ELSEVIER

Steroids 64 (1999) 510–517

Steroids

Testosterone sulfotransferase: Evidence in the guinea pig that this reaction is carried out by 3α-hydroxysteroid sulfotransferase

Byoung C. Park, Young C. Lee, Charles A. Strott*

Section on Steroid Regulation, Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, Bldg 49, Rm 6A36, National Institutes of Health, Bethesda, MD 20892-4510, USA

Received 4 November 1998; received in revised form 21 December 1998; accepted 28 January 1999

Abstract

During the course of isolating, characterizing, and cloning estrogen and 3-hydroxysteroid sulfotransferases from the guinea pig adrenal gland, it was noted that cytosolic preparations from this tissue would also sulfonate testosterone. Therefore, we set out to isolate and clone the enzyme that performs this reaction. Testosterone sulfotransferase (TST) was isolated from the guinea pig adrenal by using the standard procedures of ion exchange, affinity, and high-performance liquid chromatography. When purified, TST was examined by liquid-phase nondenaturing isoelectric focusing, it was found that the TST activity profile completely overlapped with the activity profile of the 3α-hydroxysteroid sulfotransferase (3αHST) isoform, but not the 3β-hydroxysteroid sulfotransferase (3βHST) isoform. This finding was further investigated by overexpressing the cDNAs for 3αHST and 3βHST in *Escherichia coli* and examining the expressed proteins for TST activity. This experiment confirmed that 3αHST does indeed function as a TST. In addition, 3αHST was also found to sulfonate estradiol but not estrone, a finding that further suggested that 3αHST may function as a general 17β-hydroxysteroid sulfotransferase. Published by Elsevier Science Inc.

Keywords: 3α-/3β-Hydroxysteroid; 17β-Hydroxysteroid; Sulfotransferase; Androsterone; Testosterone; Estradiol

1. Introduction

The sulfonation or sulfoconjugation of steroids is a major metabolic route in the biotransformation of these biologically important compounds [1]. Steroid sulfonation, which occurs widely across species [2], is catalyzed by a family of enzymes termed sulfotransferases [3], enzymes typically isolated from the cytosolic fraction of tissue preparations [4]. Moreover, the enzymes that sulfoconjugate other relatively small endogenous compounds such as catecholamines, iodothyronines, ascorbate, cholesterol, vitamin D, and bile acids, as well as drugs and xenobiotics, are also present in the cell sap [5,6]. This is in contrast to the subclass of sulfotransferases that catalyzes the sulfonation of macromolecules, i.e. proteins, proteoglycans, and glycosaminoglycans as well as sulfolipids, which are associated with cell membranes and the Golgi complex [7]. Until recently, reliable biochemical and structural characterization of individual steroid sulfotransferases had been sparse, primarily because of a general lack of adequate enzyme purification. This situation has now greatly changed as a result of the development of more powerful tools for isolating and resolving proteins and the strength of recombinant DNA technology. Furthermore, with the availability of bacterial and yeast overexpression systems, these enzymes can be produced in large quantities and purified for biochemical characterization and structural analysis.

The sulfonation of steroids has a profound influence on their biological activity [8]. For this reason, the sulfonation of estrogens has been extensively studied, particularly in uterine and mammary target tissues (for review, see Ref. [3]); specific estrogen sulfotransferases have been cloned and expressed from cow [9], rat [10], guinea pig [11], human [12], and mouse [13]. In contrast to the case with estrogens, the sulfonation of androgens has not received as much attention, although this process is thought to play a major role in age-related changes in androgen sensitivity in the rat liver [14]. The sulfoconjugation of testosterone is known to occur in several tissues including the testis [15–18], adrenal [19], and liver [20–23]. Although a specific

* Corresponding author. Tel.: +1-301-496-3025; fax: +1-301-496-7435.

E-mail address: chastro@box-c.nih.gov (C.A. Strott)

0039-128X/99/$ – see front matter Published by Elsevier Science Inc.
PII: S0039-128X(99)00027-6

testosterone sulfotransferase (TST) has not been isolated or cloned, hydroxysteroid sulfotransferases (HSTs) have been cloned and expressed from several species that will sulfoconjugate testosterone, e.g. rat [24], rabbit [25], and human [26,27]. These enzymes appear to have a broad substrate specificity and will sulfonate a wide range of 3β- and 3α-hydroxylated steroid substrates, e.g. 3β-hydroxy-5-androsten-17-one (dehydroepiandrosterone), 3β-hydroxy-5-pregnen-20-one (pregnenolone), 3α-hydroxy-5α-androstan-17-one (androsterone), and 3α-hydroxy-5α-pregnan-20-one (allopregnanolone); in addition, they will also sulfonate 17β-hydroxy-4-androsten-3-one (testosterone) and the estrogens, 3-hydroxy-1,3,5 [10] estratrien-17-one (estrone) and 1,3,5 [10] estratriene-3, 17β-diol (estradiol).

During the course of isolating and cloning several steroid sulfotransferases from the guinea pig adrenal gland [11,28, 29], we noted the presence of TST activity in cytosolic preparations from this tissue. Therefore, we set out to isolate and characterize TST from the guinea pig adrenal; this report describes our experience in doing so. During the purification scheme, which included ion exchange, 3′-phosphoadenosine 5′-phosphate (PAP) affinity chromatography, and gel-permeation high-performance liquid chromatography (HPLC), as well as during analysis with liquid-phase nondenaturing isoelectric focusing, it was noted that the TST activity profile overlapped completely with the activity profile of 3α-hydroxysteroid sulfotransferase (3αHST) but not the 3β-hydroxysteroid sulfotransferase (3βHST) isoform. As a result of this finding, we overexpressed the 3αHST and 3βHST isoforms in *Escherichia coli* as fusion proteins and purified them by affinity column chromatography for biochemical analysis. This study confirmed that the 3αHST isoform, but not the 3βHST isoform, was capable of sulfonating testosterone. Furthermore, 3αHST was capable of sulfonating estradiol but not estrone, suggesting that the 3αHST isoform may serve as a general 17β-hydroxysteroid sulfotransferase.

2. Materials and methods

2.1. Materials

Tritium-labeled steroids were purchased from DuPont/NEN (Boston, MA, USA). Crystalline steroids (pregnenolone, dehydroepiandrosterone, androsterone, allopregnanolone, estradiol, estrone, and testosterone) were obtained from either Steraloids, Inc (Wilton, NH, USA) or Sigma (St. Louis, MO, USA). Steroid stock solutions (0.1–10 mM) were prepared in absolute ethanol.

3′-Phosphoadenosine 5′-phosphosulfate (PAPS), 3′-phosphoadenosine 5′-phosphate (PAP), and dithiothreitol were purchased from Sigma. A PAPS stock solution (1 mM) was made up in buffer consisting of 100 mM Tris-HCl (pH 7.7) and 5 mM magnesium acetate, divided into aliquots and stored at −70°C. Restriction enzymes were obtained from New England Biolabs (Beverly, MA, USA). Unless otherwise stated, all other chemicals and reagents were purchased from Sigma and used as obtained from the supplier.

2.2. Animals and tissue preparation

Male National Institutes of Health inbred strain 2 guinea pigs, obtained from the National Cancer Institute (Frederick, MD, USA) were maintained on guinea pig chow with added greens and water ad libitum in a controlled light/dark environment at constant temperature and humidity. The experimental protocol and all animal procedures used in this investigation were approved by the National Institute of Child Health and Human Development Animal Care and Use Committee.

Animals were anesthetized with CO_2 gas and decapitated. The adrenal glands were quickly removed and placed in homogenization buffer (10 mM Tris-HCl, pH 7.5, containing 1 mM EDTA, and 1.5 mM dithiothreitol) on ice. The adrenals were cleaned of fat and fibrous material, weighed, and minced. Samples were kept on ice and homogenized in 2 vol (wt/vol) of the homogenization buffer by using a glass/Teflon apparatus (five strokes). The cytosolic fraction was prepared by centrifuging homogenates at 105 000 × *g* for 90 min at 4°C, after which the floating lipid layer was carefully suctioned off, and the supernatant decanted.

2.3. Enzyme purification

Adrenal cytosol was applied to a 20 × 1.6-cm column of DE52 anion-exchange resin (Whatman Labsales, Hillsboro, OR, USA) equilibrated with 20 mM Tris-HCl, pH 7.4, and 1 mM EDTA (TE). The column was washed extensively with TE until the 280-nm absorbance returned to baseline. Adsorbed proteins were eluted with a linear gradient of NaCl in TE (0–300 mM, 1000 ml total volume) at a flow rate of 50 ml/h, and 1 ml fractions were collected. Fractions containing the highest TST specific activity were pooled and concentrated by Centriprep 30 (Amicon, Beverly, MA, USA). The concentrate was applied to a 16 × 1.6-cm PAP-agarose (Sigma) affinity column equilibrated with TE. After washing with TE, adsorbed proteins were eluted with 10 ml of the 1 mM PAPS solution (see Section 2.1) followed by a chase with TE buffer. Fractions containing the highest TST activity were pooled and concentrated by Centriprep 30. The concentrate was applied to an HPLC Superdex 200 HR 10/30 column (Pharmacia, Piscataway, NJ, USA) equilibrated and run with TE containing 100 mM NaCl. Chromatography was performed at a flow rate of 1 ml/min, and 1-ml fractions were collected. The most active TST fractions were pooled and concentrated as described above and reapplied to the same HPLC column under the same conditions; this step was then repeated once more.

2.4. Bacterial overexpression and purification of 3αHST and 3βHST

The cDNAs for guinea pig 3αHST [28] and 3βHST [29] were amplified and inserted into the pProEx-HTc expression vector (Life Technologies, Grand Island, NY, USA) at the *Sal*I--*Not*I restriction sites, and competent *E. coli* DH5α cells were transformed with these constructs. A his-tag and a tobacco etch virus protease site are located in front of the HST coding region. The *E. coli* cells were placed in Luria–Bertani (LB) medium and incubated at 30°C for 3 h. After induction with 1 mM isopropylthiogalactoside (IPTG), incubations were continued for another 4 h at 30°C, after which the cells were centrifuged at 3000 × *g* for 10 min at 4°C. Cell pellets were lysed at 4°C with 1 vol (w/v) TE buffer (pH 7.5) containing 10 mM imidazole and 1 mg/ml lysozyme. Lysates were centrifuged at 105 000 × *g* for 1 h at 4°C, and the supernatants were applied to nickel-affinity columns (Qiagen, Valencia, CA, USA) and washed with 20 mM imidazole in TE (pH 7.5). Bound HSTs were eluted with 200 mM imidazole in TE, diluted with excess TE, and concentrated with Centriprep 30. To remove his-tags, purified fusion proteins (0.1 mg) were incubated with 500 U of recombinant tabacco etch virus (Life Technologies) for 16 h at 4°C, and the preparations were again applied to nickel-affinity columns for chromatography.

2.5. Liquid-phase nondenaturing isoelectric focusing

A Rotofor preparative electrofocusing cell (Bio-Rad Laboratories, Hercules, CA, USA) was used to separate purified HST proteins by charge in an ampholyte-generated pH gradient as initially reported by Driscoll et al. [30]. The aqueous focusing medium consisted of (vol/vol) 25% glycerol, 3% ampholine, pH 5.0 to 7.0 (Pharmacia), and 2% Bio-Lyte 3/10 (Bio Rad). A purified HST preparation was thoroughly mixed with 50 ml of ice-cold focusing solution before loading into the Rotofor chamber. The anode and cathode electrolytes were 0.1 M H_3PO_4 and 0.1 M NaOH, respectively. During the operation, the chamber was cooled by a recirculating water bath set at 1°C. The focusing was performed at 12-W constant power for 4 to 6 h. After the voltage plateaued and remained constant for ≈60 min, the fractions were collected. The pH of each fraction was measured by using a digital Model 3890 pH meter (Beckman Instruments), and an aliquot of each fraction was removed for enzymatic and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses. The fractions were made 100 mM in Tris-HCl by adding the appropriate volume of 1 M Tris-HCl (pH 7.7) and concentrated by Centriprep 30.

2.6. SDS-PAGE and Western blot analyses

Proteins were resolved in 15% polyacrylamide gels as previously reported and visualized by Coomassie staining [31]. For immunochemical analysis, proteins were electrophoretically transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH, USA) as previously detailed [32]. Western blotting and colorimetric visualization of immunoreactive proteins were performed exactly as described previously [33].

2.7. Protein determination

Protein concentrations were determined by the Bradford method with bovine serum albumin as a standard [34].

2.8. Sulfotransferase assay

Steroid sulfotransferase assays were performed in a solution composed of 100 mM Tris-HCl (pH 7.7), 5 mM magnesium acetate, 0.1 mM PAPS, and varying concentrations of ^{3}H-labeled steroids (40 nM to 50 μM). The total reaction volume was generally 100 μl. For the routine assay of column fractions, incubations were performed at room temperature, whereas kinetic and specific activity analyses were performed at 37°C. Reactions were stopped by the addition of 100 μl of 0.1 N NaOH, followed by 800 μl of dichloromethane. Unconjugated steroids were extracted by vigorous Vortex mixing followed by centrifugation at 13 000 rpm for 5 min. The amount of radioactivity in an aliquot (typically 100 μl) of the aqueous phase containing sulfoconjugated steroid was determined by liquid scintillation. To correct for extraction efficiency, background blanks (labeled substrate added after the addition of 0.1 N NaOH to

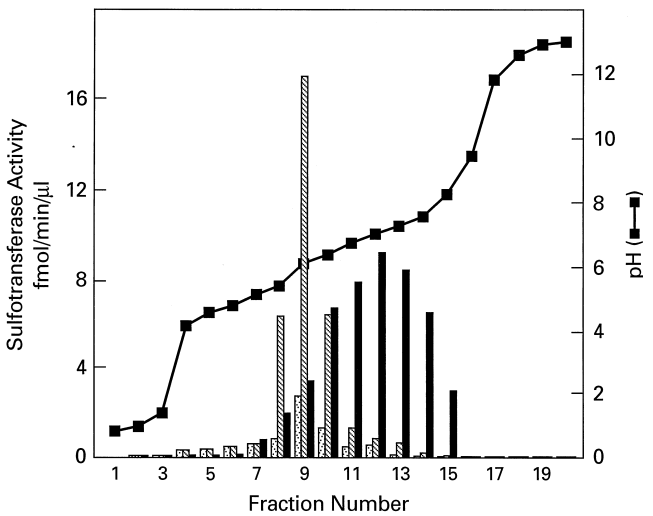

Fig. 1. Sulfotransferase activity profiles after liquid-phase nondenaturing isoelectric focusing. Adrenal cytosol was prepared, and the testosterone sulfotransferase activity was fractionated by ion-exchange chromatography; the peak activity fractions were pooled, concentrated, and applied to a Rotofor apparatus as described in Section 2. Sulfotransferase activity in 50 μl of the eluted fractions was assayed essentially as described in Section 2, by using testosterone (stippled columns), androsterone (hatched columns), and pregnenolone (solid columns) as substrates; assays were performed at 37°C for 10 min at steroid concentrations of 2 μM.

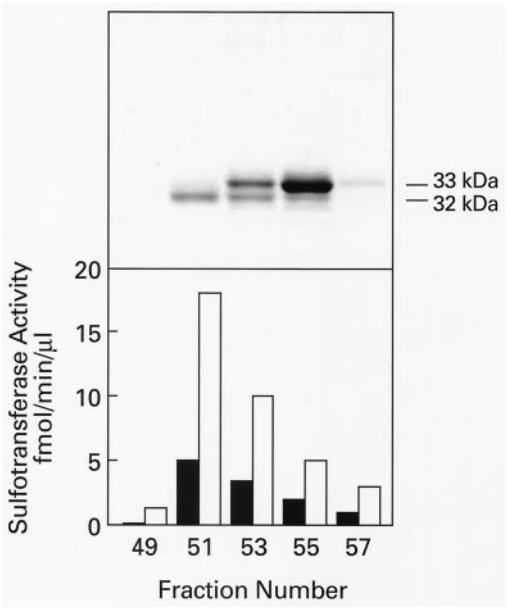

Fig. 2. Proteins and sulfotransferase activity in fractions eluting from a Superdex 200 HR 10/30 high-performance liquid chromatography (HPLC) column. Testosterone sulfotransferase activity purified by ion-exchange and 3′-phosphoadenosine 5′-phosphate (PAP) affinity chromatography was subjected to HPLC as described in Section 2. HPLC fractions (5 μl) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie blue (top) and assayed for sulfotransferase activity (bottom) essentially as described in Section 2, by using testosterone (solid columns) and androsterone (open columns) as substrates; assays were performed at 37°C for 10 min at steroid concentrations of 4 μM. Molecular mass markers refer to 3α-hydroxysteroid sulfotransferase (32 kDa) and 3β-hydroxysteroid sulfotransferase (33 kDa).

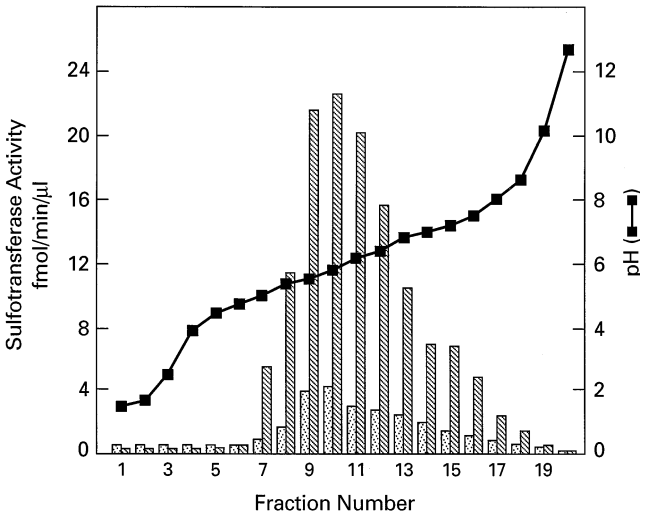

Fig. 3. Sulfotransferase activity profiles after liquid-phase nondenaturing isoelectric focusing of the material eluting in the high-performance liquid chromatography fraction containing the peak sulfotransferase activities noted in Fig. 2. Sulfotransferase activity in 50 μL of the eluted fractions was assayed essentially as described in Section 2, by using testosterone (stippled columns) and androsterone (hatched columns) as substrates; assays were performed at 37°C for 10 min at steroid concentrations of 1 μM.

the enzyme mixture) were routinely processed in parallel with the complete reactions, and the values obtained were substracted. The rate of sulfonation was calculated from the corrected amount of ^{3}H-labeled sulfoconjugated steroid that partitioned into the aqueous phase. Kinetic data were analyzed by using the Sigma Plot program (version 4.0) (SPSS, Chicago, IL, USA).

3. Results

3.1. Isolation of guinea pig adrenal TST activity

Fractions eluting from an anion-exchange column, after the application of adrenal cytosol, that contained the highest TST activity were also found to contain high activities for 3αHST (androsterone) and 3βHST (pregnenolone). Because we had previously shown that 3αHST and 3βHST could be clearly separated by liquid-phase nondenaturing isoelectric focusing, this procedure was used to examine the partially purified TST preparation. Thus, fractions containing the TST peak off the ion-exchange column were pooled, concentrated, and an aliquot was applied to the Rotofor apparatus. The eluted fractions were assayed for sulfotransferase activity by using testosterone, androsterone, and pregnenolone as substrates (Fig. 1). The highest 3αHST activity eluted at a pH of 5.5 to 6.0, whereas 3βHST activity eluted at a pH of ≈6.5 to 7.0. This result is consistent with our previous finding on the resolution of these two enzymes by using this procedure [30]; of interest, however, is that TST specific activity coeluted with 3αHST activity and was clearly separated from 3βHST activity (Fig. 1).

TST was further isolated by PAP affinity chromatography and gel-permeation HPLC. Purified TST activity eluting from the Superdex 200 HR 10/30 HPLC column behaved like a protein with an apparent M_r of 130 000, indicating that, in solution, TST behaves as a multimeric complex. This behavior is similar to the behavior of 3αHST and 3βHST when analyzed by gel-permeation chromatography [30]. When HPLC-purified TST was analyzed by SDS-PAGE, two protein bands with apparent M_r values of 32 000 and 33 000 were noted [Fig. 2 (top)]. The 32 000 M_r protein corresponds to 3αHST, whereas the 33 000 M_r protein corresponds to 3βHST [30]. Again, of interest, the TST activity profile eluting from the HPLC column completely coincided with the activity profile for 3αHST [Fig. 2 (bottom)]. The latter preparation was further analyzed by liquid-phase nondenaturing isoelectric focusing, again revealing that the two activity profiles completely overlap (Fig. 3).

3.2. Bacterial expression and purification of 3αHST and 3βHST

To further investigate the overlapping activity profiles of TST and 3αHST, the cDNA clones of 3αHST [28] and

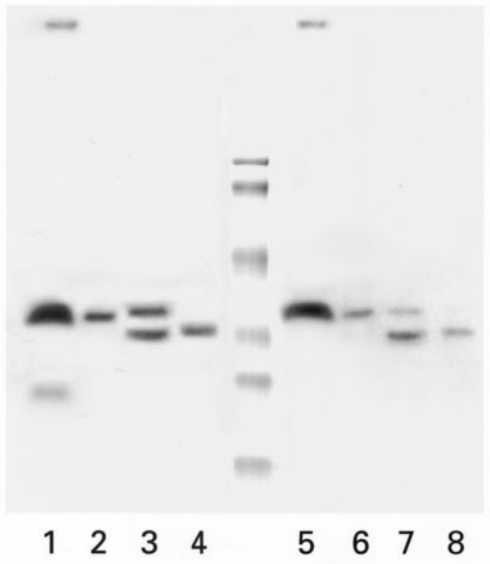

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot analyses of 3α-hydroxysteroid sulfotransferase (lanes 1–4) and 3β-hydroxysteroid sulfotransferase (lanes 5–8) overexpressed in *E. coli* and purified as described in Section 2. Lanes 1 and 5, crude *E. coli* extracts of expressed proteins (15 μg); lanes 2 and 6, affinity-purified expressed proteins (1 μg); lanes 3 and 7, affinity-purified expressed proteins after treatment with tobacco etch virus (TEV) protease (1 μg); lanes 4 and 8, flow through of affinity column after treatment of expressed proteins with recombinant TEV protease (1 μg). Prestained protein molecular weight markers are shown in the center of the figure.

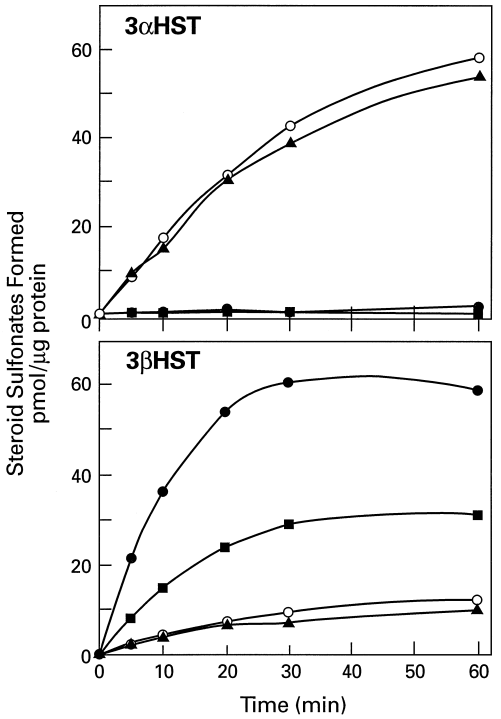

Fig. 5. Purified overexpressed 3α-hydroxysteroid sulfotransferase (3αHST) and 3β-hydroxysteroid sulfotransferase (3βHST) were analyzed (0.1 μg each) for sulfotransferase specificity by using androsterone (o), allopregnanolone (▲), pregnenolone (●), and dehydroepiandrosterone (■) as substrates (1 μM) essentially as described in Section 2.

3βHST [29] were overexpressed in *E. coli* as fusion proteins. The expressed proteins were nickle column-purified before and after removal of the his-tags with recombinant tabacco etch virus protease digestion. The purified proteins were subjected to SDS-PAGE and Western analysis by using an anti-guinea pig 3α/3βHST polyclonal antibody preparation [30,35]. Based on immunoreactivity, the Western analysis clearly demonstrated expression of the 3αHST and 3βHST proteins (Fig. 4).

3.3. Steroid specificity of the bacterially overexpressed and purified 3αHST and 3βHST

Overexpressed and purified 3αHST and 3βHST (compare Fig. 4, respectively, lanes 4 and 8) were assayed by using androsterone, allopregnanolone, pregnenolone, and dehydroepiandrosterone as substrates (Fig. 5). Consistent with our previous findings, 3αHST used only 3α-hydroxylated steroids as substrates, whereas 3βHST primarily used 3β-hydroxylated substrates. When the overexpressed and purified enzyme preparations were assayed by using testosterone as a substrate, the 3αHST isoform but not the 3βHST isoform exhibited TST activity (Fig. 6), confirming the previous finding with partially purified TST activity (compare with Fig. 3). We were curious as to whether 3αHST would also sulfonate the 17β-hydroxy group of estradiol. This was thus examined and found to indeed be the case (Fig. 6). The positional specificity of estradiol sulfonation was essentially established by the failure of 3αHST to sulfonate estrone (Fig. 6). The two enzyme preparations were further analyzed kinetically with the same substrates (Table 1). From using 3αHST, the V_{max} for allopregnanolone and androsterone was shown to be 10 to 30 times greater than that for estradiol and testosterone, whereas the K_m values for all four steroids were more or less similar, with estradiol having the lowest K_m of the group. When the V_{max}/K_m ratios were examined for 3αHST and 3βHST, androsterone was used by 3αHST $\approx$25 times more efficiently than either estradiol or testosterone, and pregnenolone was used by 3βHST about five times more efficiently than dehydroepiandrosterone (Table 1).

4. Discussion

Originally, the finding that testosterone was sulfonated by guinea pig adrenocortical cytosol led us to believe that a specific sulfotransferase acting on testosterone was present in this tissue. It was not surprising, however, that during purification it was difficult to separate the TST activity from other neutral steroid sulfotransferases, because these enzymes are similar in size and will bind to a PAP affinity column. Nevertheless, it was thought that it might be pos-

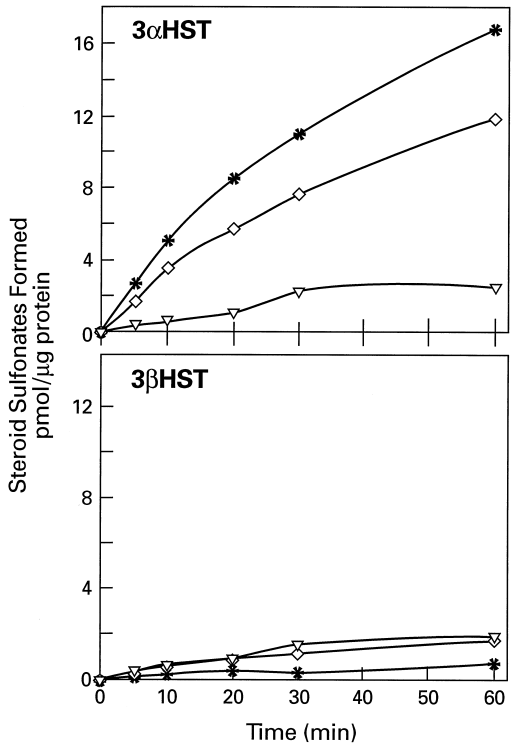

Fig. 6. Purified overexpressed 3α-hydroxysteroid sulfotransferase (3αHST) and 3β-hydroxysteroid sulfotransferase (3βHST) were analyzed (0.1 μg each) for sulfotransferase specificity, by using estradiol (*), estrone ($\triangledown$), and testosterone ($\diamond$) as substrates (0.5 μM), essentially as described in Section 2.

sible to resolve TST from 3αHST and 3βHST by charge with the Rotofor apparatus, because this procedure so clearly separates 3αHST and 3βHST, enzymes that are otherwise quite similar in size and rather difficult to separate. At first, it was assumed that the complete overlap in activities for TST and 3αHST probably meant that these two enzymes were not only similar in size but also in charge. If this was true, then isolating TST would indeed be a daunting undertaking. Therefore, to determine whether this was the case, it was decided to overexpress 3αHST and 3βHST and examine the purified enzymes for TST activity. By doing so, it is now clear that 3αHST can function as a TST. This finding suggests that, in the guinea pig, a specific sulfotransferase acting on testosterone may not exist, although it does not necessarily rule the possibility out. Even more interesting was the finding that 3αHST will not only sulfonate testosterone, it will also sulfonate estradiol. It is noteworthy that the fact that 3αHST would not sulfonate estrone indicated that estradiol was sulfonated only at the 17β-hydroxy position. Thus, these findings are interpreted to mean that 3αHST can function as general 17β-hydroxysteroid sulfotransferase. This conclusion must, however, be considered tentative, because 17α-hydroxylated steroids, particularly epitestosterone and epiestradiol, have not as yet been examined as substrates; therefore, stereospecificity for the 17β-hydroxy configuration remains to be fully established.

Table 1
Kinetic analysis of overexpressed and purified 3α- and 3β-hydroxysteroid sulfotransferase[a]

	Preg	DHEA	Allopreg	Andro	Testo	Estrad
3αHST						
V_{max} (fmol/min)	ND	ND	390	800	27	18
K_m (nM)	ND	ND	190	260	200	125
V_{max}/K_m	ND	ND	2.1	3.1	0.13	0.14
Rel.%	ND	ND	68.2	100	4.4	4.7
3βHST						
V_{max} (fmol/min)	310	212	ND	ND	ND	ND
K_m (nM)	250	788	ND	ND	ND	ND
V_{max}/K_m	1.24	0.27	ND	ND	ND	ND
Rel.%	100	21.8	ND	ND	ND	ND

[a] Preg, pregnenolone; DHEA, dehydroepiandrosterone; Allopreg, allopregnanolone; Andro, androsterone; Testo, testosterone; Estrad, estradiol; 3αHST, 3α-hydroxysteroid sulfotransferase; 3βHST, 3β-hydroxysteroid sulfotransferase; ND, not detectable; Rel.%, percentage of highest V_{max}/K_m.

It is known that estradiol can be sulfonated at both the 3-phenol and 17β-hydroxy positions [1]. Cloned guinea pig estrogen sulfotransferase, however, will only sulfonate estradiol at the 3-phenol position and will not transfer a sulfonate group to the 17β-hydroxy position of estradiol [36]. It now appears that estradiol-17β-yl sulfonate, at least in the guinea pig, may be formed by 3αHST. That 3αHST but not 3βHST will sulfonate steroids at the 17β-hydroxy position, although intriguing, is not easily understood (the two enzymes are 87% identical [29]). Nevertheless, there is precedent for this unexpected behavior of 3αHST. For instance, the steroid metabolizing enzyme 3α-hydroxysteroid dehydrogenase (3αHSD), type 2, which has been cloned and characterized, has been reported to also function as a 17β-hydroxysteroid dehydrogenase (17βHSD) [37]. As commented, the ability of 3αHSD to be positionally selective and stereospecific on the one hand and indiscriminate on the other invokes the subject of steroid pocket architecture [37]. It was suggested that, for 3α/17βHSD, steroid substrates would have to bind backward to maintain stereochemistry of hydride transfer; ie, the D-ring would enter the active site first instead of the A-ring and be upside down with the β-face of the steroid in the α-face orientation [37]. Conceivably, 3αHST can be thought to function in a similar manner. Furthermore, this suggests that the 3βHST isoform can function in a comparable manner, ie, the 3βHST isoform will sulfonate 17α-hydroxysteroids (this provocative idea is being pursued).

The finding that 3αHST also functions as TST in the guinea pig suggests the idea of tissue expression of 3αHST and 3βHST. To investigate this issue, cytosol was prepared from multiple guinea pig tissues and assayed for 3αHST- and 3βHST-specific activities (Fig. 7). In the adrenal, 3αHST specific activity was about six times that of 3βHST specific activity. In contrast, the 3βHST-specific activity clearly exceeded that for 3αHST in liver (by $\approx$75%) and

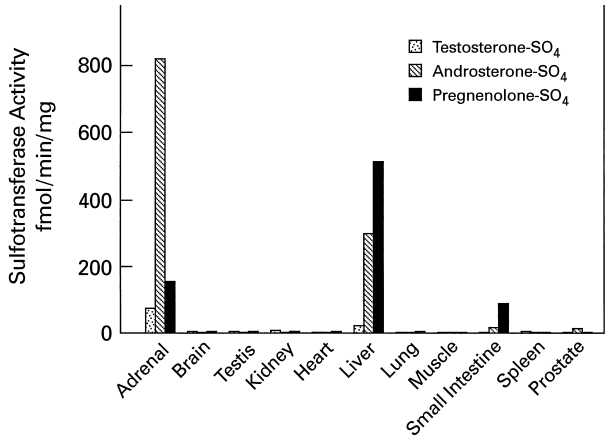

Fig. 7. Sulfotransferase activities in the cytosol of various guinea pig tissues. Cytosol was prepared and sulfotransferase activity was assayed (0.2 mg) by using testosterone (2 μM, stippled columns), androsterone (4 μM, hatched columns), and pregnenolone (8 μM, solid columns) as substrates, essentially as described in Section 2.

small intestine (by about fivefold); there was a small but detectable amount of 3αHST activity in the prostate. In several other tissues, including the testis, 3αHST and 3βHST activities were essentially undetectable with this procedure (Fig. 7).

References

[1] Bernstein S, Solomon S. Chemical and biological aspects of steroid conjugation. New York: Springer-Verlag, 1970.
[2] Hobkirk R. Steroid sulfotransferases and steroid sulfatases: characteristics and biological roles. Can J Biochem Cell Biol 1985;63:1127–44.
[3] Strott CA. Steroid sulfotransferases. Endocr Rev 1996;17:670–97.
[4] Roy AB. Enzymological aspects of steroid conjugation. In: Bernstein S, Solomon S, editors. Chemical and biological aspects of steroid conjugation. New York: Springer-Verlag, 1970. pp. 74–130.
[5] Roy AB. Sulfotransferases. In: Mulder GJ, editor. Sulfation of drugs and related compounds. Boca Raton: CRC Press, 1981. pp. 83–130.
[6] Mulder GJ, Jakoby WB. Sulfation. In: Mulder GJ, editor. Conjugation reactions in drug metabolism: an integrated approach. New York: Taylor and Francis, 1990. pp. 107–61.
[7] Huxtable RJ. Biochemistry of sulfur. New York: Plenum, 1986.
[8] Roy AK. Regulation of steroid hormone action in target cells by specific hormone-inactivating enzymes. Proc Soc Exp Biol Med 1992;199:265–72.
[9] Nash AR, Glenn WK, Moore SS, Kerr J, Thompson AR, Thompson EOP. Oestrogen sulfotransferase: molecular cloning and sequencing of cDNA for bovine placental enzyme. Aust J Biol Sci 1988;41:507–16.
[10] Demyan WF, Song CS, Kim DS, Her S, Gallwitz W, Rao TR, Slomczynska M, Chatterjee B, Roy AK. Estrogen sulfotransferase of the rat liver: complementary DNA cloning and age- and sex-specific regulation of messenger RNA. Mol Endocrinol 1992;6:589–97.
[11] Oeda T, Lee YC, Driscoll WJ, Strott CA. Molecular cloning and expression of a full-length complementary DNA encoding the guinea pig adrenocortical estrogen sulfotransferase. Mol Endocrinol 1992;6:1216–26.
[12] Aksoy IA, Wood TC, Weinshilboum R. Human liver estrogen sulfotransferase: identification by cDNA cloning and expression. Biochem Biophys Res Commun 1994;200:1621–9.
[13] Song W-C, Moore R, McLachlan JA, Negishi M. Molecular characterization of a testis-specific estrogen eulfotransferase and aberrant liver expression on obese and diabetogenic C57BL/KsJ-db/db mice. Endocrinology 1995;136:2477–84.
[14] Chatterjee B, Roy AK. Changes in hepatic androgen sensitivity and gene expression during aging. J Steroid Biochem Mol Biol 1990;37:437–45.
[15] Laatikainen T, Laitinen EA, Vihko R. Secretion of neutral steroid sulfates by the human testis. J Clin Endocrinol 1969;29:219–24.
[16] Laatikainen T, Laitinen EA, Vihko R. Secretion of free and sulphate conjugated neutral steroids by the human testis: effect of administration of human chorionic gonadotropin. J Clin Endocrinol 1971;32:59–64.
[17] Ruokonen A, Laatikainen T, Laitinen EA, Vihko R. Free and sulfate-conjugated neutral steroids in human testis tissue. Biochemistry 1972;11:1411–6.
[18] Vihko R, Ruokonen A. Steroid sulphates in human adult testicular steroid synthesis. J Steroid Biochem 1975;6:353–6.
[19] Jaffe RB, Payne AH. Gonadal steroid sulfates and sulfatase. IV. Comparative studies on steroid sulfokinase in the human fetal testis and adrenal. J Clin Endocrinol Metab 1971;33:592–6.
[20] Payne AH. Testicular steroid sulfotransferases: comparison to liver and adrenal steroid sulfotransferases of the mature rat. Endocrinology 1980;106:1365–70.
[21] Lyon ES, Jakoby WB. The identity of alcohol sulfotransferases with hydroxysteroid sulfotransferases. Arch Biochem Biophys 1980;202:474–81.
[22] Marcus CJ, Sekura RD, Jakoby WB. A hydroxysteroid sulfotransferase from rat liver. Anal Biochem 1980;107:296–304.
[23] Falany CN, Vazquez ME, Kalb JM. Purification and characterization of human dehydroepiandrosterone sulphotransferase. Biochem J 1989;260:641–6.
[24] Ogura K, Satsukawa M, Okuda H, Akira H, Watabe T. Major hydroxysteroid sulfotransferase STa in rat liver cytosol may consist of two microheterogeneous subunits. Chem Biol Interact 1994;92:129–44.
[25] Yoshinari K, Nagata K, Shiraga T, Iwasaki K, Hata T, Ogino K, Ueda R, Fujita K, Shimada M, Yamazoe Y. Molecular cloning, expression, and enzymatic characterization of rabbit hydroxysteroid sulfotransferase AST-RB2 (ST2A8). J Biochem 1998;123:740–6.
[26] Falany CN, Wheeler J, Oh TS, Falany JL. Steroid sulfation by expressed human cytosolic sulfotransferases. J Steroid Biochem Mol Biol 1994;48:369–75.
[27] Falany CN, Comer KA, Dooley TP, Glatt H. Human dehydroepiandrosterone sulfotransferase. Ann NY Acad Sci 1995;774:59–72.
[28] Lee YC, Park C-S, Strott CA. Molecular cloning of a chiral-specific 3α- hydroxysteroid sulfotransferase. J Biol Chem 1994;269:15838–45.
[29] Luu NX, Driscoll WJ, Strott CA. Molecular cloning and expression of a guinea pig 3-hydroxysteroid sulfotransferase distinct from chiral-specific 3β- hydroxysteroid sulfotransferase. Biochem Biophys Res Commun 1995;217:1078–86.
[30] Driscoll WJ, Martin BM, Chen H-C, Strott CA. Isolation of two distinct 3- hydroxysteroid sulfotransferases from the guinea pig adrenal. J Biol Chem 1993;268:23496–503.
[31] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 1970;227:680–5.
[32] Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350–4.

[33] Lee YC, Driscoll WJ, Strott CA. Charge isoforms of the adrenocortical pregnenolone-binding protein: influence of phosphorylation on isoformation and binding activity. Proc Natl Acad Sci USA 1990;87:2003–7.
[34] Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[35] Whitnall MH, Driscoll WJ, Lee YC, Strott CA. Estrogen and hydroxysteroid sulfotransferases in guinea pig adrenal cortex: cellular and subcellular distributions. Endocrinology 1993;133:2284–91.
[36] Tomizuka T, Oeda T, Tamura Y, Yoshida S, Strott CA. Characterization of guinea pig estrogen sulfotransferase expressed by Chinese Hamster Ovary cell-K1 stable transfectants. Endocrinology 1994; 135:938–43.
[37] Lin H-K, Jez JM, Schlegel BP, Peehl DM, Pachter JA, Penning TM. Expression and characterization of recombinant type 2 3α-hydroxysteroid dehydrogenase (HSD) from human prostate: demonstration of bifunctional 3α/17β-HSD activity and cellular distribution. Mol Endocrinol 1997;11:1971–84.