ELSEVIER

Comparative Biochemistry and Physiology Part B 121 (1998) 299–307

CBP

5β-Scymnol sulfotransferase isolated from the tissues of an Australian shark species

Nicole E. Pettigrew [a], Paul F.A. Wright [b], Theodore A. Macrides [a,*]

[a] *Natural Products Unit, Department of Medical Laboratory Science, RMIT-University, GPO Box 2476V, Melbourne, Victoria 3001, Australia*
[b] *Key Centre for Applied and Nutritional Toxicology, Department of Medical Laboratory Science, RMIT-University, Melbourne, Victoria, Australia*

Received 5 January 1998; accepted 3 September 1998

Abstract

The enzyme system involved in the sulfation of 5β-scymnol [(24*R*)-5β-cholestane-3α,7α,12α,24,26,27-hexol] has been investigated in the shark species *Heterodontus portusjacksoni*. The liver enzyme was partially purified by column chromatography and chemically characterized. In its partially purified form the enzyme showed typical Michaelis–Menten kinetics for 5β-scymnol and the sulfonate donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) with an apparent K_m of 3.04 μM for 5β-scymnol and 4.35 μM for PAPS. The reaction product adenosine-3′,5′-diphosphate and the substrates tested all inhibited the liver enzyme at concentrations above 10 μM. Substrate specificity of the enzyme was investigated using 5α-cyprinol, dehydroepiandrosterone, 17β-estradiol and testosterone. Only 5α-cyprinol was as efficiently sulfated as 5β-scymnol and suggests that the presence of the chiral alcohol group at C-24 is not essential for binding of the C_{27} bile steroids in the active site of the enzyme. A survey of tissue cytosolic fractions revealed that sterol sulfotransferase activity was present in the liver, kidney and testis; however, it was absent from the spleen, pancreas, brain, duodenum, heart and ovary.

Keywords: Bile salt; Elasmobranch; *Heterodontus portusjacksoni*; 3′-Phosphoadenosine 5′-phosphosulfate; 5β-Scymnol; Sulfotransferase

1. Introduction

Sulfation is catalyzed by a family of enzymes, the sulfotransferases (EC 2.8.2), which possess varying distribution, function and degree of specificity. Sulfotransferases transfer the sulfonate group of the high energy compound 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to several different compounds increasing their water solubility and facilitating excretion. Compounds that are sulfated include bile acids, steroids, phenols, aromatic amines, proteins, lipids, neurotransmitters, and many drugs and xenobiotics [13,17,18,37].

The major bile salts found in 'primitive' vertebrates are sulfated bile alcohols, in contrast to the taurine or glycine conjugated bile acids of higher vertebrates. The chondrichthyes (sharks, rays and chimeras) are considered to be one such class of primitive vertebrates, and have sulfated C_{27} bile alcohols in their bile [15]. Scymnol and chimaerol (which lacks the C-27 alcohol moiety of scymnol) have not been found in any other vertebrate group, which supports the close relationship between the chondrichthyes group and also the phylogenic distance between chondrichthyes and other vertebrates. The elasmobranchs (sharks and rays) have 5β-scymnol sulfate as the predominant bile salt [15].

Our interest in 5β-scymnol and its sulfation stems from the therapeutic potential of these natural marine products. Crude shark bile (containing 5β-scymnol sulfate) has been used in traditional Japanese and Chinese

Abbreviations: DHEA, dehydroepiandrosterone; PAP, adenosine-3′,5′-diphosphate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PAP[^{35}S], 3′-phosphoadenosine 5′-phospho[^{35}S]sulfate.

* Corresponding author. Tel.: +61 3 99252112; fax: +61 3 99253015; e-mail: macrides@rmit.edu.au

0305-0491/98/$ - see front matter
PII S0305-0491(98)10103-7

folk medicine as a treatment for liver disease and for skin aliments [19]. 5β-Scymnol has potent antioxidant and free radical scavenging properties, which are thought to be conferred by the unusual trialcohol-substituted aliphatic side-chain [27]. In addition, 5β-scymnol can significantly lower blood ethanol levels, and protects the mouse liver against acetaminophen (paracetamol) overdoses, carbon tetrachloride exposure and the mushroom toxin α-amanitin [8,26,35,39]. 5β-Scymnol has also been shown to competitively inhibit the transport of the common bile acids, cholic and taurocholic acids, in isolated rat hepatocytes [30]. As rat liver homogenates do not catalyze the sulfation of 5β-scymnol, further investigation of elasmobranch sulfotransferases is important in order to identify candidate enzymes that can be utilized for the commercial production of 5β-scymnol sulfate in bacterial expression systems.

Knowledge of the catalytic nature of bile alcohol or sterol sulfotransferases in lower vertebrates is limited, with only one elasmobranch and two amphibian studies previously described [6,25,33]. In 1957 Bridgwater and Ryan [6] found that the liver homogenate obtained from frogs (*Rana temporaria*) could sulfate ranol (the natural frog bile alcohol), 5β-scymnol, 3α,7α,12α,24-tetrahydroxycholane and cholan-24-ol. Later, Scully et al. [33] found that a liver homogenate from the toad *Xenopus laevis* enzymically sulfated the bile alcohols 5β-scymnol and 5α-cyprinol, as well as the simple aliphatic alcohols propan-1-ol and butan-1-ol. No attempt to purify or characterize the sulfotransferase was made in either of the studies.

More recently we reported preliminary investigations of the liver sterol sulfotransferase from the shark species *Heterodontus portusjacksoni* [25]. *H. portusjacksoni* (Meyer, 1793) is commonly called the Port Jackson shark and belongs to the Heterodontidae family, which are considered primitive, due to their similarity to fossils found in rocks of the Carboniferous age 350 million years ago [38]. *H. portusjacksoni* are distributed along the southern edge of Australia from Queensland to Western Australia, including Tasmania and New Zealand [22].

In this study we determined the tissue distribution of sterol sulfotransferase activity in an Australian shark species and performed partial purification and characterization of the liver enzyme.

2. Materials and methods

2.1. Preparation of cytosolic fractions

The *H. portusjacksoni* sharks (two adult females, two juvenile females and one adult male) were caught in Port Phillip Bay, Victoria, Australia. The specimens were anaesthetized (MS-222, 150 mg l^{-1}; Sandoz Ltd., Basle, Switzerland) then killed.

The organs (liver, kidney, spleen, pancreas, duodenum, heart and gonads) were excised, weighed and chilled on ice. All tissue and sample processing was conducted at 4°C. The homogenization buffer (5 mM Tris–HCl, 250 mM sucrose, 1 mM EDTA, 10 mM mercaptoethanol, pH 7.5) was added to the tissues in a 1:3 (w/v) ratio [10] and homogenized by two 20-s bursts of a Ystral T1500 homogenizer (Ystral, Germany). The homogenate was then centrifuged at 4°C, $100000 \times g$ for 60 min. The cytosolic fraction (supernatant) was then removed and stored at -80°C.

The marker enzyme NADPH cytochrome *c* reductase (endoplasmic reticulum) was assayed by the method of Sottocasa et al. [34] and was not found to contaminate the cytosolic fraction.

2.2. Enzyme assay

The sulfotransferase assay method is a modified version of the procedure by Macrides et al. [25]. The assay mix consisted of 200 μl of organ cytosolic fraction, 10 μl of 1.10 mM 5β-scymnol (provided by the Natural Products Unit, RMIT-University, Melbourne, Australia), 10 μl of 1.10 mM PAPS (in 50 mM sodium phosphate buffer, pH 6.5) and 0.5 μl of 3′-phosphoadenosine 5′-phospho[^{35}S]sulfate (PAP[^{35}S], specific activity 2.34 Ci $mmol^{-1}$, at a concentration of 0.451 μmol ml^{-1}; Dupont/NEN® Research Products, Wilmington, DE, USA). The assay mixture was incubated at room temperature for 20 min, then terminated by the addition of 200 μl of methanol. The assay mix was evaporated to dryness under vacuum, dissolved in 200 μl of methanol and applied to a $100 \times 200 \times 0.5$ mm silica gel thin-layer chromatography (TLC) plate. The TLC plate was developed twice using a solvent system of chloroform–methanol–water (70:30:5). The standard, 5β-scymnol sulfate, was visualized by spraying the plate with phosphomolybdic acid spray and heating at 100°C for 5 min [36]. The R_f values for PAPS, 5β-scymnol sulfate and 5β-scymnol were 0, 0.27 and 0.77, respectively. The radiolabeled 5β-scymnol sulfate was scraped off the TLC plate and dissolved in 10 ml of scintillant (Starscint™; Packard, Meriden, CT, USA). The amount of radioactivity was measured as counts per minute using a liquid scintillation counter. To determine the percentage incorporation in the assay, 0.5 μl PAP[^{35}S] was counted (as a standard that is a representative of 100% incorporation of radiolabel) for comparison. The reaction was linear with respect to time (0–20 min) and protein concentration (0–3 mg ml^{-1}).

This method was used to assay enzyme activity over a range of substrate concentrations (0, 0.5, 1, 1.5, 2.5, 5, 10, 20, 40, 60 and 80 μM for 5β-scymnol and PAPS)

and temperatures. The enzyme was further characterized by the addition of inhibitors (adenosine-3′,5′-diphosphate, sodium azide, iodoacetic acid, *p*-chloromercuribenzoic acid and EDTA), the effect of magnesium chloride (5 or 50 mM) and for its substrate specificity (5β-scymnol, 5α-cyprinol, dehydroepiandrosterone, testosterone and β-estradiol).

2.3. Purification procedures

The shark liver sulfotransferase was partially purified by sequential column chromatography. The *H. portusjacksoni* liver cytosolic fraction (75–100 mg of protein) was initially loaded onto a Macro-Prep® ceramic hydroxyapatite (Bio-Rad Laboratories, Hercules, CA, USA) adsorption chromatography column (2×10 cm) that was packed and equilibrated with buffer A (10 mM potassium phosphate, 250 mM sucrose, 1 mM EDTA, 3 mM mercaptoethanol, pH 6.8). Protein was eluted from the column with a linear gradient established between buffer A and buffer B (as for buffer A except 700 mM potassium phosphate). Active fractions were pooled and concentrated between columns with an Amicon ultrafiltration unit (Amicon Inc., Beverly, MA, USA) using a XM-50 ultrafilter (50-kDa cut-off). The active sample was then loaded onto a DEAE-Sephacel (Sigma Chemical Co., St. Louis, MO, USA) ion-exchange chromatography column (1.5×10 cm) equilibrated in buffer C (10 mM Tris–HCl, 250 mM sucrose, 1 mM EDTA, 3 mM mercaptoethanol, pH 7.5). Bound protein was eluted from the column using a linear gradient of buffer C and buffer C containing 1 M NaCl. Sephadex G-100 gel filtration chromatography was used as the final purification step, with columns (2×60 cm; Pharmacia, Uppsala, Sweden) swollen and eluted with buffer D (50 mM sodium phosphate, 250 mM sucrose, 2 mM EDTA, 3 mM mercaptoethanol, pH 6.5). The void volume was estimated with Dextran blue (2000 kDa) and the molecular weight of the enzyme was determined using the method of Andrews [1].

Protein concentration was determined by a modified version of the Lowry method [29] with bovine serum albumin as the standard.

3. Results

3.1. Tissue distribution of sulfotransferase activity

The cytosolic fraction of the liver, kidney and testis exhibited sulfotransferase activity towards 5β-scymnol; however, little or no activity was found in the spleen, pancreas, brain, duodenum, heart or ovary (Fig. 1). The liver was found to have the highest capacity to synthesize 5β-scymnol sulfate (Fig. 2).

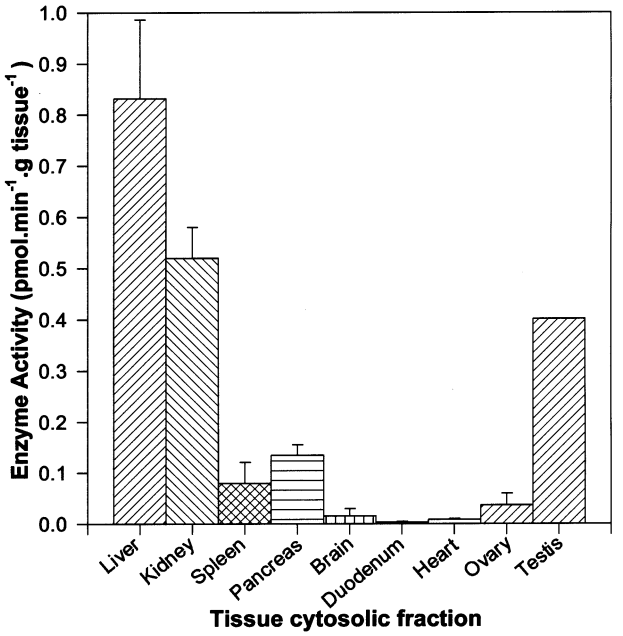

Fig. 1. Sulfotransferase activity in the cytosolic fractions of the tissues from *H. portusjacksoni*. The amount of enzyme activity per gram of tissue was determined for each tissue in duplicate for each of five specimens and is presented as the mean ± S.E.M.

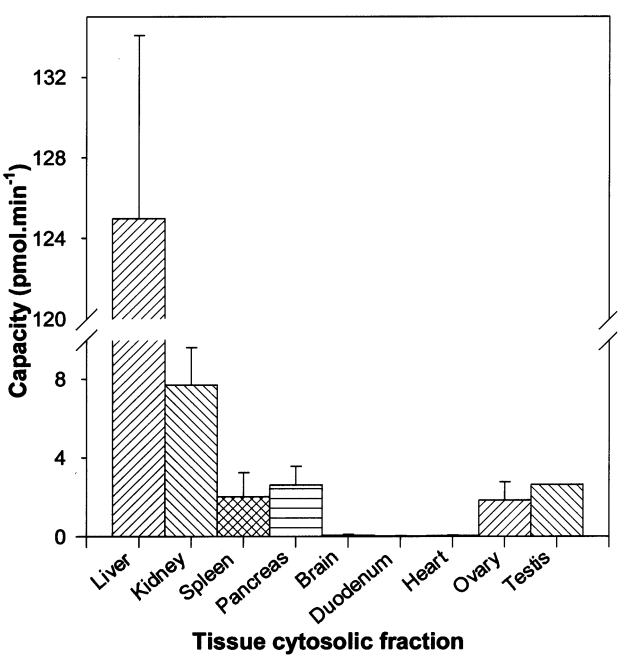

Fig. 2. Capacity of tissue cytosolic fractions obtained from *H. portusjacksoni* to sulfate 5β-scymnol. The assay reactions were performed in duplicate on tissues obtained from five shark specimens. The average activity (pmol min^{-1} g^{-1} tissue) multiplied by the total weight of the tissue (g) gave the capacity of each tissue to sulfate 5β-scymnol.

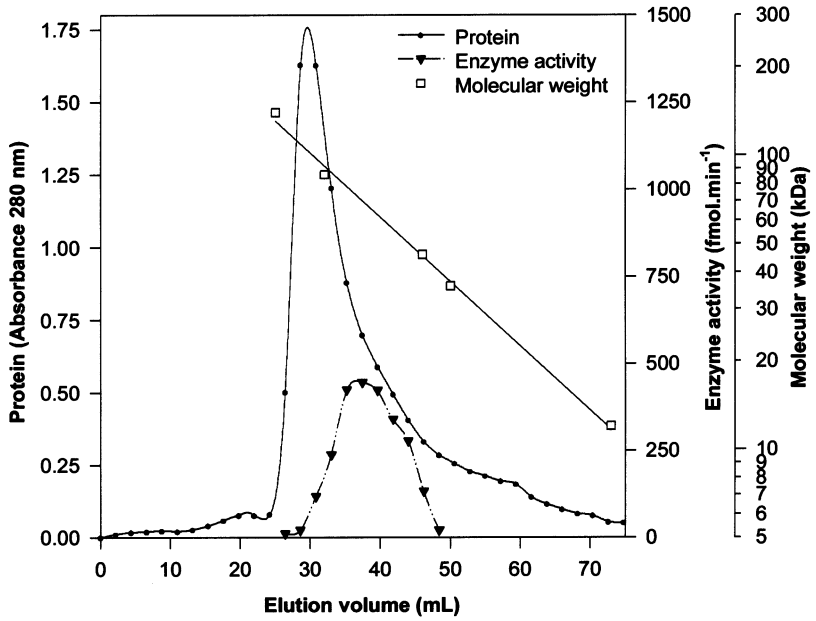

Fig. 3. Elution profile of the *H. portusjacksoni* liver from a Sephadex G-100 column. The final stage of purification of the liver sulfotransferase was a gel filtration column (2 × 50 cm). Fractions (2.2 ml) were collected at 10-min intervals with protein elution measured by absorbance at 280 nm. The native molecular weight was estimated from a standard curve (overlaid on the graph) to be 84 ± 2.3 kDa for the liver enzyme (standards eluted: lactate dehydrogenase, 140 kDa with 36.5 kDa subunits; bovine serum albumin 82 kDa; ovalbumin 49 kDa; cytochrome *c* 12.4 kDa).

3.2. Isolation of the liver sulfotransferase

The method that partially purified the sterol sulfotransferase from *H. portusjacksoni* liver tissue was a combination of three chromatography columns (hydroxyapatite, DEAE-Sephacel and Sephadex G-100) and ultrafiltration. The enzyme eluted with 0.12 M phosphate buffer from a hydroxyapatite column, while the enzyme was bound to a DEAE-Sephacel anion-exchange column until eluted with the buffer containing 0.1 M NaCl. The enzyme was eluted from a Sephadex G-100 column (Fig. 3) at a calibrated molecular mass of 84 ± 2.3 kDa (average of four experiments, ± S.E.M.). After passage through the three columns and ultrafiltration, 14.6% of the total activity was recovered with a 91-fold purification (Table 1).

An alternative strategy to the column procedures described in this study, is the use of an agarose affinity column with bound adenosine-3′,5′-diphosphate (PAP). However, when this purification procedure was performed no sulfotransferase activity from the shark liver sample could be eluted from the affinity gel. Likewise, other protein purification methods, such as the Bio-Rad model 491 Prep Cell continuous elution electrophoresis system or native polyacrylamide gel electrophoresis, were ineffective as the active enzyme would not enter the gel even at low polyacrylamide concentrations. Several compounds were added, separately or in combination, to the sample or gel buffer to solublize (2% Triton X-100, 20% glycerol) or inhibit aggregation (6 M urea, 1% formamide, 10% sodium dodecyl sulfate); however, no improvement in the experimental procedure was observed.

3.3. Sulfotransferase stability

The liver sterol sulfotransferase was stable at − 80°C for at least 12 months without significant losses in activity. However, freeze–thawing reduced activity and the enzyme had very low stability at 4°C, especially during concentration procedures, where the sulfotransferase tended to aggregate (visualized on ultrafiltration filter) and lose catalytic activity. Several compounds were added to the sulfotransferase buffers to aid in enzyme stabilization, such as sucrose, EDTA, mercaptoethanol and phosphate, which have all been reported to stabilize activity.

3.4. Sulfotransferase kinetics

The partially purified liver enzyme had typical Michaelis–Menten kinetics for 5β-scymnol (Fig. 4) and PAPS (Fig. 5) with an apparent K_m of 3.04 μM for 5β-scymnol (Fig. 6) and apparent K_m of 4.35 μM for PAPS (Fig. 7). The partially purified sulfotransferase had a V_{max} for 5β-scymnol of 1360 fmol min^{-1} mg^{-1} and for PAPS of 507 fmol min^{-1} mg^{-1}. Substrate inhibition was observed at substrate concentrations above 10 μM.

Table 1
Purification of the sterol sulfotransferase from *H. portusjacksoni* liver

Purification stage	Volume (ml)	Total activity (fmol min^{-1})	Total protein (mg)	Specific activity (fmol min^{-1} mg^{-1})	Yield (%)	Fold purification
Cytosolic fraction	3.0	1661	75.2	22.1	100.0	1.0
Hydroxyapatite	2.2	1409	15.9	88.6	84.8	4.0
DEAE-Sephacel	2.0	1121	6.0	188.1	67.4	8.5
Sephadex G-100	1.2	242	0.1	2017.5	14.6	91.3

3.5. Effect of various compounds and assay conditions on sulfotransferase activity

The reaction product PAP competitively inhibited the shark liver sulfotransferase. The apparent K_m for the substrates were increased by a factor of $(1 + I/K_I)$ where $K_I = 0.84$ μM for 5β-scymnol (Fig. 6) and $K_I =$ 0.37 μM for PAPS (Fig. 7).

The liver sulfotransferase was found to sulfate equivalent amounts of 5β-scymnol, 5α-cyprinol and testosterone (at 50 μM) under similar conditions, but not dehydroepiandrosterone (DHEA) or β-estradiol (Table 2). Exogenous magnesium ions were not found to be essential for enzyme activity and did not significantly enhance catalysis (Table 2). The sulfotransferase activity was inhibited by the metal ion complexing agents sodium azide and EDTA, suggesting the metal ions may already be bound in the enzyme (Table 3). Enzyme activity was also inhibited by iodoacetic acid and *p*-chloromercuribenzoic acid, indicating the possible requirement of sulfhydryl group(s) for activity (Table 3).

At 37°C the highest enzyme activity was measured for the liver sulfotransferase, with an activity range of 20–45°C.

4. Discussion

5β-Scymnol sulfotransferase activity was identified in the *H. portusjacksoni* shark liver, kidney and testis cytosolic fraction. Sulfotransferase enzymes, which sulfate steroids, bile acids and sterols, are generally found in the cytosolic or soluble fraction of the cell [13], with the liver as the major site of bile salt synthesis in all vertebrates [16].

The function of the elasmobranch kidney sulfotransferase may be as a secondary site for detoxication—as is the case in rodents, where the renal sulfation of bile acids and other compounds is thought to play a role in the removal of toxic compounds during liver dysfunction [10]. The enzymes involved in bile acid conjugation in mammals (bile acid CoA synthetase and bile acid–CoA:amino acid *N*-acyltransferase) are primarily located in the liver, but have also been found in the kidney of rats [20,21]. This co-distribution of the bile acid conjugating enzymes and sulfotransferases suggests that the kidney has an important role in bile metabolism, and thus the presence of a sulfotransferase in the elasmobranch kidney may indicate an important secondary site of bile salt biosynthesis in these animals.

Testicular sterol sulfotransferase activity was found in the cytosolic fraction, which is consistent with a steroid sulfotransferase previously identified in the testis of the shark *Squalus acanthias* [11,12]. The sterol sulfotransferase in *H. portusjacksoni* testes may be either a steroid sulfotransferase with broad specificity, a sterol sulfotransferase involved in spermatazoa membrane synthesis [23] or a previously undefined sulfotransferase.

We have previously reported the presence of a sulfotransferase involved in the synthesis of the bile alcohol 5β-scymnol sulfate in the liver of *H. portusjacksoni* [25], which has been further characterized in this study. The purification procedure developed for the *H. portusjacksoni* liver sterol sulfotransferase resulted in only partial

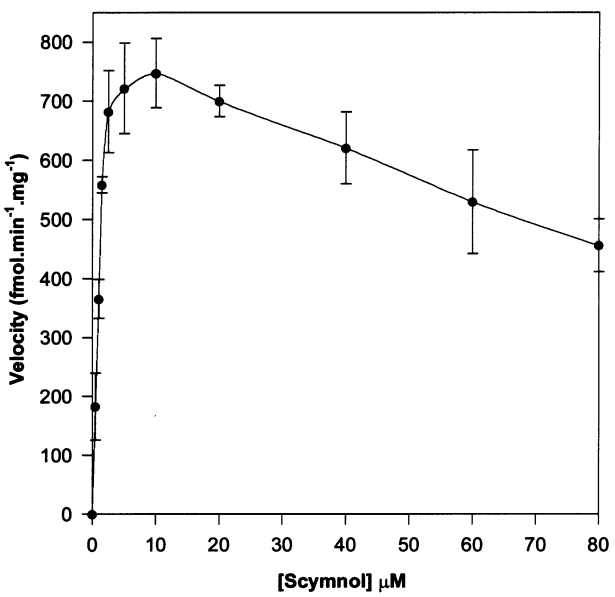

Fig. 4. The effect of varying the concentration of 5β-scymnol on the activity of the *H. portusjacksoni* liver sterol sulfotransferase. The assay mixture consisted of partially purified preparation, 50 μM PAPS, 1.02 nM PAP[^{35}S] and 5β-scymnol (at final concentrations of 0–80 μM). Each point is an average ± S.E.M. of triplicate assays.

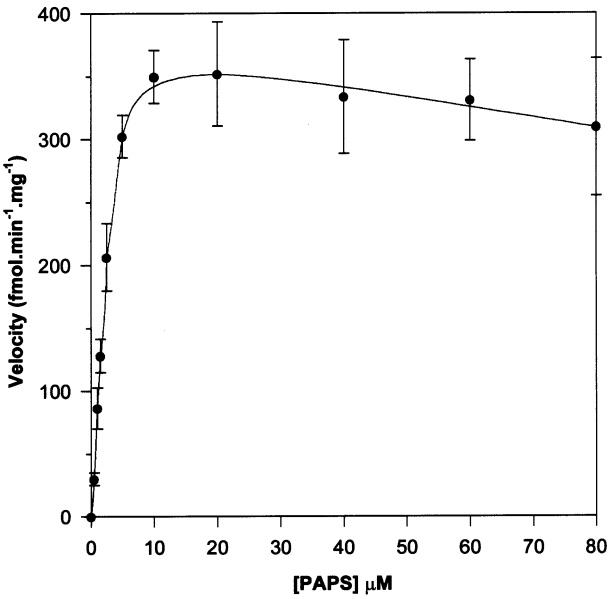

Fig. 5. The effect of varying the concentration of PAPS on the activity of the *H. portusjacksoni* liver sterol sulfotransferase. The assay mixture consisted of partially purified preparation, 50 μM 5β-scymnol, 1.02 nM PAP[^{35}S] and PAPS (at final concentrations of 0–80 μM). Each point is an average $\pm$ S.E.M. of triplicate assays.

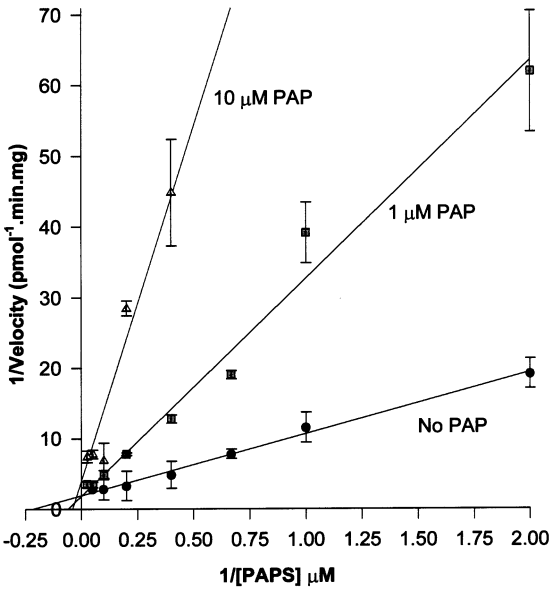

Fig. 7. Inhibition of the *H. portusjacksoni* liver sterol sulfotransferase by PAP at increasing PAPS concentrations. The PAPS concentration was varied between 0 and 40 μM. PAP was kept constant at 1 or 10 μM (or not added). Each point is an average $\pm$ S.E.M. of triplicate assays (points exhibiting substrate inhibition were excluded).

purification of the enzyme, due to its low stability levels and heavy losses occurring during purification, which seems to be a common feature of sulfotransferases [31,37].

Ultrafiltration of the liver enzyme tended to cause aggregation of the proteins and loss of enzyme activity. It appears that sulfotransferases have a high tendency to form aggregates as they have been described with liver sulfotransferases from rat, hamster, human and rhesus monkey [3–5,7,9,28]. The conditions that caused aggregation of the elasmobranch enzyme could not be determined; however, it appears to be related to the concentration of protein, as significant enzyme activity was lost during the ultrafiltration stage. Temperature may also be a factor as storage in the cold, freezing and thawing have been found to cause aggregation of sulfotransferases [2,7,24].

Purification of the shark enzyme by native polyacrylamide gel electrophoresis was unsuccessful, as the enzyme did not enter the gel, suggesting either a very

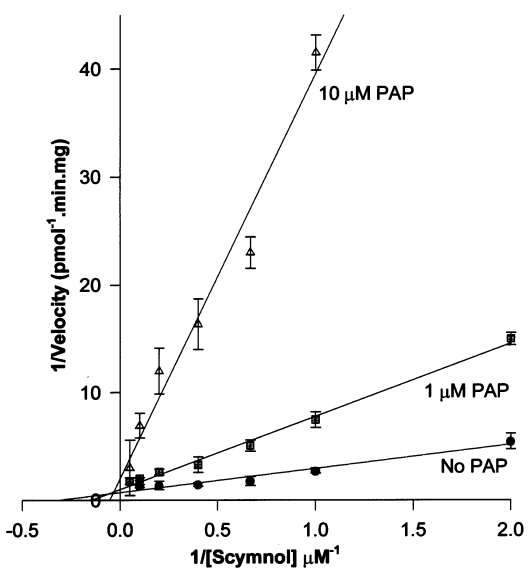

Fig. 6. Inhibition of the *H. portusjacksoni* liver sterol sulfotransferase by PAP at increasing 5β-scymnol concentrations. The PAPS concentration was varied between 0 and 40 μM. PAP was kept constant at 1 or 10 μM (or not added). Each point is an average $\pm$ S.E.M. of triplicate assays (points exhibiting substrate inhibition were excluded).

Table 2
Effect of magnesium ions and various substrates on the activity of *H. portusjacksoni* liver sulfotransferase

Substrate	Final substrate concentration	
	10 μM	50 μM
5β-Scymnol (control)	100.0 $\pm$ 2.1	100.0 $\pm$ 6.8
5β-Scymnol + 5 mM $MgCl_2$	–	92.5 $\pm$ 2.1
5β-Scymnol + 50 mM $MgCl_2$	–	85.8 $\pm$ 8.9
5α-Cyprinol	106.8 $\pm$ 8.9	96.1 $\pm$ 5.2
DHEA	15.4 $\pm$ 2.1	19.5 $\pm$ 0.2
Testosterone	24.5 $\pm$ 2.7	107.3 $\pm$ 16.5
β-Estradiol	22.2 $\pm$ 0.5	21.1 $\pm$ 1.8

All assays were performed in triplicate. The blank was subtracted from the test samples before standardization to the 5β-scymnol sulfation activity (taken as 100%).
Values are given as % of the control, average $\pm$ S.E.M., where 100% is equivalent to a 2.44% incorporation of PAP[^{35}S].

Table 3
The effect of inhibitors on the activity of the partially purified liver sterol sulfotransferase from *H. portusjacksoni*

Inhibitor	Pre-incubation time (min)	Inhibition (%)	
		10 μM	100 μM
None	0	0.00 ± 1.4	0.0 ± 1.4
PAP	10	68.2 ± 1.3	84.8 ± 2.1
	0	56.6 ± 5.5	83.9 ± 2.2
Iodoacetic acid	10	26.8 ± 2.9	36.3 ± 1.9
	0	19.9 ± 6.2	28.6 ± 3.2
p-Chloromercuribenzoic acid	10	38.7 ± 5.1	58.8 ± 1.1
	0	40.1 ± 6.5	37.5 ± 6.0
Sodium azide	10	17.5 ± 1.0	17.7 ± 9.0
	0	21.3 ± 8.0	38.6 ± 1.1
		10 mM	50 mM
EDTA	10	23.9 ± 8.5	95.9 ± 1.3
	0	−12.0 ± 3.4	89.0 ± 0.5

Inhibitor (10 μl) was added to the assay mix either 10 min prior to addition of the substrates or at the same time as the substrates. The blank was subtracted from the test values prior to standardization to the 5β-scymnol assays (without inhibitor added) to determine the percentage inhibition. Assays were performed in triplicate and results presented as the mean ± S.E.M., where 100% is equivalent to a 1.52% incorporation of PAP[^{35}S].

large molecular weight protein or the formation of an aggregate. Previous studies have also encountered a similar problem using native gel electrophoresis isolation procedures for the purification of mammalian sulfotransferases, where the sulfotransferases were unable to enter a 7% polyacrylamide gel, without the use of a detergent [5,28]. Affinity chromatography enzyme purification using the PAP-agarose column was investigated for its potential to purify the elasmobranch sulfotransferase; however, the enzymes either did not bind to the column, or were not eluted from the column in an active form.

The liver sterol sulfotransferase exhibited typical Michaelis–Menten kinetics at the substrate concentrations investigated, with similar kinetic constants (K_m) for 5β-scymnol and PAPS (3.04 and 4.35, respectively) found. Substrate inhibition is a common feature of sulfotransferases, and for the liver enzyme was observed as a decrease in activity above 10 μM concentrations of 5β-scymnol or PAPS. Inhibition is thought to be caused by the formation of a non-productive reversible enzyme–substrate–PAP complex [14]. PAP was found to be an inhibitor of both substrates (5β-scymnol and PAPS), and it may therefore play an important role as a regulatory mediator in the in vivo production of 5β-scymnol sulfate via feedback inhibition.

Similar to many mammalian sulfotransferases, the *H. portusjacksoni* liver sulfotransferase was found to require sulfhydryl group(s) for activity; however, their functional importance has not been determined [32].

Decreased formation of product with the addition of EDTA and sodium azide, suggests that these agents are chelating important divalent cations (e.g. Mg^{2+}) required for sulfotransferase activity. Mg^{2+} requirement and activation have been identified for many sulfotransferase enzymes [32]. The *H. portusjacksoni* liver sulfotransferase was active without the addition of Mg2 + and not activated by its addition; however, Mg2 + or another divalent cation may already be bound within the enzyme.

Substrate specificity was briefly investigated using 5α-cyprinol, DHEA, β-estradiol and testosterone. The side chain of 5α-cyprinol is very similar to 5β-scymnol, lacking only the chiral C-24 hydroxyl group. The A/B ring junction of the steroid nucleus can be oriented in two different conformations as either a curved or flat structure, and therefore 5β-scymnol and 5α-cyprinol are structurally quite different. The ability of the sulfotransferase to readily sulfate 5α-cyprinol suggests that the hydroxyl group at C-24 is not essential for binding within the active site of the sulfotransferase. It also shows that the different orientation of the steroid rings does not hinder binding of the side chain within the active site of the sulfotransferase.

To estimate the evolutionary significance of these enzymes, the amino acid or DNA sequences are required for comparison. The commercial production of 5β-scymnol sulfate will require further research into other potential sources and bacterial expression systems, as the elasmobranch liver sulfotransferases cannot be purified in large enough amounts to be economically viable.

Acknowledgements

The authors thank N. Bridge and staff from the Victorian Fisheries Research Institute, Queenscliff, Victoria, for providing the specimens. This work was supported by a grant from McFarlane Laboratories (Australia) Pty Ltd., Melbourne and the RMIT-University Faculty of Biomedical and Health Sciences Research Committee. N.E.P. was the recipient of an Australian Postgraduate Award and a RMIT-University Faculty of Biomedical and Health Sciences Writing-up award. A more detailed account of the work described in this paper is in the Ph.D. thesis of N.E.P., RMIT-University, Melbourne.

References

[1] Andrews P. Estimation of the molecular weights of proteins by sephadex gel-filtration. Biochem J 1964;91:222–33.
[2] Barford DJ, Jones JG. Thiol-dependent changes in the properties of rat liver sulfotransferases. Biochem J 1971;123:427–34.
[3] Barnes S, Spenney JG. Evidence for heterogeneity of hepatic bile salt sulfotransferases in female hamsters and rats. Biochim Biophys Acta 1982;704:353–60.
[4] Barnes S, Waldrop R, Crenshaw J, King RJ, Taylor KB. Evidence for an ordered reaction mechanism for bile salt:3′-phosphoadenosine-5′-phosphosulfate:sulfotransferase from rhesus monkey liver that catalyzes the sulfation of the hepatotoxin glycolithocholate. J Lipid Res 1986;27:1111–23.
[5] Barnes S, Buchina ES, King RJ, McBurnett T, Taylor KB. Bile acid sulfotransferase I from rat liver sulfates bile acids and 3-hydroxy steroids: purification, N-terminal amino acid sequence, and kinetic properties. J Lipid Res 1989;30:529–40.
[6] Bridgwater RJ, Ryan DA. Sulphate conjugation with ranol and other steroid alcohols in liver homogenates from *Rana temporaria*. Biochem J 1957;65:24P–5P.
[7] Carroll J, McEvoy FA. The activation of liver phenol sulphotransferase. Biochem J 1970;119:27P.
[8] Carter FM, Wright PFA, Kalafatis N, Macrides TA. A mechanistic investigation of 5β-scymnol's protection against paracetamol-induced liver damage in mice. Proc Aust Soc Clin Exp Pharmacol Toxicol 1995;2:116.
[9] Chen LJ, Segel IH. Purification and characterization of bile salt sulfotransferase from human liver. Arch Biochem Biophys 1985;241:371–9.
[10] Chen LJ, Imperato TJ, Bolt RJ. Enzymatic sulfation of bile salts II. Studies on bile salt sulfotransferase from rat kidney. Biochim Biophys Acta 1978;522:443–51.
[11] Cuevas ME, Callard G. Characterization and stage-dependent distribution of an estrogen sulfotransferase in the testis of the dogfish *Squalus acanthias*. Bull Mt Desert Island Biol Lab 1986;26:40–2.
[12] Cuevas ME, Miller W, Callard G. Sulfoconjugation of steroids and the vascular pathway of communication in dogfish testis. J Exp Zool 1992;264:119–29.
[13] Falany CN. Sulfation and sulfotransferases 3. Enzymology of human cytosolic sulfotransferases. FASEB J 1997;11:206–16.
[14] Falany CN, Comer KA, Dooley TP, Glatt H. Human dehydroepiandrosterone sulfotransferase. Purification, molecular cloning, and characterization. Ann New York Acad Sci 1995;774:59–72.
[15] Haslewood GAD. The biological importance of bile salts. In: Neuberger A, Tatum EL, editors. Frontiers of Biology, vol. 47. Amsterdam: North-Holland, 1978:1–206.
[16] Hofmann AF. Bile acids. In: Arias IM, Jakoby WB, Popper H, Schachter D, Shafritz DA, editors. The Liver: Biology and Pathobiology, 2nd ed. New York: Raven Press, 1988:553–572.
[17] Jakoby WB, Sekura RD, Lyon ES, Marcus CJ, Wang J. Sulfotransferases. In: Jakoby WB, editor. Enzymatic Basis of Detoxication, vol. II. New York: Academic Press, 1980:199–228.
[18] Klaassen CD, Boles JW. Sulfation and sulfotransferases 5. The importance of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in the regulation of sulfation. FASEB J 1997;11:404–18.
[19] Kosuge Y, Kosuge T, Tsuji K, Ishida H, Broadbent JM. Scymnol sulphate salts isolated from shark tissues for the treatment of liver and skin ailments. Chem Abstr 1989;110:88640g.
[20] Kwakye JB, Barnes S, Diasio RB. Identification of bile acid coenzyme A synthetase in rat kidney. J Lipid Res 1993;34:95–9.
[21] Kwakye JB, Johnson MR, Barnes S, Grizzle WE, Diasio RB. Identification of bile acid–CoA:amino acid *N*-acyltransferase in rat kidney. Biochem J 1991;280:821–4.
[22] Last PR, Stevens JD. Sharks and Rays of Australia. Australia: CSIRO, 1994.
[23] Legault Y, Bouthillier M, Bleau G, Chapdelaine A, Roberts KD. The sterol sulfate content of the male hamster reproductive tract. Biol Reprod 1979;20:1213–9.
[24] Lööf L, Hjertén S. Partial purification of a human liver sulfotransferase active towards bile salts. Biochim Biophys Acta 1980;617:192–204.
[25] Macrides TA, Faktor DA, Kalafatis N, Amiet RG. Partial purification and characterization of an enzyme from the liver of the shark *Heterodontus portusjacksoni* that catalyzes the sulfation of the shark bile steroid 5β-scymnol. Comp Biochem Physiol 1994;107B:461–9.
[26] Macrides TA, Naylor LM, Kalafatis N, Shihata A, Wright PFA. Hepatoprotective effects of the shark bile salt 5β-scymnol on acetaminophen-induced liver damage in mice. Fund Appl Toxicol 1996;33:31–7.
[27] Macrides TA, Shihata A, Kalafatis N, Wright PFA. A comparison of the hydroxyl radical scavenging properties of the shark bile steroid 5β-scymnol and plant pycnogenols. Biochem Mol Biol Int 1997;42:1249–60.
[28] Marcus CJ, Sekura RD, Jakoby WB. A hydroxysteroid sulfotransferase from rat liver. Anal Biochem 1980;107:296–304.
[29] Markwell MAK, Haas SM, Bieber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 1978;87:206–10.
[30] Mock MR, Macrides TA, Kalafatis N, Wright PFA. Effect of the shark bile steroid 5β-scymnol on bile acid transport in isolated rat hepatocytes. Proc Aust Soc Clin Exp Pharmacol Toxicol 1995;2:170.
[31] Powell GM, Roy AB. Sulphate conjugation. In: Gram TE, editor. Extrahepatic Metabolism of Drugs and Other Foreign Chemicals. Lancaster: MTP Press, 1980:389–425.
[32] Roy AB. Sulfotransferases. In: Mulder GJ, editor. Sulfation of Drugs and Related Compounds. Boca Raton, FL: CRC Press, 1981:83–130.
[33] Scully MF, Dodgson KS, Rose FA. Sulphation of simple aliphatic alcohols and bile alcohols by the toad *Xenopus laevis*. Biochem J 1970;119:29P–30P.
[34] Sottocasa GL, Kuylenstierna B, Ernster L, Bergstrand A. An electron-transport system associated with the outer membrane of liver mitochondria. J Cell Biol 1967;32:415–38.
[35] Spurrell B, Wright PFA, Kalafatis N, Macrides TA. 5β-Scymnol reduces blood ethanol levels in the mouse after acute ethanol ingestion. Proc Aust Soc Clin Exp Pharmacol Toxicol 1996;3:145.

[36] Usui T. Thin layer chromatography of bile acids with special reference to separation of keto acids. J Biochem Tokyo 1963;53:283–6.
[37] Weinshilboum R, Otterness D. Sulfotransferase enzymes. In: Kauffman FC, editor. Handbook of Experimental Pharmacology, vol. 112. Berlin: Springer-Verlag, 1994:45–78.
[38] Whitley GP. Australian Sharks. Melbourne: Lloyd O'Neil, 1983.
[39] Wright PFA, Naylor LM, Kalafatis N, Macrides TA. The shark bile steroid 5β-scymnol is a broad spectrum hepatoprotective agent. Proc Aust Soc Clin Exp Pharmacol Toxicol 1994;1:129.