GCDC (100 μM) was first determined. Then the assay was repeated (GCDC 50–500 μM) at this inhibitor concentration. Control incubations were performed for each fraction by deletion of the PAPS; deletion of PAPS and the tissue fraction were used to determine radiotracer purity. Methanol (0.8 ml) was added to terminate the reaction and to precipitate the protein. After centrifugation (1,500 *g* × 10 min) the pellet was re-extracted with methanol (0.8 ml) and recentrifuged. The supernatants were combined, evaporated to dryness, reconstituted in 100 μl of methanol–water 1:1 (v/v), and 20 μl was applied across a 13-mm track on a 20 × 20 cm, 0.25 mm thick, silica gel G plate (Rediplate, Fisher Chemical Co., Atlanta, GA). Standard markers of GCDC and its sulfates were run on both sides and the middle of each plate. The plates were developed for 15 cm in chloroform–methanol–acetic acid–water 65:24:15:6 (by vol) at 25°C. The standard lanes were sprayed with Usui's (15) reagent to locate the bile acids. The zones corresponding to glycochenodeoxycholate monosulfates (R_f 0.34, the 3- and 7-isomers do not separate) and glycochenodeoxycholic acid (R_f 0.91) were marked, and the silica was carefully scraped off into separate plastic scintillation vials. Methanol (0.5 ml) and scintillant (5.0 ml) NE-260, Nuclear Enterprises, Palo Alto, CA) were added to each vial and the radioactivity was measured in a Nuclear Chicago scintillation spectrometer. Protein pellets were dissolved in 1 M NaOH and quantitated by an automated protein procedure (16) using a bovine serum albumin standard.

Calculation of BAST activity

Enzyme activity expressed as pmol min^{-1} mg $protein^{-1}$ was determined from the PAPS-dependent conversion of glyco-[11,12-$^3H_2$]chenodeoxycholic acid to its sulfate. The fractional conversion at each concentration of GCDC was corrected for the small conversion observed both in the absence of PAPS alone and in the absence of PAPS and the tissue fraction; this was corrected to pmol by multiplying by the mass of GCDC present (in pmol). This amount was related to the total protein in the incubation and to the incubation time.

Identification of product of incubation with glycochenodeoxycholic acid

In preliminary experiments silica from tracks after TLC separation of the products of incubation was scraped off in 0.5-cm zones. Radioactivity, other than that corresponding to glycochenodeoxycholic acid, was found to be associated only with glycochenodeoxycholate monosulfate.

Further identification was achieved by extraction of the material corresponding to glycochenodeoxycholic acid monosulfate from the TLC plate. This was then hydrolyzed with cholylglycine hydrolase in 1 ml of 500 mM sodium phosphate buffer, pH 5.6, containing 10 mM mercaptoethanol for 8 hr at 37°C. Two ml of 1 M NaOH was added to terminate the reaction, and the mixture was extracted with XAD-7 resin (17). The extract was rerun in the same TLC system. The monosulfate fraction was eluted and solvolyzed in acetyl chloride in methanol (0.5%, v/v) at room temperature for 16 hr. The methyl ester thus

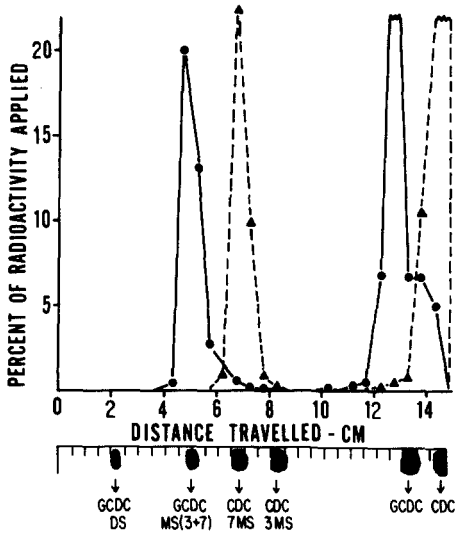

Fig. 1. Thin-layer chromatographic separation of the incubation products of hepatic BAST activity and glycochenodeoxycholic acid. In the upper part of the figure the solid line represents the radioactivity in the incubate and the dotted line is the radioactivity after hydrolysis with cholylglycine hydrolase. The solvent system was chloroform–methanol–acetic acid–water 65:24:15:6 (by vol.). Both samples were cochromatographed with standards to ensure correct identification. In the lower part of the figure the separation of glycochenodeoxycholate-3,7-disulfate, glycochenodeoxycholate monosulfates (3- and 7-isomers), chenodeoxycholate-7-monosulfate, chenodeoxycholate-3-monosulfate, glycochenodeoxycholate, and chenodeoxycholate is shown.

formed was converted to its trimethylsilyl (TMS) ether by 100 μl of pyridine–1,1,1,3,3,3-hexamethyldisilazane–chlorotrimethylsilane 4:2:1 (by vol). It was analyzed, after extraction into hexane, by capillary gas–liquid chromatography on a 30 m, 0.2 mm i.d. SP-2250 column (SGE, Houston, TX) in a Hewlett Packard HP-5831 gas chromatograph. The chromatographic conditions were oven temperature 250°C, injector temperature 270°C, detector temperature 300°C, helium flow rate 2 ml min^{-1}, nitrogen make-up gas 30 ml min^{-1}, hydrogen 30 ml min^{-1}, and air 250 ml min^{-1}. A 10:1 split ratio was used in the injector. Gas–liquid chromatography–mass spectrometry was performed on the derivatives using a 15-m SP-2250 glass capillary column mounted in a Hewlett Packard HP-5985 GLC–MS–DS instrument.

Synthesis of chenodeoxycholic acid sulfates

The 3-monosulfate ester was prepared via the 7-formate ester of chenodeoxycholic acid (18) and the 7-monosulfate ester was prepared via the 3-hemisuccinate ester of chenodeoxycholic acid (19). Glycochenodeoxycholic acid-7-monosulfate was prepared by reaction between equimolar amounts of GCDC and sulfuric acid, the reaction being caused by dicyclohexylcarbodiimide (10 M excess) in dry dimethylformamide; to prepare glycochenodeoxycholic acid-3,7-disulfate, a 3-fold excess of sulfuric acid (20, 21) was used.

Determination of kinetic parameters

The apparent maximum rate (V_{max}) and the concentration(s) at half V_{max}, i.e., K_m, were calculated from a linear transformation (22) of the Michaelis-Menten equation

$$V = -K_m{*}V/S + V_{max}$$

by least-mean-square regression analysis. The values calculated are reported as the means and their standard errors. An unpaired Student t test was used to test for significance of difference between enzyme activities at individual GCDC concentrations.

Competitive inhibition of GCDC sulfation by other bile acids was tested for by comparision of the V_{max} in the presence and absence of the inhibitor. The inhibition constant (K_i) was then determined in the following double reciprocal plot (23):

$$\frac{1}{V} = \frac{K_m\left(1 + \frac{[I]}{K_i}\right)}{V_{max}}\left(\frac{1}{S}\right) + \frac{1}{V_{max}}$$

where $[I]$ is the fixed inhibitor concentration and K_i is the inhibitor constant.

Competitive inhibition is characterized by an unchanged V_{max}. This was tested for each of the bile acids and subsequently a pooled estimate of the intercept was used to calculate V_{max}. The inhibitor constant K_i and its asymptotic standard error were determined using statistical differentials (24).

RESULTS

Product of the enzymic reaction

The product of sulfation of glycochenodeoxycholic acid by each of the tissue supernatant fractions having activity had the TLC mobility of glycochenodeoxycholic acid-monosulfate, no disulfate being found (**Fig. 1**). Hydrolysis with cholylglycine hydrolase gave a product with the mobility of chenodeoxycholic acid-7-monosulfate (Fig. 1) for BAST activity from both male and female hamsters. Solvolysis yielded a bile acid methyl ester which on silylation eluted on GLC with the same retention time as chenodeoxycholic acid methyl ester TMS ether. This compound, when analyzed by GLC–MS, gave major fragments at *m/e* 255 ($M^+ - 2 \times 90 + 115$), 355 ($M^+ - 2 \times 90 + 15$),

and 370 ($M^+ - 2 \times 90$) which were identical with and in similar proportions to those of the chenodeoxycholic acid methyl ester trimethylsilyl derivative. On the basis of this comparison with known spectra, we have confirmed that the compound is the methyl ester trimethylsilyl ether of chenodeoxycholic acid.

Location of enzyme activity

BAST activity was found only in the supernatant (105,000 *g*) fractions of liver, proximal intestine, and adrenal glands. Unlike the rat (7), no activity was detected in the kidney homogenate fraction. No activity was found in brain, heart, spleen, skeletal muscle, distal small intestine, or the large intestine. When expressed per mg protein, the greatest enzyme activity was found in the liver, with a significantly higher activity in female than in male hamsters ($P < 0.001$). The intestinal activity was found consistently in male animals but in only 5 of 18 females, and was confined to the proximal third of the small intestine (**Table 1**).

Optimization of conditions for hepatic enzyme

No sex differences in the conditions for optimum enzyme activity were found, except for the concentration of GCDC (see below).

The effect of pH was measured over the pH range 6.0–8.0 and the pH optimum was found to be 7.0 in 50 mM sodium phosphate buffer containing 5 mM $MgCl_2$, 180 μM PAPS, and 100 μM GCDC (**Fig. 2*A***). In the same assay system at pH 7.0 the PAPS concentration was varied between 22.5 and 540 μM. The peak activity was observed at 180 μM PAPS; above that concentration, inhibition occurred (Fig. 2*B*). The enzyme activity required $MgCl_2$ and reached a maximum at 2.5 mM $MgCl_2$; at 0.5 mM the activity was one sixth of the maximum (Fig. 2*C*). Variation of ionic strength in sodium phosphate buffer, pH 7.0, over the range 25–125 mM did not alter the activity (Fig. 2*D*).

The reaction rate ($pmol \cdot min^{-1}$) was directly related to the amount of the soluble fraction added over a range of total protein from 0.1 to 0.8 mg; above 0.8 mg nonlinearity occurred (**Fig. 3*A***). Even in fractions with the highest activity (female animals) the reaction rate was linear for at least 90 min (Fig. 3B).

The reproducibility of the assay was assessed on female liver soluble fraction at 100 μM GCDC. No significant difference was observed between measurements made on two separate TLC plates (71.6 ± 2.2 vs. 72.2 ± 1.6 $pmol \cdot min^{-1} \cdot mg\ protein^{-1}$). Hepatic and intestinal soluble fractions, which were stored at −20°C, showed no loss of activity over a 6 month period.

TABLE 1. Bile acid sulfotransferase activity (mean ± SEM) in 105,000 *g* supernatant fraction from hamster tissue

Tissue	Sex	Activity
		pmol min^{-1} mg protein^{-1}
Liver	Female (4)[a]	61.8 ± 5.1
	Male (6)	16.5 ± 2.1
Proximal small intestine	Male (6)	4.7 ± 0.3
	Female (5)	3.8 ± 0.5[b]
Adrenals	(3)	2.6 ± 0.7

[a] Number of animals in parentheses.

[b] 18 female animals were studied. Only 5 had measurable activity, as shown.

Tissue homogenate fractions were incubated at 37°C for 60 min in a total volume of 200 μl containing 10 μmol of sodium phosphate buffer, pH 7.0, 1 μmol of $MgCl_2$, 36 nmol of PAPS, 10 nmol of GCDC, and 0.15 μCi of glyco-[11,12-$^3H_2$]chenodeoxycholic acid; the products were assayed by TLC as described in Methods. No enzyme activity was found in any of the particulate fractions. No activity was found in the brain, heart, spleen, kidney, skeletal muscle, distal small bowel, or large bowel soluble fractions.

Effect of increasing glycochenodeoxycholic acid concentration

Over the range 50–500 μM GCDC each fraction studied showed a saturable response (**Fig. 4**). Analysis of the data using a V against V/S plot showed that highly significant correlations ($r > 0.95$, $P < 0.05$) could be obtained. A marked difference was observed between male and female hamsters. The apparent K_m for the female animals (79.0 ± 7.3 μM, mean ± SEM) was one quarter of that in male animals (317 ± 47 μM). There was also a significant difference ($P < 0.001$) in the calculated V_{max}; in females the apparent maximal velocity was one third greater than in males (**Table 2**). The factor $V^*(V_{max}/K_m)$, the apparent rate constant for concentrations well below the K_m, i.e., close to normal physiological concentrations, was six times higher in the females (1.42 ± 0.13, mean ± SEM) than in males (0.23 ± 0.03).

Inhibition of sulfation by other bile acids

Because other workers (6, 7) have studied sulfation of monohydroxy bile acids, it was pertinent to this study to determine the type of inhibition of GCDC sulfation by these and other bile acids. Preliminary studies revealed that glycolithocholate (40 μM) caused 50% inhibition of enzyme activity from female hamsters at a GCDC concentration of 100 μM. For chenodeoxycholate and ursodeoxycholate 50% inhibition occurred at an approximate concentration of 100 μM of these bile acids. Statistical analysis of saturation curves for GCDC performed in the presence and absence of these bile acid inhibitor concentrations then showed that the inhibition was competitive because the estimated intercepts in the double reciprocal plot were not significantly different

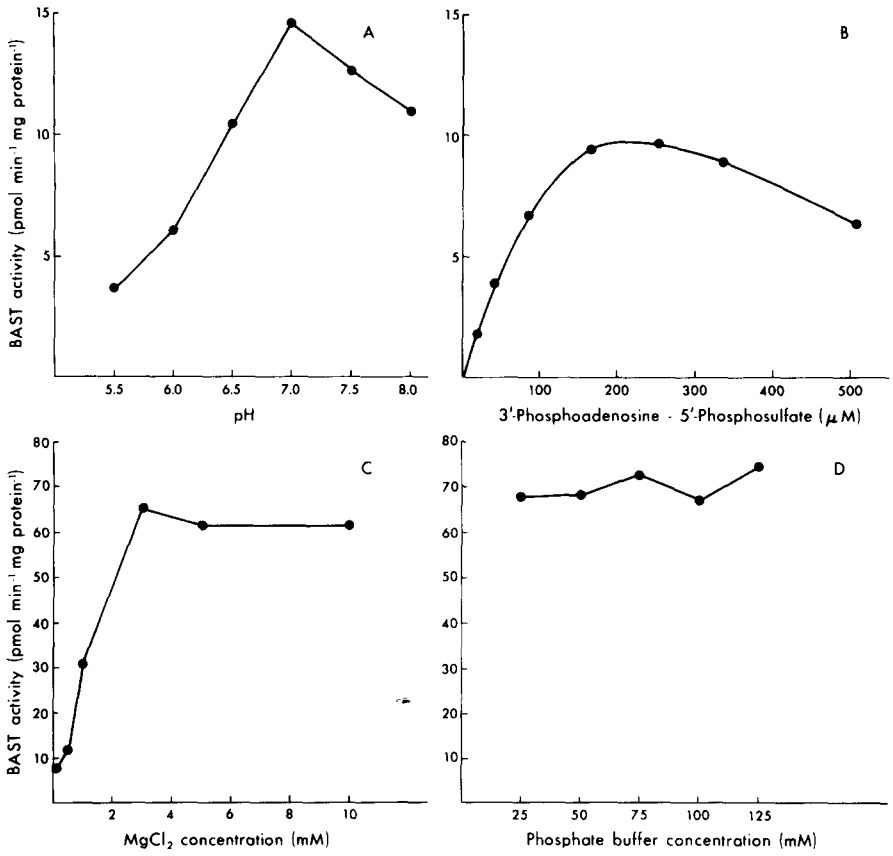

Fig. 2. Dependence of BAST activity on pH (*A*), PAPS (*B*), Mg^{2+} (*C*), and buffer concentration (*D*). Aliquots of hamster liver 105,000 *g* supernatant were assayed in a final volume of 200 μl. Except where stated each tube contained 10 μmol of sodium phosphate buffer, pH 7.0, 1 μmol of $MgCl_2$, 36 nmol of PAPS, 20 nmol of GCDC, and 0.15 μCi of glyco-[11,12-$^3H_2$]chenodeoxycholic acid. Tubes were incubated for 60 min at 37°C. Each datum point was the mean of two separate determinations. The data in the upper two figures (*A* and *B*) were obtained using supernatants from male hamsters, and those in the lower figures (*C* and *D*) were from female hamsters.

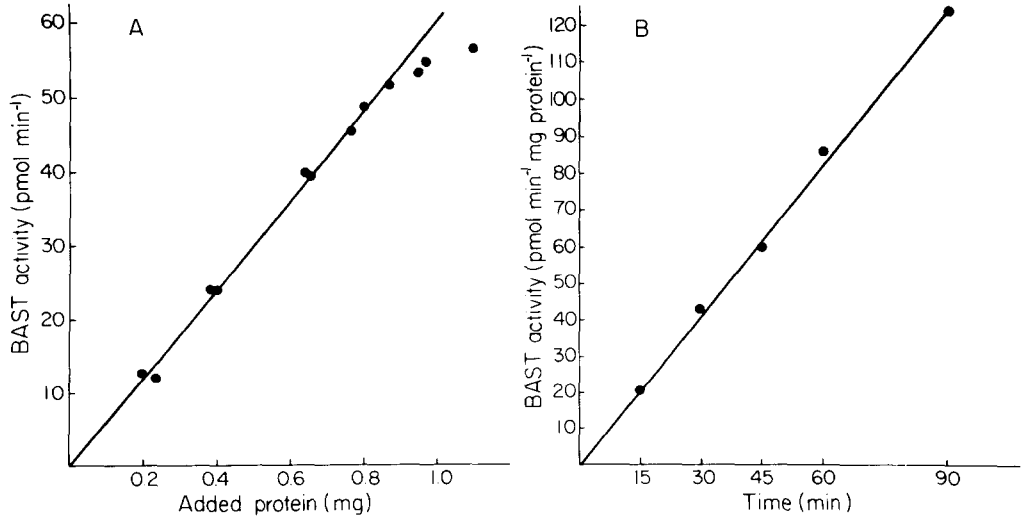

Fig. 3. Linearity of the assay with respect to added protein (*A*) and time (*B*). Aliquots of female hamster liver 105,000 *g* supernatant were assayed in a final volume of 20 μl, containing 10 μl of sodium phosphate buffer, pH 7.0, 1 μmol of $MgCl_2$, 36 nmol of PAPS, 20 nmol of GCDC, and 0.15 μCi of glyco-[11,12-$^3H_2$]chenodeoxycholic acid. Each datum point was the mean of two separate determinations.

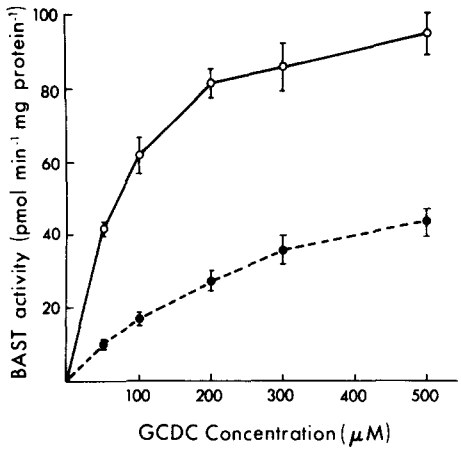

Fig. 4. Effect of GCDC concentration on BAST activity. The GCDC concentration was varied between 50 and 500 μmol. Aliquots of male (●) and female (○) hamster liver 105,000 *g* supernatant were incubated in a total volume of 200 μl, containing 10 μmol of sodium phosphate buffer, pH 7.0, 1 μmol of $MgCl_2$, 36 nmol of PAPS, and 0.15 μCi of glyco-[11,12-$^3H_2$]chenodeoxycholic acid as described in Methods. The data are the mean ± SEM of values obtained from six male and four female hamsters.

($P = 0.275$) (**Fig. 5**). The inhibitor constants were then calculated to be 18 ± 3 μM (constant ± SEM) for glycolithocholate, 71 ± 12 μM for chenodeoxycholate, and 83 ± 15 μM for ursodeoxycholate.

DISCUSSION

These studies have shown that, using a radiometric in vitro assay, there is bile acid:3′-phosphoadenosine-5′-phosphosulfate sulfotransferase activity in the soluble fraction of hamster liver homogenates. Lesser activities were found in the proximal small intestine and the adrenals, whereas none was found in the kidney. Thus the hamster is different from the rat (7) but similar to man (25). The absence of the kidney activity is consistent with the finding that thioacetamide, a specific hepatotoxin, markedly reduced the urinary excretion of bile acid sulfates in the bile duct-ligated hamster (14). BAST activity in the adrenals and the small intestine has not been reported before, although Rachmilewitz and Saunders (26) observed an unidentified polar metabolite of chenodeoxycholic acid in incubates of rat jejunum with a TLC mobility similar to chenodeoxycholic acid-7-monosulfate. The distribution of this enzyme activity is thus similar to that of the steroid sulfotransferases which, like BAST, are also soluble enzymes (27).

The product of sulfation of GCDC by hamster liver soluble fractions has been shown to be the monosulfate on the basis of TLC of it and its deconjugated product. GLC–MS analysis of the deconjugated, solvolyzed product indicated that CDC was not further metabolized during incubation. Studies using the isolated rat kidney (19) and BAST activity obtained from rat kidney (7) have found that it is the 7-monosulfate which is formed. The present study confirms that it is the 7-monosulfate, although a more specific method than TLC separation would be preferable. In cholestatic man, the main urinary sulfate is glycochenodeoxycholic acid-monosulfate (28). No 3,7-disulfate of glycochenodeoxycholic acid was formed by any of the soluble fractions studied. Whether the disulfate of chenodeoxycholic acid conjugates is really present in biological fluids is yet to be confirmed. Using Sephadex LH-20 columns, workers have isolated bile acid "disulfate" fractions (28), but these may have arisen from incomplete elution of the monosulfate band since "ghosting" is a problem using Sephadex LH-20. If the disulfate is a biological metabolite, then it is possible that other yet-undefined reaction conditions for this enzyme have to be determined or that a second bile acid sulfotransferase remains to be found.

TABLE 2. Apparent kinetic parameters (mean ± SEM) of hepatic bile acid sulfotransferase

Sex	Apparent V_{max}	Apparent K_m
	pmol min⁻¹ mg protein⁻¹	*μM*
Female (4)	109.7 ± 5.0	79.0 ± 7.3
Male (6)	68.2 ± 4.8[a]	316.6 ± 46.9[a]

[a] Significantly different from females ($P < 0.001$), unpaired Student *t* test.

These parameters were determined for each soluble fraction by linear regression analysis using the equation $V = -V/S*K_m + V_{max}$, where V is the observed activity at each S concentration.

The use of glyco-[11,12-$^3H_2$]chenodeoxycholic acid with nonradioactive PAPS has several advantages

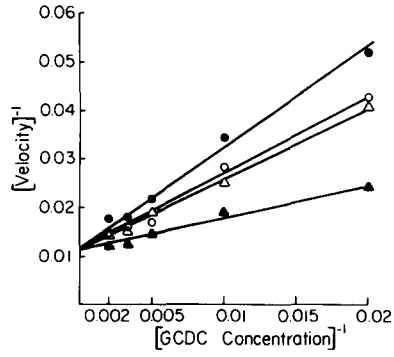

Fig. 5. Demonstration of competitive inhibition of sulfation of GCDC by BAST. BAST activity on GCDC in the presence (●, glycolithocholic acid; ○, chenodeoxycholic acid; △, ursodeoxycholic acid) and absence (▲) of added bile acids was analyzed using a double reciprocal plot. The least-mean-square regression lines for each inhibitor passed through the y-axis at the same point as that for the control (GCDC alone).

over the use of ^{35}S-labeled PAPS and TLCA (6, 7). GCDC is freely water soluble, unlike TLCA which is insoluble above 100 μM at pH 7.0. Labeling of non-bile acid substrates (steroids, phenols, etc.) with ^{35}S-labeled PAPS can be avoided by the use of labeled bile acid. The high specific radioactivity of the tracer (40 mCi/μM) enables the concentration of the unlabeled GCDC to be varied as required (subject to the limitation of the tissue concentration of GCDC). The only disadvantage of this approach is that a separate radiolabeled bile acid tracer is required for the study of the sulfation of other bile acids. Since monohydroxy bile acids are not found in the blood in concentrations above 5 μM even in cholestasis (9–12), GCDC would appear to be the more relevant substrate.

The conditions for optimum activity of the hamster hepatic BAST activity were similar but not identical to those reported for the partially purified hepatic and renal BAST enzymes from the rat (6, 7). In the hamster the pH optimum was at pH 7.0 as opposed to pH 6.5 in rats (6, 7). In the hamster Mg^{2+} was essential and had an optimum at 2.5 mM as opposed to 0.5 mM in rats (6, 7). The use of 0.5 mM $MgCl_2$ would have underestimated BAST activity by a factor of six. The optimal concentration of PAPS for the hamster hepatic enzyme was 180 μM, which is similar to that reported for non-bile acid sulfotransferases (29–31). The apparent K_m for PAPS could not be directly calculated, but was clearly much greater than that observed in purified enzyme preparation from rat liver, 1 μM (6), and rat kidney, 8 μM (7), or for cytosolic preparations from human liver (25).

Hepatic cytosolic enzyme activity in female hamsters measured with GCDC (100 μM) as substrate, 60 pmol·min^{-1}·mg protein^{-1}, is 2.5-fold greater than that measured with TLCA in rats (12), suggesting that either the assay is inherently more sensitive because of the concentration of Mg^{2+} used in this study, or that the hamster has a greater enzyme activity than the rat. The rate of sulfation of GCDC with respect to the GCDC concentration showed saturation kinetics; analysis of each curve revealed that the apparent K_m was much lower in female animals (79 μM) than in males (317 μM) although it was higher than that reported for TLCA (50 μM) with partially purified BAST (6, 7). The apparent V_{max} was only 30% higher in females. Since the BAST activity was measured in the unpurified cytosol, it is possible that multiple BAST enzymes occur, possibly with different substrate specificities. This emphasizes the need for a specific method for identification and quantitation of the monosulfate isomers. These studies using GCDC as substrate are consistent with the observed sex difference in the enzymatic sulfation of TLCA in vitro and in vivo by rat liver (8). Much larger amounts of fecal bile acid sulfates in females compared to males have also been found in rats and mice (32) and this raises the possibility of estrogen control of bile acid sulfation. Increased sulfation of bile acids by administration of ethinyl estradiol (33) and of TLCA by estradiol propionate after oophrectomy (8) has been reported in rats in vivo.

Inhibition by 3β-hydroxy-5β-cholanoate of sulfation of TLCA by rat kidney BAST activity has been observed (7). In this study it has been shown that each bile acid tested competitively inhibited sulfation of GCDC by BAST from female hamster liver. Glycolithocholate had an apparent K_i of 18 μM which is one quarter of the K_m of GCDC (79 μM), those of chenodeoxycholate (71 μM) and ursodeoxycholate (83 μM) being similar to the K_m of GCDC. The latter result suggests that there is little difference between the ability of the 7α and 7β epimers to occupy the active site of the enzyme and that conjugation was not important. Despite the greater affinity for glycolithocholic acid, the hepatic GCDC concentration ought to substantially exceed that of glycolithocholate, which makes it possible that sulfation of GCDC occurs in vivo. If the K_i for glycolithocholate is the same in males as in females (this was not studied), then in vivo sulfation of GCDC may show sex differences.

These studies do not in themselves prove that in vivo sulfation of dihydroxy bile acids occurs in health. Indeed, proof is not straightforward. If it is assumed that any sulfates formed are not recovered by intestinal absorption (3, 34), then the proportion of dihydroxy bile acid sulfates in bile to account for daily turnover of dihydroxy bile acids (about 20% of the pool) need only be 2% of the total dihydroxy bile acids. Such a small proportion is hard to detect analytically because of uncertainty in existing techniques. Confirmation of the presence of dihydroxy bile acid sulfates in vivo in normal hamsters awaits further studies.

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