Identification by Chimera Formation and Site-selected Mutagenesis of a Key Amino Acid Residue Involved in Determining Stereospecificity of Guinea Pig 3-Hydroxysteroid Sulfotransferase Isoforms*

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The 3-hydroxysteroid sulfotransferases that have been isolated and cloned from humans and rodents appear to have broad substrate specificities. In the guinea pig, however, two 3-hydroxysteroid sulfotransferases have been isolated that function according to an innate stereospecificity: the α-isofrom acts on steroids with a 3-hydroxyl group oriented in the α position, whereas the β-isofrom acts on steroids where the 3-hydroxyl group is in a β orientation. To examine the structural basis for this remarkable stereoselectivity, chimeras of the two enzymes, which are 87% identical, were constructed. A chimera consisting of the NH₂-terminal 91 amino acids of the α-isofrom and the COOH-terminal 196 amino acids of the β-isofrom displayed activity similar to that of the α-isofrom. Site-selected mutagenesis of this 3αβ-hydroxysteroid sulfotransferase chimera involving the 12 amino acid differences that exist between the two isoforms within the 91 amino acid NH₂-terminal region revealed that the amino acid residue at position 51 plays a fundamental role in determining the stereospecificity exhibited by the α- and β-isofroms, i.e. if residue 51 is an asparagine, α activity predominates, whereas if an isoleucine is in that position, β activity prevails.

A major metabolic route in the biotransformation of steroidal compounds is via sulfoconjugation or sulfonation (1). Steroid sulfonation, which occurs widely across species (2), is catalyzed by a family of enzymes termed sulfotransferases, enzymes typically isolated from the cytosolic fraction of tissue preparations (3). Sulfotransferase enzymes are of immense biological significance in that they are involved in both genomic and non-genomic steroid action (4).

Steroid sulfotransferases acting on neutral steroids have been isolated and cloned from several species (4); these enzymes, although not always carefully examined, appear for the most part to have broad substrate specificities. An apparent exception to this rule was thought to be the guinea pig; in this species, two physically distinct forms of 3-hydroxysteroid sulfotransferase (HST), specifically a 3α-HST and a 3β-HST, were isolated from the adrenal gland (5). The guinea pig 3α-HST and 3β-HST enzymes demonstrated remarkable substrate selectivity with respect to the spatial orientation of the ring A 3-hydroxyl group (5). Furthermore, these enzymes were considered to be not only stereoselective but also site-specific in that they acted only on a 3-hydroxyl group. It was recently discovered, however, that the 3α-HST isoform, in contrast to the 3β-HST isoform, would also sulfonate the 17β-hydroxyl group of testosterone and estradiol (6). Nevertheless, with respect to the 3-hydroxyl group, the 3α-HST enzyme acts only on 3α-hydroxylated steroids such as androsterone and allopregnanolone, whereas the 3β-HST enzyme acts on 3β-hydroxylated steroids such as pregnenolone and dehydroepiandrosterone. Thus, regarding the 3-carbon position of neutral steroids, the guinea pig HST isoforms appear to behave quite differently from their rodent and human counterparts by exhibiting a more precise substrate stereospecificity. The 3α-HST isoform with a calculated molecular mass of 33,637 daltons and pl of 6.3 (7) and the 3β-HST isoform with a calculated molecular mass of 34,033 daltons and pl of 7.3 (8) have been cloned and kinetically analyzed.

The structural basis for the stereoselectivity of the 3α-HST and 3β-HST isoforms, which are 87% identical, is not known. Therefore, we set out to examine this question by creating chimeras of the two 3-HST isoforms in combination with site-selected mutagenesis. The results of these studies, which form the basis for this report, suggest that the 3-hydroxy stereospecificity shown by guinea pig 3α-HST and 3β-HST largely resides within the NH₂-terminal 91 amino acids of the proteins. In particular, the amino acid residues at position number 51 plays a crucial role.

EXPERIMENTAL PROCEDURES

Construction of Wild Type and Chimeric cDNAs

Full-length cDNAs encoding the 3α-HST and 3β-HST isoforms were subcloned into the bacterial expression vector pProEX HTc (Life Technologies, Inc.) at SaI and NotI sites. Briefly, the HST cDNAs (nucleotides −7 to +896) were amplified by PCR using primers designed to contain SaI and NolI sense primer (5'-ACGACTCGAGGACACATCTGATGATAACACTC-3') and antisense primer (5'-ATAACAATTGCGGCCACGAGATCTCCACATAATACACCCT-3') restriction enzyme sites. Amplified DNAs were purified by Wizard PCR Prep purification system (Promega, Madison, WI), digested with SaI and NolI, and purified by 1% agarose gel electrophoresis followed by Gene Clean II (Bio 101 Inc., Vista, CA). The restricted DNAs were ligated into the expression vector that was similarly digested and purified. The resulting plasmids (HST-pProEX HTc) were amplified using Max Efficiency DH5α compe-
The guinea pig 3α/β-hydroxysteroid sulfotransferase (3α/β-HST) chimera consists of amino acids 1–91 of 3α-hydroxysteroid sulfotransferase and amino acids 92–297 of 3β-hydroxysteroid sulfotransferase (cf. Fig. 2B). Location of amino acid substitutions are indicated for each numbered construct of the 3α/β-HST chimera (A) and 3β-hydroxysteroid sulfotransferase (3β-HST) wild type (B). For substitutions involving the 3α/β-HST chimera, α-HST amino acid residue substitutions precede the residue numbers, whereas the β-HST amino acid substitutions follow the residue numbers. Conversely, for the 3β-HST wild type enzyme, β-HST amino acid residue substitutions precede the residue numbers, whereas the α-HST amino acid substitutions follow the residue numbers (cf. amino acid sequences for 3α-HST and 3β-HST in Fig. 2A).

| Construct | Substitutions |
|-----------|---------------|
| A. 3α/β-HST chimera: |  |
| 1 | C17G; I22L |
| 2 | C17G; I22L; F80T; T81Q; G82H; Y84N |
| 3 | C17G; I22L; F80T; T81Q; G82H; Y84N; P6L |
| 4 | C17G; I22L; F80T; T81Q; G82H; Y84N; L65N; N71L |
| 5 | C17G; I22L; F80T; T81Q; G82H; Y84N; Y87M; G89S |
| 6 | C17G; I22L; F80T; T81Q; G82H; Y84N; N61L |
| 7 | I51N |
| 8 | I71N |

| B. 3β-HST wild type: |  |

*Table I*

**Primers used in site-selected mutagenesis**

| Construct No. | Primers |
|---------------|---------|
| 1 | 5'-CCTATGTTAGGTTCCACGATCTCCGATTATTTAAGGAAGTAGCCGGAC-3' |
| 2 | 5'-GTCCTGATCTCTAGGATTTCAACACAGAGTGGTTTGTTG-3' |
| 3 | 5'-GGACCACTTGCTTCTGCTCTGCTCTTCTC-3' |
| 4 | 5'-GGGATCCCCCAGTTGGGTCACTGACTGCCCCATGTTGGGATC-3' |
| 5 | 5'-GATCCCGAGATGGGCACTGATTGAAACTCCACTTGGGATCCCC-3' |
| 6 | 5'-GGTTAATGAACTAATGAAGAGTCAGGAGGACC-3' |
| 7 | 5'-GAAACCACTGTTGATTAAATGGTAGTTGCTGCC-3' |
| 8 | 5'-CCATGGACAACTTCAATTAAACCGTGGTCTC-3' |
| 9 | 5'-GAAACCACTGTTGATTAAATGGTAGTTGCTGCC-3' |
| 10 | 5'-GAAACCACTGTTGATTAAATGGTAGTTGCTGCC-3' |

**Site-selected Mutagenesis**

Site-selected mutagenesis was performed by PCR using Phu DNA polymerase according to the method of Quick Change site-directed mutagenesis (Stratagene, La Jolla, CA). Based on the amino acid sequence alignment of 3α-HST and 3β-HST, primers were designed to make amino acid substitutions at residues sites where a difference existed between the two isomers. In all, eight constructs were prepared as outlined in Table I; primers used to produce the eight constructs are depicted in Table II.

**Construct Numbers 1–6 Involved the 3α/β-HST Chimera (Table I)**

Each construct was produced using the appropriate primers (as denoted in Table II) and template (as indicated below). The aim here was to substitute β-HST-specific amino acid residues for α-HST-specific amino acid residues in the α-HST portion of the 3α/β-HST chimera. For construct number 1, the codons for amino acids Cys13 and Ile-22 in the chimera were mutated to yield Gly and Leu residues using the Self-β-HST chimera as template. For construct number 2, in addition to the mutations carried out for construct number 1, the codons for amino acids Phe-80, Thr-81, Gly-82, and Tyr-84 were altered to generate Thr, Gln, His, and Asn residues at those respective sites using construct number 1 as template. For construct numbers 3–6, construct number 2 was used as a template; in construct number 3, the codons for Pro-6 was changed to produce a Leu; in construct number 4, codons for Leu-65 and Asn-71 were mutated to yield Trp and Ile, respectively, at those sites; in construct number 5, codons for Val-87 and Gly-89 were altered to create Met and Ser, respectively, at those sites; in construct number 6, the codon for Asn-51 was changed to produce an Ile at that site.

**Construct Numbers 7 and 8 Involved Single Mutations of 3β-HST Wild Type (Table I)**

Each of these constructs was produced using the appropriate primers as detailed in Table II and 3β-HST wild type as template. For construct number 7, the codon for Ile-51 was altered to create an Asn at that position; for construct number 8, the codon for Ile-71 was mutated to produce an Asn at that site.

**3-Hydroxysteroid Sulfotransferase Expression and Purification**

The HST chimeras and mutant constructs were used to transform Max Efficiency DH5α competent bacteria (Life Technologies, Inc.). Positive colonies were selected by PCR using HST-specific primers and bacteria in the PCR reaction mixture. Plasmin were purified from positive colonies and sequenced using Thermo Sequenase Radiolabeled Terminator Cycle Sequencing kit (Amersham Pharmacia Biotech). The selected bacteria were grown at 32 °C to an A590 of 0.5 at which time 1 ml isopropanol-1-thio-β-galactopyranoside was added to induce fusion protein synthesis, and the incubations were continued for an additional 4 h. Bacterial cells were harvested by centrifugation and lysed in 1:1 (v/v) of column buffer (50 mM sodium phosphate, 300 mM NaCl, pH 8.0) containing 10 mM imidazole and 1 mg/ml lysozyme followed by brief sonication. Bacterial lysates were centrifuged at 100,000 × g for 1 h at 4 °C, and the supernatants were applied to nickel affinity columns (Qiagen, Inc., Valencia, CA). Columns were washed with 20 ml imidazole in column buffer, and the bound fusion proteins were eluted with 250 mM imidazole in column buffer. Eluted proteins were concentrated and equilibrated with 20 mM Tris-HCl, pH 7.5, containing 15% glycerol using a Centriprep 30 (Amicon, Inc., Beverly, MA). To remove the fused protein from HST, the concentrated fusion protein preparation (0.1 mg) was incubated with 500 units of recombinant TEV protease (Life Technologies, Inc.) for 16 h at 4 °C and resubjected to nickel affinity column chromatography. Unbound protein in the flow-through was collected,
concentrated, and analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting as described previously (9). Protein concentrations were determined by the Bradford method (10).

**Enzymatic Assays and Kinetic Analyses**

The radiolabeled steroids, [3H]androsterone (54 Ci/mmol) and [3H]pregnenolone (21 Ci/mmol), were purchased from NEN Life Science Products; crystalline steroids were obtained from Steraloids, Inc. (Wilton, NH). 3'-Phosphoadenosine 5'-phosphosulfate was obtained from Sigma. Sulfotransferase activity was assayed in a buffer consisting of 100 mM Tris-HCl, pH 7.5, and 5 mM magnesium acetate to which 0.1 mM 

RESULTS AND DISCUSSION

The aim of these studies was to determine which amino acid residues are responsible for the striking stereospecificity demonstrated by the guinea pig 3α-HST and 3β-HST isoforms. These HST isoforms are distinguished by the fact that the α-isofrom will sulfonate 3-hydroxylated steroids such as androstosterone, which have the 3-hydroxyl group spatially oriented in the α position, but will not sulfonate 3-hydroxylated steroids such as pregnenolone, which have the 3-hydroxyl group in a β orientation (Fig. 1A); on the other hand, the β-isofrom sulfonates 3β-hydroxylated steroids (pregnenolone), in contrast to 3α-hydroxylated steroids (androsterone) (Fig. 1B).

**Sequence Analysis and Chimera Formation—**The 3α-HST and 3β-HST isoforms are 87% identical (Fig. 2A). Although most of the amino acid differences between the two isoforms are scattered throughout the proteins, there are three regions where a substantial number of differences are concentrated (Fig. 2A). To examine the most NH2-terminal of these regions, chimeras were constructed whereby the NH2-terminal 91 amino acids of one isoform was joined to the COOH-terminal 196 amino acids of the other isoform. For reasons that are not clear, the chimera consisting of the upstream 91 amino acid region of the α-isofrom fused to the downstream 196 amino acid region of the β-isofrom (Fig. 2B) was more highly expressed by a considerable measure than the reverse construct; therefore, the so-called 3α/β-HST chimera was employed in these investigations. Interestingly, the 3α/β-HST chimera demonstrated a substrate specificity (Fig. 1C) that was nearly identical to the 3α-HST wild type (cf. Fig. 1A), as distinct from the 3β-HST wild type (cf. Fig. 1B). This result suggests that the structural determinants regulating the 3-hydroxysteroid substrate stereoselectivity, which characterizes the guinea pig 3-HST isoforms, largely resides within the NH2-terminal 91 amino acid region of the proteins. It should be noted that although poorly expressed, the reverse 3β/α-HST chimera demonstrated a preference for 3β-hydroxylated steroids (data not presented).

**Amino Acid Substitutions Involving the 3α/β-HST Chimera—**The α portion of the 3α/β-HST chimera contains 12 amino acid residues that are dissimilar from those in the corresponding region of the 3β-HST isoform (the location of these differences are indicated by arrows in Fig. 2B). Therefore, we set out to examine the relative importance of each amino acid difference in determining the stereoselection manifested by the 3β-HST enzyme. These experiments involved selectively substituting 3β-HST amino acid residues for corresponding 3α-HST amino acid residues in the 3α/β-HST chimera. Six substituted constructs of the 3α/β-HST chimera were generated as presented in Table I. Four of the substituted constructs involving 9 of the 12 amino acid differences continued to exhibit steroid stereospecificity behavior akin to the 3α-HST wild type and essentially the same as the unaltered 3α/β-HST chimera (Figs. 3–6). A fifth substituted construct, however, displayed a clear difference from the previous constructs. That is, a distinction between 3α-HST and 3β-HST activity was not evident, and the 3α-hydroxylated and 3β-hydroxylated steroid substrates were sulfonated in an essentially equivalent manner (Fig. 7). This result suggested that amino acid residues at position 87 and/or 89 of the 3-HST isoforms might be playing a role in determining substrate specificity. The amino acid residues at position 87 (valine in 3α-HST and methionine in 3β-HST) are conservative, suggesting that the amino acids residues at position 89 might be the more critical. The 6th construct, which involved the 12th and final amino acid difference, demonstrated behavior that, for the first time, was more akin to the unreacted samples (NaOH addition to enzyme mixture prior to substrate) was used as a blank, and the value was subtracted from each sample.
3β-HST wild type and clearly distinct from the unaltered 3α/β-HST chimera, i.e. it sulfonated the 3β-hydroxylated steroid substrate more actively than the 3α-hydroxylated steroid substrate (Fig. 8). This last result strongly suggested that the amino acid residue at position 51 plays an especially important role in conferring the characteristic stereospecificity demonstrated by the 3α/β-HST chimera. The α-HST portion of the chimera (black lettering on white background) consists of the NH₂-terminal 91 amino acids; the β-HST portion of the chimera (black lettering on gray background) consists of the COOH-terminal 196 amino acids. In the α-HST portion of the chimera, the location of the amino acid dissimilarities existing between the α- and β-HST isomers in that region of the protein (refer to the upper panel) is indicated by arrows.

**Fig. 2.** Amino acid sequence of 3α-HST wild type, 3β-HST wild type, and the 3α/β-HST chimera. Panel A, alignment of 3α-HST (black lettering on white background) and 3β-HST (black lettering on gray background). Amino acid identities are boxed, and regions of high amino acid differences are indicated by the numbered brackets. The vertical dashed line between amino acid residues 91 and 92 locates the demarcation of chimera formation. Panel B, amino acid sequence of the 3α/β-HST chimera. The α-HST portion of the chimera (black lettering on white background) consists of the NH₂-terminal 91 amino acids; the β-HST portion of the chimera (black lettering on gray background) consists of the COOH-terminal 196 amino acids. In the α-HST portion of the chimera, the location of the amino acid dissimilarities existing between the α- and β-HST isomers in that region of the protein (refer to the upper panel) is indicated by arrows.

**Fig. 3.** Sulfotransferase activity curves for mutated 3α/β-HST chimera (construct number 1 in Table I). The sequence at the top of the figure represents the NH₂-terminal 91 amino acid α-HST portion of the 3α/β-HST chimera denoting location of the α-HST amino acid residues (arrows) to be replaced by corresponding β-HST amino acid residues (bold type above arrows). The 3α-hydroxylated steroid, androsterone, and the 3β-hydroxylated steroid, pregnenolone, substrates are indicated. The mutated 3α/β-HST chimera cDNA was prepared and overexpressed in bacteria; the fusion protein was column-purified, cleaved, and assayed for sulfotransferase activity as described under “Experimental Procedures.”

**Fig. 4.** Sulfotransferase activity curves for mutated 3α/β-HST chimera (construct number 2 in Table I). The sequence at the top of the figure represents the NH₂-terminal 91 amino acid α-HST portion of the 3α/β-HST chimera denoting location (arrows) of the α-HST amino acid residues to be replaced by corresponding β-HST amino acid residues (bold type above arrows). The 3α-hydroxylated steroid, androsterone, and the 3β-hydroxylated steroid, pregnenolone, substrates are indicated. The mutated 3α/β-HST chimera cDNA was prepared and overexpressed in bacteria; the fusion protein was column-purified, cleaved, and assayed for sulfotransferase activity as described under “Experimental Procedures.”
FIG. 5. Sulfotransferase activity curves for mutated 3α/β-HST chimera (construct number 3 in Table I). The sequence at the top of the figure represents the NH2-terminal 91 amino acid α-HST portion of the 3α/β-HST chimera denoting location (arrows) of the α-HST amino acid residues to be replaced by corresponding β-HST amino acid residues (bold type above arrows). The 3α-hydroxylated steroid, androsterone, and the 3β-hydroxylated steroid, pregnenolone, substrates are indicated. The mutated 3α/β-HST chimera cDNA was prepared and overexpressed in bacteria; the fusion protein was column-purified, cleaved, and assayed for sulfotransferase activity as described under “Experimental Procedures.”

FIG. 6. Sulfotransferase activity curves for mutated 3α/β-HST chimera (construct number 4 in Table I). The sequence at the top of the figure represents the NH2-terminal 91 amino acid α-HST portion of the 3α/β-HST chimera denoting location (arrows) of the α-HST amino acid residues to be replaced by corresponding β-HST amino acid residues (bold type above arrows). The 3α-hydroxylated steroid, androsterone, and the 3β-hydroxylated steroid, pregnenolone, substrates are indicated. The mutated 3α/β-HST chimera cDNA was prepared and overexpressed in bacteria; the fusion protein was column-purified, cleaved, and assayed for sulfotransferase activity as described under “Experimental Procedures.”

FIG. 7. Sulfotransferase activity curves for mutated 3α/β-HST chimera (construct number 5 in Table I). The sequence at the top of the figure represents the NH2-terminal 91 amino acid α-HST portion of the 3α/β-HST chimera denoting location (arrows) of the α-HST amino acid residues to be replaced by corresponding β-HST amino acid residues (bold type above arrows). The 3α-hydroxylated steroid, androsterone, and the 3β-hydroxylated steroid, pregnenolone, substrates are indicated. The mutated 3α/β-HST chimera cDNA was prepared and overexpressed in bacteria; the fusion protein was column-purified, cleaved, and assayed for sulfotransferase activity as described under “Experimental Procedures.”

FIG. 8. Sulfotransferase activity curves for mutated 3α/β-HST chimera (construct number 6 in Table I). The sequence at the top of the figure represents the NH2-terminal 91 amino acid α-HST portion of the 3α/β-HST chimera denoting location (arrows) of the α-HST amino acid residues to be replaced by corresponding β-HST amino acid residues (bold type above arrows). The 3α-hydroxylated steroid, androsterone, and the 3β-hydroxylated steroid, pregnenolone, substrates are indicated. The HST cDNA was prepared and overexpressed in bacteria; the fusion protein was column-purified, cleaved, and assayed for sulfotransferase activity as described under “Experimental Procedures.”
strated by the guinea pig 3-HST isomers.

Amino Acid Substitutions Involving the 3β-HST Wild Type—To further examine the relative importance of the amino acid residues at position 51 of the guinea pig 3-HST isoforms for determining stereoselectivity, two constructs consisting of single amino acid substitutions in the 3β-HST wild type were made (Table I). In one construct, asparagine, which is present at residue position 51 in 3α-HST, was substituted for the isoleucine that normally is present at that position in 3β-HST. When tested, this construct exhibited activity that was now similar to the first four constructs described above; that is, it displayed behavior more akin to the 3α-HST wild type and the unaltered 3α/β-HST chimera (Fig. 9). As a control, the second construct consisted of a similar substitution, i.e. an asparagine for an isoleucine, made at position 71 of 3β-HST. When tested, this construct exhibited enzymatic behavior that was indistinguishable from the 3β-HST wild type enzyme (Fig. 10). The results of these latter experiments strengthen the case for the amino acid residue at position 51 as playing a crucial role in determining the 3-hydroxy stereospecificity that characterizes the guinea pig 3α-HST and 3β-HST isomers.

Summary—Table III summarizes the kinetic values determined for the 3α-hydroxylated steroid, androsterone, and the 3β-hydroxylated steroid, pregnenolone, the substrates used to evaluate the stereospecificity of each described guinea pig HST preparation. It is concluded, based on the data presented, that the structural determinants underlying the stereoselectivity exhibited by the guinea pig 3α-HST and 3β-HST isomers largely resides within the NH2-terminal 91 amino acid region of the proteins. Specifically, the amino acid residue at position 51 is crucial; if asparagine is present at that position, 3α-HST activity prevails, whereas if isoleucine is present, 3β-HST activity is dominant. In addition, the amino acid residues at position 87 and/or 89 might also play a significant role, although that possibility has not been tested. Finally, although these recombinant structure-function studies have yielded interesting and provocative results, a more precise understanding of the structural determinants involved in imparting the exceptional stereosepecificity that is characteristic of the guinea pig 3-HST enzymes must await crystallization and final resolution of their three-dimensional structures.

The only steroid sulfotransferase whose structure has been solved is mouse estrogen sulfotransferase; however, it is only 31% identical to the guinea pig 3α-HST and 3β-HST enzymes (11). Furthermore, the amino acid residues, particularly those within the NH-terminal portion of mouse estrogen sulfotransferase that are found near the estradiol-17β substrate, although conserved in guinea pig estrogen sulfotransferase (12),

![Fig. 9. Sulfotransferase activity curves for the I51N mutant of 3β-HST wild type (construct number 7 in Table I). The 3α-hydroxylated steroid, androsterone, and the 3β-hydroxylated steroid, pregnenolone, substrates are indicated. The mutated β-HST cDNA was prepared and overexpressed in bacteria; the fusion protein was column-purified, cleaved, and assayed for sulfotransferase activity as described under “Experimental Procedures.”](image1)

![Fig. 10. Sulfotransferase activity curves for the I71N mutant of 3β-HST wild type (construct number 8 in Table I). The 3α-hydroxylated steroid, androsterone, and the 3β-hydroxylated steroid, pregnenolone, substrates are indicated. The mutated β-HST cDNA was prepared and overexpressed in bacteria; the fusion protein was column-purified, cleaved, and assayed for sulfotransferase activity as described under “Experimental Procedures.”](image2)

**TABLE III**

Kinetic values for androsterone and pregnenolone substrates

Wild type guinea pig 3α-HST and 3β-HST, 3α/β-HST chimera, site-selected mutants of 3α/β-HST chimera, and 3β-HST wild type were overexpressed in *Escherichia coli*, purified, and assayed for sulfotransferase activity as described under “Experimental Procedures.” Values represent the average of triplicate determinations.

| Construct* | αWT | βWT | α/β | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|------------|-----|-----|-----|---|---|---|---|---|---|---|---|
| Androsterone | Vmax (pmol/min/mg) | 1200 | 600 | 650 | 600 | 1100 | 800 | 900 | 500 | 1200 | |
| Km (nM) | 250 | 330 | 350 | 200 | 400 | 500 | 400 | 500 | 650 | |
| Vmax/Km | 4.8 | 1.8 | 1.9 | 3.0 | 2.7 | 1.6 | 2.2 | 1.0 | 2.3 | |
| Pregnenolone | Vmax (pmol/min/mg) | 1100 | 130 | 240 | 160 | 420 | 330 | 920 | 800 | 800 | 1150 |
| Km (nM) | 250 | 250 | 330 | 190 | 500 | 400 | 400 | 400 | 500 | 400 | 400 |
| Vmax/Km | 5.5 | 0.5 | 0.7 | 0.8 | 0.8 | 0.8 | 2.3 | 2.0 | 2.0 | 2.8 |

*Construct: αWT, βWT, and α/β refer to 3α-HST wild type, 3β-HST wild type, and 3α/β-HST chimera, respectively; numbers 1–8 correspond to the mutant constructs listed in Table I.
are not conserved in the guinea pig 3-HST isoforms. The amino acid residue in mouse estrogen sulfotransferase that would appear comparable with residue 51 in the guinea pig 3-HST isoforms is a serine, and this serine is not considered to be involved in the binding of the estradiol-17β substrate (11). Based on analysis of the mouse estrogen sulfotransferase structure, the most highly conserved region of the molecule is involved in interaction with the sulfonate donor compound, phosphoadenosine 5’-phosphosulfate, whereas the region interacting with substrate appears to be much more variable (11).

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