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Steroid Sulfation by Expressed Human Cytosolic Sulfotransferases

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The human cytosolic sulfotransferases (STs), dehydroepiandrosterone sulfotransferase (DHEA-ST) and the phenol-sulfating form of phenol sulfotransferase, (P-PST), have been expressed in bacteria and used to investigate the ability of the cloned enzymes to conjugate steroids and related compounds. DHEA-ST was capable of sulfating all of the 3-hydroxysteroids, testosterone and estrogens tested as substrates. The 3-hydroxysteroids, androsterone, epiandrosterone and androstenediol, were conjugated at 50–60% of the rate of DHEA. Of the steroids tested, P-PST was capable of conjugating only the estrogens. The catechol estrogens, 2-hydroxyestradiol, 4-hydroxyestradiol and 4-hydroxyestrone, and compounds with estrogenic activity such as 17α-ethynylestradiol and *trans*-4-hydroxytamoxifen, were also tested as substrates. DHEA-ST showed little or no sulfation activity with these compounds; however, all of these compounds were sulfated by P-PST. These results indicate that the expressed human STs are valuable in analyzing the overlapping substrate specificities of these enzymes and that P-PST may have an important role in the metabolism of estrogens and estrogenic compounds in human tissues.

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INTRODUCTION

Sulfate conjugation is a major pathway in humans for the biotransformation of steroids and bile acids. Large amounts of steroid sulfates, such as dehydroepiandrosterone sulfate and pregnenolone sulfate, are synthesized and secreted from the adrenals and serve as precursors for the synthesis of androgens and estrogens [1, 2]. Sulfation of steroids and bile acids in the liver is considered to have both an inactivation and protection function [3, 4]. Sulfation may also be a mechanism for controlling steroid hormone levels in target tissues, as the sulfated steroid conjugates are less biologically active than the unconjugated forms, possibly because sulfation renders the steroids incapable of combination with specific hormone receptors.

Sulfation is catalyzed by a family of enzymes termed sulfotransferases (STs) which represent a family of enzymes involved in the biotransformation of a variety of endogenous and exogenous substrates via conjugation with a sulfonate moiety. The STs catalyze the transfer of the sulfonate moiety from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to a hydroxyl group of the steroid, to form a sulfate ester.

The purification and characterization of at least two different human liver cytosolic STs involved in the conjugation of steroids has been reported. These enzymes are dehydroepiandrosterone ST (DHEA-ST) and the phenol-sulfating form of phenol sulfotransferase (P-PST) [5–7]. DHEA-ST has been reported to conjugate a number of different steroids and is responsible for most of the bile acid sulfation activity in human liver [7, 8]. Included among the steroids sulfated by DHEA-ST are androgens, estrogens, testosterone and pregnenolone, whereas P-PST has been reported to sulfate only estrone and β-estradiol [6, 9]. In addition, the cDNAs for both DHEA-ST and P-PST have been isolated from human liver cDNA libraries and the active enzymes have been expressed in mammalian cells [10–12]. These expressed STs are involved in steroid sulfation in the same manner as are the native human liver forms of the enzymes. Although P-PST has been expressed relatively well by mammalian cells, DHEA-ST has been expressed only at much lower levels. The low level of DHEA-ST expression hinders the ability to characterize the properties of the enzyme.

This report describes the expression of both DHEA-ST and P-PST in bacteria and the initial characterization of their kinetic properties. Bacterial expression of these STs is advantageous because the STs are expressed at much higher levels than in mammalian cells and it is possible to express and purify significant amounts of both DHEA-ST and P-PST

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from bacteria without the interference of any other mammalian STs. Expressed DHEA-ST and P-PST have been used to study the sulfation of steroids and therapeutic estrogenic compounds. In addition to endogenous steroids, we examined the ability of the expressed forms of P-PST and DHEA-ST to sulfate several catechol estrogens, 17α-ethynylestradiol, and 4-hydroxytamoxifen (4-OH-Tam) which is a biological metabolite of the drug tamoxifen used in the treatment of breast cancer.

MATERIALS AND METHODS

Oligonucleotide primers were purchased from Midland Certified Reagent Company (Midland, TX). Polymerase chain reaction (PCR) reagents were purchased from Perkin-Elmer Cetus (Norwalk, CT). Restriction enzymes were purchased from New England Biolabs (Beverly, MA). T4 ligase was purchased from Promega (Madison, WI). *E. coli* XL1-Blue cells were purchased from GIBCO-BRL (Grand Island, NY). [^{35}S]PAPS (2.2 Ci/mmol) was purchased from New England Nuclear (Boston, MA). PAPS was purchased from Dr Sanford Singer (Dayton, OH). [1,2,6,7-^{3}H]DHEA (78 Ci/mmol), [6,7-^{3}H]β-estradiol (45 Ci/mmol), [2,4,6,7-^{3}H]estrone (96 Ci/mmol), [7-^{3}H]pregnenolone (25 Ci/mmol), and [7-^{3}H]testosterone (27.7 Ci/mmol) were purchased from New England Nuclear. DHEA, estradiol, estrone, pregnenolone, androsterone, epiandrosterone, androstenediol, testosterone, 3′,5′-diphosphoadenosine (PAP)–agarose, and DEAE–Sepharose Cl-6B were purchased from Sigma Chemical Co. (St Louis, MO). *Trans* 4-OH-Tam was a gift from ICI (Paris, France). All other reagents were of reagent grade quality.

Synthesis of bacterial sulfotransferase expression vectors

In order to express relatively large quantities of the active ST enzymes, the cDNAs for P-PST and DHEA-ST were subcloned into a bacterial expression vector. To subclone P-PST into the pKK233-2 bacterial expression vector (Pharmacia, Piscataway, NJ), a primer was synthesized which incorporated a single base change to create an Nco I restriction site at the initiating methionine of the P-PST cDNA (Fig. 1). P-PST subcloned into the mammalian expression vector, pSV-SPORT-1, was used as a template for PCR amplification [12]. PCR was performed using the P-PST/Nco I primer (Fig. 1) and the T7 primer. The amplified DNA was digested with the restriction enzymes Nco I and Hind III, purified from low melting agarose, and subcloned into the Nco I and Hind III sites of pKK233-2. The mutated P-PST/pKK233-2 was transfected into *E. coli* XL1-Blue cells made competent using a calcium chloride procedure [13]. Colonies were selected by ampicillin resistance, and those colonies containing the Nco I–Hind III P-PST DNA fragment in the correct orientation were identified by restriction mapping and DNA sequencing.

A. P-PST

```
5′-AG  CTC  AGG  AAC  ATG  GAG  CTG-3′
60                   Met  Glu  Leu  79

P-PST/Nco I Primer
5′-AG  CTC  AGG  ACC  ATG  GAG  CTG-3′
60                   Met  Glu  Leu  79
```

B. DHEA-ST

```
5′-GAG  GTC  ATC  ATC  ATG  TCG  GAC  G-3′
41                   Met  Ser  Asp   61

DHEA-ST/Nco I Primer
5′-GAG  GTC  ATC  ACC  ATG  GCG  GAC  G-3′
41                   Met  Ala  Asp   61
```

Fig. 1. Generation of Nco I restriction sites incorporating the initial methionines of the cDNAs for P-PST and DHEA-ST. (A) An oligonucleotide (P-PST/Nco I primer) was synthesized identical to nucleotides 60–79 of the cDNA for P-PST [12] except that a cytosine was substituted for the adenosine at position 69. The generated Nco I restriction site is underlined. (B) An oligonucleotide (DHEA-ST/Nco I primer) was synthesized identical to nucleotides 41–61 of the cDNA for DHEA-ST [10] except that a cytosine was substituted for the thymine at position 51 and a guanine was substituted for the thymine at position 56. This resulted in the conversion of the second amino acid in the translated sequence from a serine to an alanine. The generated Nco I restriction site is underlined.

A similar procedure was used to subclone the cDNA for DHEA-ST into the Nco I and Hind III sites of pKK233-2. An oligonucleotide was synthesized as a PCR primer and two nucleotides were changed to create an Nco I site incorporating the initiating methionine of the DHEA-ST cDNA [10]. As a result of these changes, the second amino acid, Ser, was changed to an Ala (Fig. 1). The cDNA for DHEA-ST also has an internal Nco I site. The smaller 3′ Nco I–Hind III cDNA fragment (250 bp) was subcloned into pKK233-2 first. The larger 5′ Nco I–Nco I fragment (850 bp) was generated by PCR using the DHEA-ST/Nco I primer and the T7 primer. The pSV-SPORT-1-DHEA-ST8 mammalian expression vector was used as a template. The Nco I–Nco I fragment was subcloned into the Nco I site of pKK233-2 containing the smaller 3′ Nco I–Hind III fragment. The correct orientation of the complete clone was confirmed by restriction mapping and DNA sequence analysis. The DHEA-ST/pKK233-2 clone with the incorrectly oriented Nco I–Nco I fragment was used as a negative control.

Bacterial expression of sulfotransferase activity

E coli XL1-Blue cells transfected with pKK-P-PST or pKK-DHEA-ST were grown to late log phase ($O.D._{600} = 0.5$) in Luria broth (LB) with ampicillin (200 μg/ml) and induced overnight with 0.5 mM isopropy-beta-D-thioglactopyranoside. Cells were pelleted and resuspended in bacterial lysis buffer (75 mM Tris–HCl pH 8, 0.25 M sucrose, 0.25 mM EDTA, 0.02 mg/ml lysozyme) and incubated 20 min on ice. Cells were repelleted at 3000*g*, resuspended in TEA

buffer (10 mM TEA, pH 7.5, 10% glycerol, 1.5 mM DTT, 10 μg/ml phenolmethylsulfonylfluoride), and sonicated 4 × with 10 s bursts and 30 s cooling between each burst. A final centrifugation at 100,000*g* for 1 h was performed and the supernatant fraction was used for ST assays and/or purification of the ST enzymes. The ST enzymes prepared by this method were further purified using DEAE–Sepharose 4B chromatography and PAP–agarose affinity chromatography as described previously [5, 6].

P-PST activity was assayed with [^{35}S]PAPS using minoxidil as a substrate as described previously [14]. Steroid ST assays were performed using ^{3}H-labeled steroids and nonradiolabeled PAPS. The ^{3}H-steroid sulfates were separated from the ^{3}H-steroids using the alkaline–chloroform extraction procedure described by Falany *et al.* [6].

Sulfation of steroids and related compounds by expressed human liver STs

Sulfation activity was determined using steroids and related compounds as substrates. The reactions contained the appropriate substrates dissolved in ethanol, 7 mM $MgCl_2$, 50 mM Tris–HCl, pH 7.4 and 20 μM [^{35}S]PAPS in a final volume of 0.125 ml. Control reactions were identical except the substrate for sulfation were not added. Reactions were incubated for various times at 37°C and were terminated by spotting a 50 μl aliquot of each reaction on a silica gel F-250 TLC plate. The plate was developed in methylene chloride–MeOH–ammonium hydroxide (81:15:5, by vol) [15] and the radiolabeled sulfated products were localized by autoradiography. The sulfated products were scraped into scintillation fluid and the radioactivity determined by scintillation spectroscopy.

Immunoblot analysis of expressed sulfotransferases

P-PST and DHEA-ST expressed in bacteria were analyzed for immunoreactivity by immunoblot analysis as described previously using specific rabbit antihuman PST and DHEA-ST antibodies [5, 7, 16].

RESULTS

Functional expression of P-PST and DHEA-ST in bacteria

In order to more easily compare the enzymatic properties of P-PST and DHEA-ST, the two enzymes were expressed in *E coli*. For efficient expression of the ST cDNAs in the bacterial expression vector, pKK233-2, Nco I restriction enzyme sites incorporating the initial translated methionines of both cDNAs were created using PCR with the P-PST and DHEA-ST pSV-SPORT-1 expression vectors as templates as described in Materials and Methods. The amino acid sequence of P-PST was not altered by the generation of the Nco I site. However, the second amino acid in DHEA-ST was changed from a serine to an alanine. The modified P-PST and DHEA-ST cDNAs were ligated into the Nco I and Hind III restriction sites of pKK233-2 and expressed in *E coli* XL1-Blue cells.

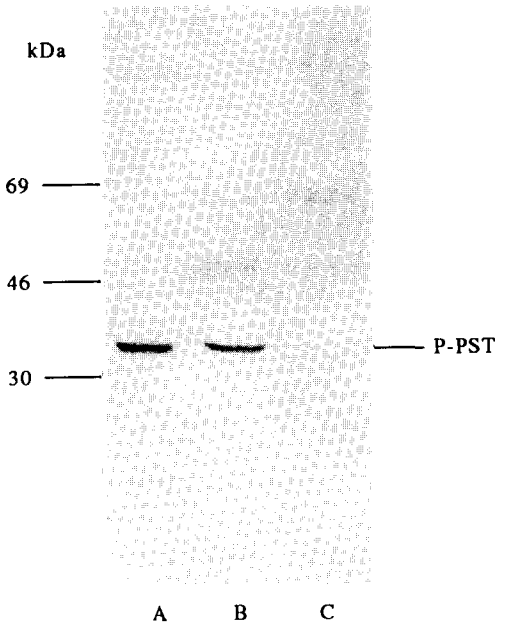

Fig. 2. Western blot analysis of P-PST expressed in *E. coli* XL1-Blue cells. Samples of cytosol prepared from bacteria transformed with the P-PST/pKK233-2 expression vector or the pKK233-2 vector alone were resolved in a 12.5% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane and reacted with rabbit antihuman PST IgG [5, 16]. Lane A contained 20μg human liver cytosol. Lane B contained 120 μg of cytosol from XL1-Blue cells transformed with the pKK233-2/P-PST vector and lane C contained 120 μg of cytosol from XL1-Blue cells transformed with the pKK233-2 alone. The arrows in the margin denote the migration of molecular weight standards.

To confirm that the expressed STs are similar to the liver forms of the enzymes, the expressed proteins were analyzed by immunoblot analysis and kinetic characterization. Figures 2 and 3 show the presence of immunoreactive P-PST and DHEA-ST in cytosol prepared from bacteria transformed with pKK233-2 containing the P-PST or DHEA-ST cDNAs, respectively. No immunoreactive protein was detected by either rabbit antihuman DHEA-ST or antihuman PST antibodies in cells transformed with pKK233-2 alone. Both bacterially expressed P-PST and DHEA-ST migrated with the same subunit molecular mass as the liver forms of the enzymes. Variable amounts of PAPS degradative activities were present in the bacterial cytosolic preparations of the expressed STs so the enzymes were routinely purified as described previously to remove these contaminating activities [5, 6]. Figure 4 shows an SDS-polyacrylamide gel of expressed P-PST purified from bacterial cytosol by DEAE–Sepharose CL-6B and PAP–agarose chromatography. No apparent size modification or proteolytic degradation was observed during or following expression of both enymes.

Sulfation activities of the expressed STs

The ability of the expressed STs to sulfate a variety of different steroids and related compounds was tested

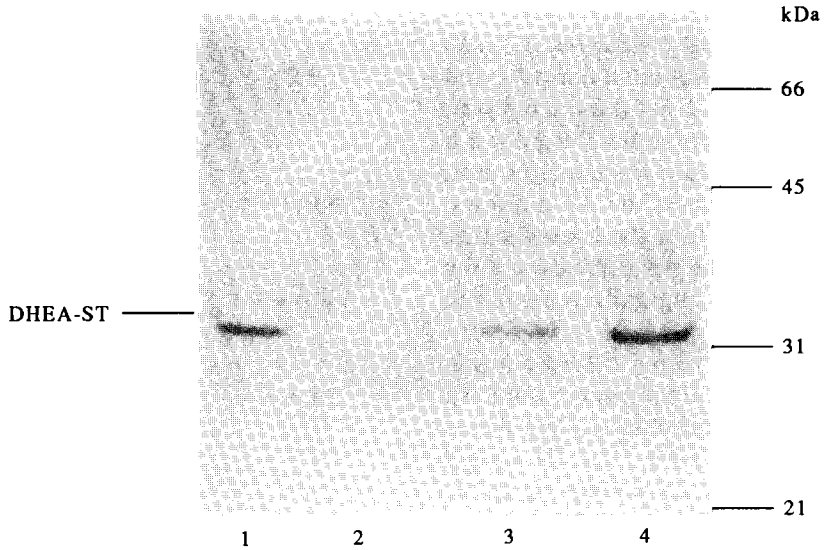

Fig. 3. Western blot analysis of DHEA-ST expressed in *E. coli* XL1-Blue cells. Samples of cytosol prepared from bacteria transformed with the DHEA-ST/pKK233-2 expression vector or the pKK233-2 vector alone were resolved in a 12.5% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane and reacted with rabbit antihuman DHEA-ST IgG [7]. Lanes 1 and 4 contain 10 and 20 μg human liver cytosol, respectively. Lane 3 contained 120 μg of cytosol from XL1-Blue cells transformed with the pKK233-2/DHEA-ST vector and lane 2 contained 120 μg of cytosol from XL1-Blue cells transformed with the pKK233-2 alone. The arrows in the margin denote the migration of molecular weight standards and immunoreactive DHEA-ST.

to determine their roles in the conjugation of these compounds in the absence of contaminating ST activities. Sulfation activity was initially assayed with both enzymes in the presence of increasing concentrations of each substrate to determine the concentration giving maximal sulfation activity. This was necessary as the STs frequently display substrate inhibition with increasing steroid concentrations [6]. Figure 5 shows that P-PST and DHEA-ST activities expressed in bacteria are both capable of conjugating estrogenic steroids. P-PST showed maximum activity at 6 μM for β-estradiol and substrate inhibition was observed at concentrations above 6 μM. DHEA-ST expressed in bacteria had maximal activity for β-estradiol sulfation at 20 μM and substrate inhibition was observed for β-estradiol concentrations above 20 μM.

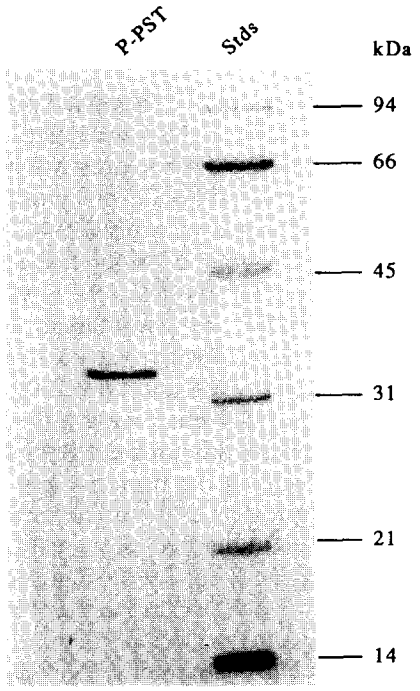

Fig. 4. SDS-polyacrylamide gel of expressed P-PST purified from bacterial cytosol. P-PST was purified from bacterial cytosol by DEAE–Sepharose CL-6B and PAP–agarose chromatography [5]. SDS-polyacrylamide gel electrophoresis was performed in a 12.5% gel and the migration of pure P-PST (2 μg) was compared to molecular weight standards (Bio-Rad).

Table 1 shows that of the steroids and related compounds tested, DHEA-ST was capable of conjugating all of the steroids tested including 3-hydroxysteroids such as DHEA and androsterone, as well as the 17β-hydroxyl of testosterone and the 3-phenolic hydroxyl of estrone. A comparison of the relative sulfation rates of the different steroids assayed at the concentrations giving maximal sulfation rates shows that DHEA was the preferred substrate and that a relatively low concentration was required for maximal activity. DHEA-ST sulfated estrone at a slightly higher rate than β-estradiol; however, the concentration of estrone required for maximal activity was higher than that of β-estradiol. The 3-hydroxysteroids, androsterone, epiandrosterone and androstenediol, were conjugated at rates 50–60% that of DHEA, but the concentrations required for maximal activity were significantly greater. DHEA-ST was also capable of conjugating 17α-ethynylestradiol at approx. 15% the rate of DHEA although the concentration of 17α-ethynylestradiol giving maximal activity was lower than that of DHEA. DHEA-ST did not show detectable activity with the catechol estrogens, 2-hydroxyestradiol, 4-hydroxyestrone, nor with the antiestrogenic metabolite of tamo-

xifen, *trans*-4-hydroxytamoxifen, but displayed some activity towards 4-hydroxyestradiol.

In contrast to DHEA-ST, P-PST was capable of sulfating the estrogenic steroids, estrone and β-estradiol, at detectable levels. Using the steroid concentrations providing maximal sulfation activity, P-PST conjugated estrone at approximately twice the rate of β-estradiol but at a 2.5-fold higher concentration. No activity was observed with any of the 3-hydroxysteroids or testosterone as substrates. P-PST was also capable of sulfating 17α-ethynylestradiol at a greater rate than it sulfated 17β-estradiol. The catecholestrogens, 2-hydroxyestradiol, 4-hydroxyestrone and 4-hydroxyestradiol, were efficiently conjugated by P-PST as was *trans*-4-hydroxytamoxifen. 2-Hydroxyestradiol demonstrated maximal activity at the lowest concentration (0.8 μM) of any of the substrates tested.

Table 1. Sulfation of steroids and related compounds by expressed DHEA-ST and P-PST

Steroids	Relative activity[a,b] DHEA-ST %	(μM)	P-PST %	(μM)
Dehydroepiandrosterone	100	(3 μM)	ND[c]	
Testosterone	26	(5 μM)	ND	
β-Estradiol	34	(20 μM)	45	(6 μM)
Estrone	43	(25 μM)	100	(15 μM)
Androsterone	65	(6 μM)	ND	
Dexamethasone	ND		ND	
Epiandrosterone	59	(8 μM)	ND	
Androstenediol	55	(12 μM)	ND	
2-Hydroxyestradiol	ND		122	(0.8 μM)
17α-Ethynylestradiol	14	(2 μM)	151	(1.5 μM)
4-Hydroxytamoxifen	ND		15	(10 μM)
4-Hydroxyestradiol	8	(20 μM)	129	(10 μM)
4-Hydroxyestrone	ND		118	(10 μM)

[a]ST activity is expressed relative to dehydroepiandrosterone sulfation for DHEA-ST and β-estradiol sulfation for P-PST.
[b]Steroid sulfation activity was assayed with both enzymes with the steroid concentration giving maximal activity.
[c]ND represents no detectable activity.

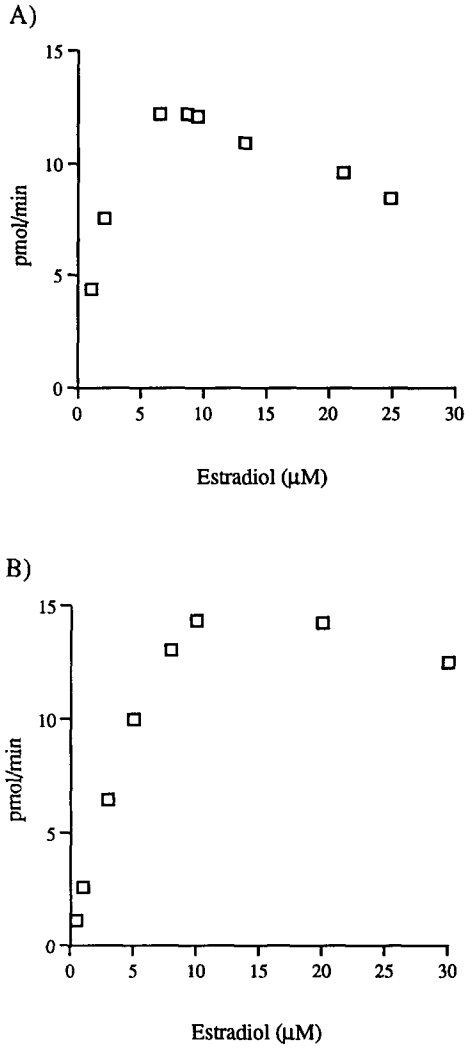

Fig. 5. Sulfation of β-estradiol catalyzed by expressed human P-PST and DHEA-ST. The effect of increasing concentrations of β-estradiol as a substrate was tested using expressed P-PST (A) and DHEA-ST (B) activity after partial purification using DEAE–Sepharose CL-6B chromatography [5, 6].

DISCUSSION

The STs are a family of enzymes involved in the biotransformation of steroids, bile acids and a large number of other types of compounds. The individual members of the ST family have broad overlapping substrate reactivities which may cause problems in determining the substrate specificities of the individual enzymes. In order to clarify the nature of the STs involved in the sulfation of steroids and related compounds, bacterially expressed DHEA-ST and P-PST were used. The advantage of using the bacterially expressed STs is that their enzymatic properties can be characterized in the absence of any contaminating mammalian ST activities.

Although P-PST is well expressed by Cos 7 cells using a vector containing the SV40 early promotor, DHEA-ST is expressed only at very low levels [10, 12]. Therefore, bacterial expression of both P-PST and DHEA-ST was advantageous in that both enzymes could be expressed in large quantities and at relatively high levels of activity as compared to the Cos 7 expression system. One disadvantage of the bacterial expression system is the presence in bacterial cytosol of an endogenous PAPS degradative activity which resulted in ST activities which were non-linear with respect to time. This was overcome by partially purifying DHEA-ST and P-PST activities from bacterial cytosol by DEAE Cl-6B Sepharose chromatography [5, 6]. Immunoblot analysis of both expressed DHEA-ST and P-PST with the appropriate antibodies gives bands which correspond completely with the bands produced by the human liver forms of these enzymes, indicating that there are no apparent post-translational modifications of the expressed proteins by the bacteria that alter their migration during SDS–PAGE. The single amino acid modification of DHEA-ST to facilitate its cloning into pKK233-2 does not alter its

apparent molecular mass, kinetic or immunogenic properties.

The importance of steroid sulfation in human tissues is well established. DHEA-ST has a prominent role in the synthesis and secretion of DHEA-sulfate from the human adrenal cortex and in the synthesis of steroid and bile acid sulfates in human liver [1, 4]. DHEA-ST has also been reported to be responsible for most, if not all, of the sulfation of bile acids in human liver [8]. Previous studies have indicated that DHEA-ST is capable of conjugating a wide range of steroids [6, 7, 17]. The data in this study confirms that expressed human liver DHEA-ST also has a relatively broad substrate specificity for steroids and is capable of conjugating a number of different steroids including estrogens and testosterone.

Bacterially expressed DHEA-ST and P-PST were used to compare the activities of these enzymes towards several common steroid substrates. P-PST was capable of conjugating only the estrogens of the steroids tested. Hernandez *et al.* [9] have reported that P-PST is the enzyme predominantly responsible for β-estradiol sulfation in human liver, whereas DHEA-ST is primarily responsible for estrone sulfation. β-Estradiol reactions were run at a substrate concentration of 100 μM and estrone reactions were run at 25 μM for both enzymes using partially purified preparations of the STs. The results of the present study indicate that DHEA-ST sulfates estrone at a slightly higher rate than β-estradiol and that P-PST sulfates estrone at more than twice the rate it sulfates β-estradiol. However, we found that both DHEA-ST and P-PST had a different optimal concentration for sulfation of each substrate so the assays were run at the appropriate optimal concentrations. The differences in steroid substrate concentrations used in these studies may account for the differences in sulfation rates.

The observation that both DHEA-ST and P-PST may sulfate estrogens suggests that previous reports of estrogen ST activity in human tissues may well be in part describing the activity of either of these two enzymes [15, 18, 19]. The existence of a distinct estrogen ST in human tissues needs to be determined at the genomic DNA level or via molecular cloning techniques since protein purification and characterization techniques have not been sufficient to confirm its existence. An estrogen ST cDNA has been cloned from both bovine and rat tissues [20, 21] and the rat estrogen ST amino acid sequence is 67% similar to the sequence of the rat PST, minoxidil ST [22]. If a separate human estrogen ST does not exist, the roles of DHEA-ST and P-PST in estrogen sulfation need to be re-evaluated with the assumption that they are the enzymes responsible for the majority of estrogen sulfation in human tissues.

The addition of the second hydroxyl group to the estrogenic A-ring apparently greatly decreases the ability of DHEA-ST to sulfate the catechol estrogens. DHEA-ST displayed little or no activity with the 2- and 4-hydroxy-catecholestrogens, whereas P-PST readily conjugated these compounds at rates greater than those for β-estradiol. Interestingly, catecholamine neurotransmitters such as dopamine, are not readily sulfated by P-PST but are rapidly conjugated by the monoamine-sulfating form of PST, M-PST [23–25]. Whether or not M-PST has the ability to sulfate catechol estrogens is not known but it has been reported that M-PST is not involved in the sulfation of estrogens in humans [9].

The ability of P-PST to conjugate 17α-ethynylestradiol and *trans*-4-hydroxytamoxifen indicates that estrogen sulfation activity may not directly correlate with the sulfation of other steroids or related estrogenic or antiestrogenic compounds in a given tissue. Tissues possessing high levels of estrogen sulfation activity due to the presence of DHEA-ST will also possess the ability to sulfate hydroxysteroids, whereas tissues containing high levels of P-PST may sulfate estrogens but not hydroxysteroids. In MCF-7 human breast carcinoma cells, P-PST is the prevalent cytosolic ST and is responsible for the majority of estrogen sulfation; the estrogen sulfation activity of DHEA-ST represents approx. 3% of the P-PST activity in these cells [26]. The presence of P-PST in MCF-7 cells also indicates that efficient sulfation of estrogen-related compounds, such as 17α-ethynylestradiol and *trans*-4-hydroxytamoxifen, is likely to occur. Therefore P-PST activity may also have a role in the response of estrogen-responsive mammary tumors to the effects of estrogenic drugs. These results indicate that understanding the kinetic properties of the individual STs will help the investigation of the presence and role of sulfation in normal human and tumor tissues.

Bacterially expressed DHEA-ST and P-PST have enabled us to characterize these STs in the absence of any other contaminating mammalian ST activities. These expressed STs have been characterized immunologically and also with respect to substrate specificity, optimal substrate concentration and relative rates towards different steroid substrates. Results correlate with and expand those previously found when these enzymes were studied from human tissue cytosolic preparations. It will be important to further elucidate the roles of these STs in steroid sulfation, both with regard to activity and localization in human tissues.

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