Identifying Androsterone (ADT) as a Cognate Substrate for Human Dehydroepiandrosterone Sulfotransferase (DHEA-ST) Important for Steroid Homeostasis

STRUCTURE OF THE ENZYME-ADT COMPLEX

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In steroid biosynthesis, human dehydroepiandrosterone sulfotransferase (DHEA-ST) in the adrenals has been reported to catalyze the transfer of the sulfonate group from 3′-phosphoadenosine-5′-phosphosulfate to dehydroepiandrosterone (DHEA). DHEA and its sulfate play roles as steroid precursors; however, the role of the enzyme in the catabolism of androgens is poorly understood. Androsterone sulfate is clinically recognized as one of the major androgen metabolites found in urine. Here it is demonstrated that this enzyme recognizes androsterone (ADT) as a cognate substrate with similar kinetics but a 2-fold specificity and stronger substrate inhibition than DHEA. The structure of human DHEA-ST in complex with ADT has been solved at 2.7 Å resolution, confirming ADT recognition. Structural analysis has revealed the binding mode of ADT differs from that of DHEA, despite the similarity of the overall structure between the ADT and the DHEA binary complexes. Our results identify that this human enzyme is an ADT sulfotransferase as well as a DHEA sulfotransferase, implying an important role in steroid homeostasis for the adrenals and liver.

Sulfonation is catalyzed by a family of sulfotransferases that conjugate a sulfonate group (SO₃⁻) from 3′-phosphoadenosine-5′-phosphosulfate (PAPS)¹ to a hydroxyl group of the recipient molecule. With desulfation by sulfatases, sulfonation has been considered as one of the major enzymatic reactions in the metabolism not only of endogenous compounds and xenobiotics, but also of steroid hormones. In most cases, the transfer of the charged sulfonate moiety to an acceptor steroid decreases the biological activity of the steroid. Indeed, steroid sulfates resulting from this reaction are not capable of binding to or activating steroid receptors. In addition, the sulfonation reaction increases water solubility of steroids and thereby enhances their excretion into the urine and/or bile (1, 2).

Human dehydroepiandrosterone sulfotransferase (DHEA-ST; SULT2A1; EC 2.8.2.2) was identified mainly from human liver and adrenals, using Northern blot analysis (3) and RT-PCR analysis (4). A single isoform of DHEA-ST from human liver and adrenal tissues was confirmed by the expression and purification of the enzyme from these organs (5, 6), molecular cloning studies (7) and the comparison study of the physical, kinetic, and immunological properties of liver and adrenal forms of the enzyme (8). Steroid sulfonation has been recognized as an important means for maintaining steroid hormone levels in their metabolism. In humans, dehydroepiandrosterone sulfate (DHEAS) is the most prodigious steroid precursor and one of the major secretory products of both adult and fetal adrenals. In the fetoplacental-maternal unit (the unique interdependence of fetus, placenta, and mother) shown in Scheme 1, DHEAS plays an important role as the major precursor for placental estrogen biosynthesis, thus maintaining pregnancy. A considerable amount of DHEAS is mainly produced from the fetal zone in the adrenal gland (9). Then DHEAS is hydroxylated primarily in the fetal liver and partly in the fetal adrenal itself (10). In the placenta, the hydroxylated DHEAS is desulfated and aromatized to form estradiol, which increases uteroplacental blood flow, and then is secreted into the maternal circulation (11). Androsterone sulfate (ADTS) is the most abundant circulating 5α-reduced androgen metabolite in serum (12), while DHEAS is the major precursor for the active steroid hormones. A fraction of dehydroepiandrosterone (DHEA) is metabolized in liver, resulting in androstenedione and the double bond of the latter compound is reduced by 5α-reductase, giving rise to 5α- and 5β-androstenedione. The reduction of the ketone and conjugation reaction at C3 produces mainly 5α-androsterone (ADT) and etiocholanolone (or 5β-ADT), glucuronono- and sulfo-conjugates, among various metabolites. More interestingly, the major portion of testosterone is oxidized to androstenedione in liver, following the same metabolism as described above (13). With other conjugation reaction, ADT sulfonation has been considered as one of the major catabolism processes of androgens in human liver before urinary excretion since a considerable amount of ADTS was identified in urine (14). Nevertheless, it is quite interesting that a steroid sulfotransferase enzyme, other than DHEA-ST,
Crystal Structure and Mechanism of ADT- and DHEA-ST

Scheme 1: The fetoplaclental-maternal unit (the unique interdependence of fetus, placenta, and mother).

responsible for ADT sulfonation in human liver has not been reported so far. This is our detailed kinetic study on DHEA-ST for various steroids, among which ADT was found to exert a similar activity and substrate inhibition pattern. The latter resulted in reexamination of specificity and substrate inhibition for ADT and DHEA by this enzyme. The two steroids seem quite similar viewing from the plane of the steroid core. However, when looking in detail, a DHEA molecule is stereospecifically distinct from an ADT molecule in their A rings: DHEA as a 3β-hydroxysteroid and ADT as a 3α-hydroxysteroid (Fig. 1). This stereospecific difference has intrigued us in view of the binding mode of both steroids in the substrate binding site of DHEA-ST.

Until now, two crystal structures of the enzyme have been available: SULT2A1 in complex with 3′-phosphoadenosine-5′-phosphate (PAP) (the PAP complex) (15) and DHEA-ST in complex with DHEA (the DHEA complex) (16).

In this study, we report the crystallographic structure of the enzyme in complex with ADT, a 3α-hydroxysteroid, and describe enzyme kinetics addressing substrate specificity and substrate inhibition patterns toward DHEA and ADT.

EXPERIMENTAL PROCEDURES

Materials—DHEA, ADT, and PAPS were obtained from Sigma Chemical Co. (9,11-3H (N))-ADT (54 Ci/mmol) and (4-14C)-DHEA (53 mCi/mmol) were purchased from PerkinElmer Life Sciences. Scintillation mixture solution, glutathione-Sepharose 4B, Q-Sepharose fast flow, and Factor Xa were from Amersham Biosciences.

Purification and Sulfitotransferase Assay—Preparation of homogeneous DHEA-ST was performed as described previously (17). In brief, human DHEA-ST expressed as a glutathione sulfitotransferase fusion from Escherichia coli was purified using glutathione-Sepharose 4B affinity chromatography, a Factor Xa cleavage step, and Q Sepharose anion exchange column chromatography. Purified protein was confirmed by SDS-PAGE and stored at −20 °C with 50% glycerol before using. No big change for the sulfating activity was observed during storage.

DHEA-ST activity assay was performed as mentioned previously (17) with little modification. DHEA-ST activity was assayed at 37 °C at various time intervals in a final reaction volume of 150 μl containing 20 mM Tris, pH 7.5, 15 mM MgCl2, 50 μM PAPS, 2% ethanol, and various amounts of steroids. The reaction was stopped by adding the equivalent volume of xylene, vortexing, and centrifuging for 10 min at 3000 rpm to divide into the aqueous and the solvent phases. The phases were completely separated with ethanol-dry ice bath. Each phase of 80 μl was used to determine the amount of sulfate-conjugated steroids by liquid scintillation counting in a Beckman LS 3801 (Irvine, CA). One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 nmol of each steroid sulfate per min under the conditions mentioned above.

Kinetic Studies and Data Processing—All reactions were performed at 37 °C and pH 7.5 using 0.1–6.25 μg of enzyme, a wide range of the steroid concentrations (0.05–40 μM) and a saturating concentration of the cofactor, PAPS, (50 μM) in the reaction mixture. The initial velocities were measured with less than 10% substrate conjugation. For the determination of all the kinetic constants, at least 2–3 independent experiments were performed and then the mean values were taken.

Initial velocity data in the range of non-inhibitory substrate concentrations (for DHEA and ADT) were first individually fitted to the Michaelis-Menten Equation 1. Michaelis constant (Km) and Maximal velocity (Vmax) for all the steroids examined were calculated using the corresponding double-reciprocal plots (Equation 2). Data for substrate inhibition were fitted to substrate inhibition in Equation 3 (18). The real maximal velocity (Vmax) and the substrate concentration at that velocity were calculated by Equations 4 and 5 that were derived mathematically from Equation 3 (19).

\[ v = \frac{[S]}{K_m + [S]} \]  
\[ \frac{1}{v} = \frac{K_m}{V_{max}} + \frac{1}{V_{max}} \]  
\[ v = \frac{[S]}{K_m + [S](1 + [S]/K_s)} \]  
\[ V_{max} = V(1 + 2 v/K_mK_s) \]  
\[ s = \sqrt{K_mK_s} \]

v is the experimentally determined initial velocity, V is the maximal velocity, [S] is the concentration of the variable substrate, Km is the concentration of substrate at half-maximal velocity, and Ks is the substrate inhibition constant.

Steady-state Kinetics and Substrate Inhibition for ADT and DHEA—The steady-state kinetics for DHEA and ADT were studied as described under “Experimental Procedures.” The initial velocity versus steroid concentration plots and the corresponding Lineweaver-Burk plots are shown in Fig. 2, A and B for DHEA and ADT, respectively. In the inserts of Fig. 2, the initial velocity increases with substrate concentrations up to 2 μM for ADT and 4–6 μM for DHEA and then decreases with increasing substrate concentrations, identifying a substrate inhibition pattern. At very high substrate concentrations, this velocity reaches a minimum level of 30 units/mg for ADT and 40
units/mg for DHEA, rather than falling to zero. As depicted in Fig. 2, the Lineweaver-Burk plots are by no means linear compared with those of general Michaelis-Menten kinetics. Three regions of the plots can generally be discerned: the region that follows Michaelis-Menten kinetics, the transition region, and the substrate inhibition region. At low concentrations of DHEA (2 μM and lower) and ADT (1 μM and lower), the double-reciprocal plot gives a linear range that follows the Michaelis-Menten equation. The sulfonation reaction was largely inhibited when higher concentrations of DHEA (20 μM and higher) and ADT (10 μM and higher) were used and 1/v value increased rapidly following the decrease of 1/S value (the increase of substrate concentration) in this range. A transition phase of 1–10 μM for ADT and 2–20 μM for DHEA showed the transfer from the Michaelis-Menten zone to the substrate inhibition area. The kinetic constants were calculated when the whole range of experimental data was analyzed by the substrate inhibition equation: 

\[ v = \frac{V_{\text{max}} [S]}{K_m} \times \frac{[S]}{[S] + [S] + 1 + [S] K_m} \]

The value of specificity was calculated as \( k_{\text{cat}} / K_m \). With ADT and DHEA compared in two regions. Table I presents the stead-state kinetics for the sulfonation reaction of DHEA-ST with ADT and DHEA.

### Table I

| Steroid | \( K_m \) | \( V_{\text{max}} \) | \( k_{\text{cat}} \) | \( k_{\text{cat}} / K_m \) | Specificity |
|---------|---------|---------|--------|----------------|------------|
| ADT     | 2.1 ± 0.5 | 221.1 ± 36.4 | 0.13 ± 0.02 | 3.8 ± 0.9 | 0.062 |
| DHEA    | 3.1 ± 0.7 | 180.3 ± 27.1 | 0.1 ± 0.015 | 10.6 ± 2.4 | 0.032 |

Overall Structure—The crystal structures of human DHEA-ST have been reported in the presence of DHEA (16) and PAP (15). Here the ADT binary complex structure is determined. The overall structure of the enzyme includes an ADT molecule and a modeled PAP, showing both substrate and expected cofactor binding sites (Fig. 3). The overall structure of the ADT complex is very similar to that of the DHEA complex except for some residues and the flexible loops. The root mean square deviation value between the two structures is 0.935 for the \( \alpha \)-carbon of 267 amino acids excluding the two flexible loops formed by residues Asn-226 to Asp-237 and Leu-246 to Val-250. All data between 20 and 2.70 Å were used in the refinement, yielding a crystallographic R-factor of 23.0% and a free R-factor of 27.0%. There is one monomer in the asymmetric unit even though the active protein is a homodimer in solution (5, 17). The main core of the ADT complex structure is composed of an \( \alpha/\beta \)-fold with a central four-stranded parallel \( \beta \)-sheet surrounded by \( \alpha \)-helices on both sides as described in a previous report (15). The refined model includes one DHEA-ST monomer, an ADT molecule and a sulfate molecule. In addition, the model includes 25 water molecules. Out of the 284 amino acids contained in the protein, 267 were modeled into the electron density in the present structure while 28 amino acids were built in the DHEA complex structure. The missing amino acids belong to loop regions that could not be built in the ADT complex structure. The Ramachandran plot showed that all residues are in allowed regions with 87.2% in the most favored regions.

Substrate Binding Site—The present ADT binary complex structure is compared with both the PAP-DHEA-ST structure...
(the PAP binary complex structure) and the DHEA binary complex structure. The $2F_o - F_c$ electron density map contoured at 1σ level enables the unambiguous localization of an ADT molecule as a whole is close to the location of the DHEA molecule in the proposed alternative orientation. In the ADT complex structure, no electron density for the proposed catalytic orientation has been found since the Cα atom of Met-137 residue is inward the substrate binding site and results in steric hindrance against the proposed catalytic location of the DHEA molecule (Fig. 5).

The active site for the sulfonation reaction is identified through the position of O-3 of ADT, which functions as a sulfonate acceptor (Fig. 5). There is little displacement of O-3 atom between the DHEA complex and the ADT complex structures ($1.04\,\text{Å}$ for the distance between O-3 of ADT and O-3 of DHEA in the alternative orientation, $1.74\,\text{Å}$ between O-3 of ADT and O-3 of DHEA in the catalytic orientation). A hydrogen bond (3.0 Å) between O-3 of ADT and Ne-2 of His-99 in DHEA-ST is identified similar to that between DHEA and His-99 in the
This histidine is strongly conserved among several sulfotransferase families, including EST, phenol sulfotransferase, and flavonol 3-sulfotransferase, suggesting its catalytic role (15). Until now, the ADT complex and the DHEA complex structures are quite similar. If so, what is the main difference between the two structures in the catalytic center of DHEA-ST? As shown in Fig. 6, the main difference between the two structures is the steroid orientation: the ADT molecule is flipped over 180° around its long axis, in comparison to the DHEA molecule of the alternative orientation. Several factors have been involved in the flip-flop of the ADT molecule. Primarily, this takes place in order to favor the making of a hydrogen bond between O-3 of ADT and N/H9280-2 of His-99 of the enzyme. If the ADT molecule is in the same plane as the DHEA molecule considering the orientation of C-18 and C-19 atoms, O-3 of ADT (α-position) would be far away from Ne-2 atom with the distance of 4.6 Å due to the stereospecific difference of A ring. Therefore the hydrogen bond between O-3 of ADT and Ne-2 of His-99 of DHEA-ST in the ADT complex structure cannot be made. The present crystallographic data indicate that human DHEA-ST does not provide stereospecific discrimination between an O-3 α steroid (ADT) and an O-3 β steroid (DHEA), thereby supporting our kinetic results (Table II). On the contrary, guinea pig DHEA-STs showed stereospecificity, based on the comparative enzymatic study with DHEA and ADT (29, 30). Secondly, O-17 of ADT establishes another hydrogen bond (3.28 Å) with the hydroxyl group of Ser-80 that stabilizes the ADT molecule in this position (Fig. 5). No hydrogen bond for the O-17 atom of DHEA has been found in the DHEA complex structure. Third, several hydrophobic residues are found in the vicinity of the substrate binding site (within 6 Å). Among them, the Phe-133, Trp-134, Phe-18, and Trp-72 residues are involved in van der Waals interactions with the ADT molecule. Fourth, the side chain of Trp-77 is juxtaposed with ADT at a distance of around 4 Å, indicating that this residue provides another important interaction for ADT orientation (Fig. 6). The A and B rings of the ADT molecule are sandwiched between the side chain of residue Trp-77 on one side and the side chains of residues Phe-133 and Trp-134 on the other. This sandwich conformation makes the orientation of the A ring of ADT stable. All these observations suggest that ADT binds to DHEA-ST at least as tightly as DHEA, as shown by the apparent affinity for ADT (K_m = 2.1 ± 0.5 μM) and DHEA (K_m = 3.1 ± 0.7 μM) (Table II).

The loop comprising the residues Asn-226 to Asp-237 is missing in the ADT complex structure while in the DHEA complex structure this loop is situated near the substrate binding site and plays a role as a roof for this site. In the PAP complex structure, the substrate binding site is occupied by the loop (Asn-226 to Asp-237) and the aromatic ring of Trp-77. Therefore, the site is more compact in the absence of a substrate. The active site extends to a proper size to accommodate the substrate when a steroid approaches the site. This is in agreement with the recent results in the study of other steroid-converting enzymes, such as the significant active site volume modification in binding different steroids found in the type 5 human 17β-hydroxysteroid dehydrogenase (HSD),2 and the conformational rearrangements found when estradiol advances in the binding site of human estrogenic 17β-HSD (31).

The catalytic orientation of the DHEA molecule of the DHEA complex structure was then chosen (16) based on the orienta-

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2 W. Qiu and S.-X. Lin, unpublished data.
tion of PAP within the DHEA-ST molecule (15). However, the true ternary complex of the enzyme with DHEA and PAP has not been obtained, thus the proposed catalytic orientation has not yet been conclusively elucidated and requires further study and evaluation.

**Cofactor Binding Site: A Gate Structure Implied in Substrate Inhibition**

All sulfotransferases use only one cofactor, PAPS, as a sulfonate donor. This may explain why the cofactor binding sites of the sulfotransferase structures are more conserved than the substrate binding sites throughout the sulfotransferase families. Our substrate binary structures (the ADT complex and the DHEA complex) are compared with the PAP containing structures. As shown in Fig. 7, the main difference in the cofactor binding site is the loop (Lys-242 to Asp-253) which is explicitly open in the DHEA complex structure, whereas it is closed in the PAP complex structure. In detail, the open entrance in the DHEA complex structure has two sides with a width of around 8.4 Å: Lys-188, Phe-220, and Lys-224 on one side (side A) and Leu-246 to Gly-252 on the other side (side B) (Fig. 7). Since the electron density of this loop is not clear in the ADT complex structure, seven residues of this loop (twelve residues) have been built on the open position. Accordingly the loop in the ADT complex structure is expected to be in the open position in the DHEA complex structure. Residues Thr-243 and Lys-242 of side A play a role as a hinge and so does residue Asp-253 in our binary complex structures. However in the PAP complex structure, side A is in the closed position on the entrance, functioning like a gate for the cofactor’s entrance, while there is only little displacement on side B to accommodate the cofactor molecule as compared with our binary complex structures (Fig. 7). This gate produces additional interactions between the enzyme and PAP: a van der Waals contact with Leu-246, hydrogen bonds with Arg-247, Lys-248, and Gly-249 and a hydrophobic interaction with Leu-245. These interactions and the closed conformation are also identified in PAP containing EST structure by our structural comparison studies. The gate structure indicates that the cofactor binds more tightly than a substrate, shown by the comparison of important interactions with the enzyme (Table III). This is in agreement with the fact that the $K_m$ value for PAPS has been determined around 0.8 μM when DHEA was used as a substrate at a fixed concentration and those for ADT and DHEA were 2.1 ± 0.5 μM and 3.1 ± 0.7 μM, respectively. This gate structure may further explain the substrate inhibition pattern of DHEA-ST. Generally the sulfonation reaction starts by taking the sulfonate group from PAPS and transferring it to a 3-hydroxyl substrate such as ADT or DHEA. The sulfated product may be released first due to the strong association of the cofactor to the enzyme.

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**FIG. 6.** Comparison of the binding mode of two 3-hydroxysteroids, DHEA, and ADT. Comparison of the binding mode was performed between the DHEA complex (yellow) and the ADT complex structures (red). The ADT molecule (magenta) is flipped over along the long axis of the steroid when compared with the DHEA molecule (green). The sulfate group introduced by the crystallization is in blue. This image was produced using O/OPLOT and Molray.

**FIG. 7.** A gate structure for the cofactor-binding site. The cofactor binding sites of the DHEA complex (left) and the PAP complex (right) are colored in green. The loop from Gln-244 to Asp-253 plays a role as a gate and is drawn in ribbon form. In the PAP complex structure, the same residues (side A) move into side B to close the entrance; PAP is in purple. This image was produced using O/OPLOT and Molray.

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ADT, or DHEA was determined from the PAP complex, the ADT co-

ferase in Adrenal? The number of important interactions between the enzyme and PAP, ADT, or DHEA was determined from the PAP complex, the ADT complex, and the DHEA complex structures respectively, using Ligplot (37).

| Hydrogen bonds | PAP | ADT | DHEA |
|---------------|-----|-----|------|
| 18            | 2   | 1   | 6    |

Hydrophobic contacta

b Only important hydrophobic contacts (less than 4 Å) were counted.

This agrees with the fact that the dissociation constant for PAP in EST is 30 ± 0.3 nM whereas that for estradiol sulfate is 270 ± 50 nM (32). Then the other reaction product, PAP, can be freed from the enzyme into the environment before a second substrate comes to the active site for next cycle of the enzyme reaction. However, at a certain high substrate concentration, the second non-sulfated substrate binds to the active site before PAP leaves, forming a dead end complex (a nonproductive enzyme-PAP-substrate complex). The formation of the dead end complex was proposed as non-reactive PAP possessing a Kcat value of 0.07 μM against phenol sulfotransferase (7).

There is one sulfate group in the cofactor-binding pocket in the present ADT complex structure (Fig. 6). The sulfate group originated from the crystallization conditions (1.6 M ammonium sulfate, 0.1 M HEPES, pH 7.5, 0.1 M sodium chloride) for the ADT binary complex as described in the materials and methods section. The sulfate group is supposed to be next to the 3'-phosphate group (P-2) of PAP, if PAPS structure is considered based on the PAP complex structure. But in the ADT complex structure, the sulfate is located in a similar position to the 3'-phosphate group of PAP. Even though it is misplaced in the position corresponding to 3'-phosphate group of PAP, the present location might thermodynamically be the best for the sulfate group.

ADT Sulfortransferase in Liver as Well as DHEA Sulfortransferase in Adrenal?—No detailed study addressing ADT sulfonation by human DHEA-ST has been reported. In former reports, ADT has not been considered as a cognate substrate for human DHEA-ST due to its lower reactivity (30–70%) when compared with DHEA sulfonation (1, 11). Let us review the reactivity of ADT again from the perspective of enzyme kinetics. The ADT concentration used for investigating the relative activity was 6–10 μM, which is significantly higher than the Kcat value of ADT (Kcat = 3.8 ± 0.9 μM) whereas the DHEA concentration used for the same experiments was 3 μM, which is much lower than the Kcat value of DHEA (Kcat = 10.6 ± 2.4 μM).

Therefore substrate inhibition occurs at these concentrations of ADT as shown in Fig. 2B. Accordingly, suffice it to say that the maximum velocity for ADT could be similar to or even higher than that for DHEA if the substrate inhibition is considered. This has been confirmed by our kinetic data (Kcat = 2.1 ± 0.5 μM, Kcat = 0.13 ± 0.02 s-1 and specificity = 0.062 μM-1 s-1 for ADT and Kcat = 3.1 ± 0.7 μM, Kcat = 0.1 ± 0.015 s-1 and specificity = 0.032 μM-1 s-1 for DHEA), indicating that ADT is at least as specific as DHEA. On top of that, human embryonic kidney (HEK)-293 cells transfected with human DHEA-ST cDNA showed sulfonation reaction toward ADT, implying that ADT sulfonation by DHEA-ST happens in cells (33).

Based on the measurements of steroid level and correlations among steroids in serum, the two sulfated steroids, ADTS and DHEAS were significantly correlated (r = 0.59) (12). This is even more interesting if we consider DHEAS the major androgen precursor and ADTS one of the main androgen metabolites in view of steroid homeostasis.

Substrate inhibition is induced at a certain high concentra-
tion range of ADT and DHEA, which is a typical phenomenon in the sulfotransferase family (26–28). The Ks level of DHEA molecule (Ks = 10.6 ± 2.4 μM) is quite high compared with the actual DHEA concentration (at most 0.79 μM) in the adrenal organ (34). This implies that substrate inhibition is not related to the physiological significance of the metabolism of steroids in human adrenals. However the substrate inhibition of ADT sulfonation (Ks = 3.8 ± 0.9 μM) produces a totally different story from a physiological point of view. The concentrations of ADT and etiocholanolone (at both sulfonated and glucuronidated form) in urine have been reported to be 6 and 2.5 μM, respectively (14). The ADT concentration in human liver might be higher than 6 μM if a precursor-to-product relationship is considered. Taking this into consideration, substrate inhibition of ADT sulfonation may control the effect of an increase of ADT levels even though there is no direct information for the levels of ADT in human liver. In steroid catabolism, this enzyme can contribute to the maintenance of the steroid level through substrate inhibition mechanism while no such controlling mechanism in producing DHEAS has been found caused by low DHEA concentration in human adrenal.

In conclusion, with glucuronidation by UDP-glucuronosyl-
transferases, DHEA-ST plays a pivotal role in sulfonating and metabolizing ADT in human liver. We have now demonstrated that the former reported human DHEA-ST is the ADT sulfotransferase in human liver as well as the DHEA sulfotransferase in human adrenal. Substrate inhibition of this enzyme plays a major role in maintaining the level of steroid hormones, especially those of androgens. At the same time, our study shows that substrate inhibition is useful in identifying cognate substrates for enzymes.

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