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Immunological Characterization of Dehydroepiandrosterone Sulfotransferase from Human Liver and Adrenal

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SUMMARY

Dehydroepiandrosterone sulfotransferase (DHEA-ST), a steroid sulfotransferase (ST), has recently been purified from human liver cytosol and partially characterized. DHEA-ST has a subunit molecular mass of 35 kDa and is responsible for the majority of the sulfation of steroids and bile acids in the liver. For these studies, polyclonal antibodies to human liver DHEA-ST were raised in rabbits. The anti-human liver DHEA-ST antibodies were used to characterize the immunoreactivity of DHEA-ST in human liver and to study the relationship of human adrenal DHEA-ST to the liver form of the enzyme. Immunoblot analysis of several different human liver cytosol samples with the rabbit anti-human liver DHEA-ST antiserum detected only a single 35-kDa protein in each liver. Anti-human liver DHEA-ST antibodies also did not react with either form of phenol sulfotransferase (PST), P-PST or M-PST, present in human liver cytosol. DHEA-ST activity was purified from the 100,000 × *g* supernatant fraction of human adrenal tissue by DEAE-Sepharose CL-6B chromatography and 3′,5′-diphosphoadenosine-agarose affinity chromatography. Human adrenal DHEA-ST was shown to have a molecular mass of 35 kDa, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunoblot analysis of human adrenal cytosol revealed that the anti-human liver DHEA-ST antibodies reacted specifically with the 35-kDa subunit of DHEA-ST. The apparent K_m values for DHEA and 3′-phosphoadenosine-5′-phosphosulfate obtained with human adrenal DHEA-ST were 1.0 μM and 1.6 μM, respectively. Adrenal DHEA-ST demonstrated the same pattern of reactivity towards different steroid substrates as did human liver DHEA-ST, and neither form of DHEA-ST was found to sulfate cortisol. The results of this study suggest that DHEA-ST is the major steroid ST present in human liver and adrenal tissue and that the physical, biochemical, and kinetic properties of adrenal DHEA-ST are similar if not identical to those of the liver form of the enzyme.

Sulfation is an important conjugation reaction for the biotransformation and detoxification of steroids and bile acids in the human liver. The sulfation of steroids and bile acids in the liver converts these compounds to more hydrophilic metabolites, which are readily excreted into the urine or bile (1). Sulfation also serves to protect tissues from the toxic detergent effects of several bile acids, as well as to prevent the accumulation of potentially toxic bile acids in the liver during cholestasis, cirrhosis, and hepatitis (2).

Sulfation is also an important process in the synthesis and secretion of several steroids from the human adrenal cortex. One of the major steroids secreted in relatively large amounts from the human adrenal gland is DHEAS. High levels of DHEAS are secreted from both the fetal and adult adrenal cortex. During fetal development, DHEAS is secreted into the fetal circulation, desulfated in the placenta, and converted into β-estradiol and estriol (3). Fetally derived DHEAS is the precursor for the majority of the estrogens observed in the maternal circulation during pregnancy. Also, it has been reported that DHEAS may function in the softening of the cervix before parturition (4). In adults, between 2 and 20 mg/day of DHEAS may be secreted from the adrenal cortex, and micromolar concentrations of DHEAS are found in the plasma (5). Until recently, DHEAS has been considered to be an "inactive" storage form of the steroid, which may be converted to both androgens and estrogens in peripheral tissues (6). A number of properties are now being attributed to DHEAS, including the ability to protect against obesity and cardiovascular disease (7, 8). In addition, DHEAS has been shown to noncompetitively inhibit glucose 6-phosphate dehydrogenase and to decrease the binding of carcinogens to DNA in rats (9, 10).

The purification and characterization of a number of different mammalian STs capable of conjugating steroids have been reported (11, 12). Recently, Falany *et al.* (13) have described the purification of a steroid ST, from human liver cytosol, that

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ABBREVIATIONS: DHEAS, dehydroepiandrosterone sulfate; ST, sulfotransferase; DHEA, dehydroepiandrosterone; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TEA, triethanolamine; PAP, adenosine 3′,5′-diphosphate; PST, phenol sulfotransferase; DHEA-ST, dehydroepiandrosterone sulfotransferase; P-PST, Phenol-sulfating form of PST; M-PST, monoamine-sulfating form of PST.

has a subunit molecular mass of 35 kDa and is capable of conjugating a variety of steroids. This enzyme is most reactive with DHEA and has been referred to as DHEA-ST. Subsequently, DHEA-ST has been shown to be responsible for most, if not all, of the bile acid sulfation activity in human liver cytosol (14). The goals of this investigation were twofold. First, polyclonal antibodies were raised against human liver DHEA-ST and used to characterize the immunoreactivity and heterogeneity of DHEA-ST in human liver. Second, the anti-DHEA-ST antibodies were used to investigate the relationship of human adrenal DHEA-ST to the liver form of the enzyme.

Materials and Methods

PAP and PAPS were purchased from Pharmacia (Piscataway, NJ). DEAE-Sepharose CL-6B, PAP-agarose, and the nonradiolabeled steroids were obtained from Sigma Chemical Co. (St. Louis, MO). Goat anti-rabbit IgG alkaline phosphatase conjugate and protein assay reagent were purchased from Bio-Rad (Rockville Center, NY). Freund's adjuvant was obtained from Difco Laboratories (Detroit, MI). Nitrocellulose paper was acquired from Schleicher & Schuell (Keene, NH). [1,2,6,7-^{3}H]DHEA (79 Ci/mmol) was purchased from Amersham Corporation (Arlington Heights, IL). [1,2-^{3}H]Hydrocortisone and β-[6,7-^{3}H]estradiol were purchased from NEN Research Products (Boston, MA). All other chemicals were of reagent grade quality.

ST assays. DHEA-ST activity was determined using the method of Falany *et al.* (13), with [^{3}H]DHEA as the sulfate acceptor. Reactions, which contained 50 mM Tris·HCl, pH 7.5, 10 mM $MgCl_2$, and 3.0 μM [^{3}H]DHEA, were started by the addition of PAPS to a final concentration of 20 μM, in a total volume of 0.25 ml. Reactions were terminated by the addition of 3.0 ml of chloroform, followed by the addition of 0.25 ml of Tris·HCl, pH 8.7, to alkalinize the mixture. The reaction mixtures were then vortexed for 30 sec and centrifuged at 600 × *g* for 5 min, to separate the aqueous and organic phases. Synthesis of DHEAS was determined by scintillation counting of the aqueous phase. Each assay was run with a control reaction, which did not contain the enzyme. Routinely <0.02% of the nonsulfated DHEA remained in the aqueous phase after extraction, and no detectable DHEAS was present in the organic phase. The apparent K_m of adrenal DHEA-ST for DHEA was determined using 10 μM PAPS and varying DHEA concentrations (1–16 μM). To determine the K_m of adrenal DHEA-ST for PAPS, reactions contained 3.0 μM DHEA and PAPS was varied (1–20 μM).

Assays using either tritiated cortisol or β-estradiol were performed as described for DHEA; however, the steroid concentrations were varied to obtain maximal rates. Steroid sulfation assays using nonradiolabeled steroids were similar to the DHEA assays. Both steroid ST and PST activities were measured by the method of Foldes and Meek (15), using nonradiolabeled steroids and 4-nitrophenol as the sulfate acceptors, respectively.

Preparation of human adrenal and liver cytosol. Samples of human adrenal and liver were obtained through the Organ Procurement Program at the University of Rochester, during the removal of other organs for transplantation. Tissue samples were immediately chilled on ice and frozen at −70° within 30 min of removal from the donor. Samples of liver or whole adrenal tissue (20%, w/v) were thawed in 10 mM TEA buffer, pH 7.5, containing 1.5 mM dithiothreitol and 10% glycerol, minced with scissors, and then homogenized with a Brinkman Polytron. The homogenates were centrifuged at 12,000 × *g* for 15 min at 4°, and I then centrifuged at 100,000 xg for 1 hr the resulting cytosol preparations were stored at −70°. Protein concentrations were estimated using the Bio-Rad protein assay originally developed by Bradford (16), with bovine serum albumin as a standard.

Purification of human liver DHEA-ST. DHEA-ST was purified from human liver cytosol using DEAE-Sepharose CL-6B chromatography and PAP-agarose affinity chromatography, according to the method of Falany *et al.* (13). All purification procedures were done at 4°. Human liver cytosol was applied to a DEAE Sepharose CL-6B column (2.5 × 12 cm), which had been previously equilibrated in TEA buffer. The column was then washed with three column volumes of TEA buffer containing 100 mM NaCl. DHEA-ST activity was eluted from the column with a 600-ml linear gradient of NaCl (100–250 mM) in TEA buffer. Fractions containing DHEA-ST activity were pooled, desalted, and concentrated to a volume of 10 ml, using an ultrafiltration concentrator with a PM-30 membrane (Amicon).

The concentrated DHEA-ST activity was applied to a PAP-agarose affinity column (1.0 × 7.0 cm), which had been previously equilibrated in TEA buffer. The column was washed with three column volumes of TEA buffer containing 50 mM NaCl, and DHEA-ST activity was eluted with a linear gradient of PAPS (0–20 μM) in TEA buffer.

DHEA-ST activity was purified from combined preparations of several different human adrenal cytosol samples, following a scaled down version of the procedure outlined for the purification of human liver DHEA-ST. For the adrenal samples, the DEAE Sepharose CL-6B column (1.5 × 15 cm) was eluted with a 300-ml linear gradient of NaCl (100–250 mM) in TEA buffer.

Immunization procedure. For the production of antibodies specific for DHEA-ST, two male New Zealand white rabbits were subcutaneously administered approximately 50 μg of purified human liver DHEA-ST, in complete Freund's adjuvant, at several sites along the back, as described by Vaitukaitus *et al.* (17). Two weeks later, each rabbit received a booster injection containing an additional 50 μg of purified DHEA-ST, in Freund's incomplete adjuvant. After 10 days, the rabbits were bled and the serum was analyzed, by immunoblot analysis, for the presence of antibodies specific for DHEA-ST.

Gel electrophoresis. SDS-PAGE was performed in a Bio-Rad Protean II unit, using the buffer system of Laemmli (18). Samples were pretreated by the addition of β-mercaptoethanol and SDS, to final concentrations of 5% and 1%, respectively, and were then heated to 100° for 10 min. To visualize protein bands after electrophoresis, the gel was stained for 2 hr in isopropanol/acetic acid/water (5:2:13, by volume), containing 0.05% Coomassie Blue R-250, and was then destained with isopropanol/acetic acid/water (1:1:8, by volume). Minimum subunit molecular masses were estimated by comparison with commercial standards (Sigma).

Immunoblot analysis. For immunoblot analysis, proteins were resolved by SDS-PAGE in a 12.5% polyacrylamide gel and were then electrophoretically transferred to nitrocellulose paper, using a Bio-Rad Transblot apparatus and the buffer system described by Towbin *et al.* (19). The nitrocellulose paper was blocked using 3% gelatin in 100 mM Tris·HCl, pH 7.5, containing 500 mM NaCl (Tris-buffered saline). The nitrocellulose paper was then incubated with rabbit anti-human liver DHEA-ST antiserum overnight at 28°, with gentle shaking, in TBS containing 0.1% Tween-20 and 1% gelatin. In most studies, a 1/10,000 dilution of rabbit anti-human liver DHEA-ST IgG fraction (0.3 μg/ml before dilution) was used as the primary antibody. The IgG fraction was prepared by Protein A-Sepharose 4B chromatography, following the method of Harlow and Lane (20). A Bio-Rad immunoblot assay kit, containing goat anti-rabbit IgG alkaline phosphatase conjugate as the secondary antibody, was used to visualize the immunoconjugates.

Results

Immunological characterization of human liver DHEA-ST. Fig. 1 shows the DHEA-ST and P-PST activities assayed in seven different human liver cytosol samples. DHEA-ST and P-PST activities vary among the individual livers by approximately 3-fold and 10-fold, respectively. A correlation of −0.09 was observed between the levels of DHEA-ST and P-PST activity in the individual cytosols. Also, there was no obvious correlation between the age or sex of the liver donors and the level of cytosolic DHEA-ST activity in the small number of samples tested for this study. The highest levels of DHEA-ST activity were found in liver cytosols prepared from a 56-year-old male (HL6) and a 6-year-old female (HL2),

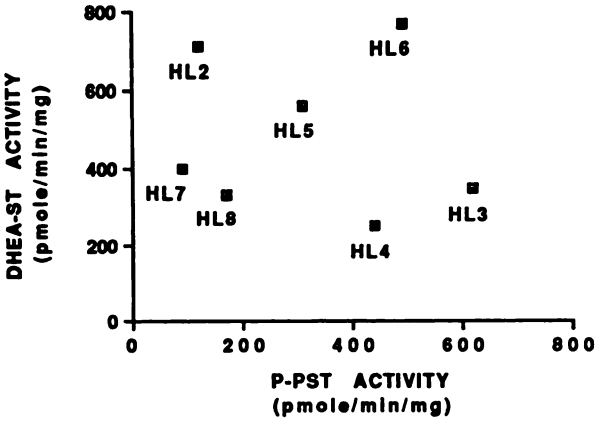

Fig. 1. Human liver DHEA-ST and P-PST activities in cytosol prepared from different normal human livers. DHEA-ST and P-PST activities were measured with 3 μM DHEA and 4 μM *p*-nitrophenol, respectively, in cytosol prepared from different human livers as described in Materials and Methods. The age (in years) and sex of the individual liver donors were as follows: HL2, 6 F; HL3, 56 F; HL4, 55 M; HL5, 34 M; HL6, 56 M; HL7, 14 F; HL8, 41 M.

whereas the lowest activities were found in cytosols prepared from a 55-year-old male (HL4) and a 41-year-old male (HL8).

The stepwise purification of DHEA-ST activity is illustrated in Fig. 2 by SDS-PAGE and immunoblot analysis of fractions obtained during the purification procedure. SDS-PAGE analysis of fractions containing DHEA-ST activity that eluted during PAP-agarose affinity chromatography revealed the presence of the 35-kDa subunit of DHEA-ST and a small amount of the 32-kDa subunit of P-PST (13). Immunoblot analysis of fractions obtained during the purification procedure using rabbit anti-human liver DHEA-ST IgG shows that the antibodies are specific for DHEA-ST. Anti-human liver DHEA-ST antibodies did not cross-react with P-PST or with the monoamine-sulfating form of PST, M-PST, present in human liver cytosol. Also, neither DHEA-ST activity nor immunoreactivity was observed in human blood platelet cytosol (data not shown), which has been demonstrated to contain M-PST and P-PST activity (21).

Immunoblot analysis of cytosol prepared from several different human livers was done to determine the specificity of rabbit anti-human liver DHEA-ST antibodies and to investigate the possible presence of size heterogeneity between immunoreactive DHEA-STs. A single 35-kDa protein was detected in each of the individual cytosol preparations, and no other immunoreactive proteins were observed (Fig. 3). In addition, no size heterogeneity was observed between the individual immunoreactive DHEA-STs in the small number of livers used for this study.

Purification of adrenal DHEA-ST. Cytosol prepared from whole adrenal glands of several different individuals was assayed for steroid ST activity. DHEA-ST activity (243 ± 137 pmol/min/mg of cytosolic protein) was present in all of the adrenal samples and was approximately 2-fold lower than DHEA-ST activity in human liver cytosol (481 ± 201 pmol/min/mg of cytosolic protein).

DHEA-ST activity was purified from human adrenal cytosol by the methods used to purify human liver DHEA-ST; however, it was necessary to combine adrenal cytosol prepared from several individuals for the purification of adrenal DHEA-ST. Fig. 4 illustrates the elution of DHEA-ST and P-PST activities during DEAE-Sepharose CL-6B chromatography of adrenal

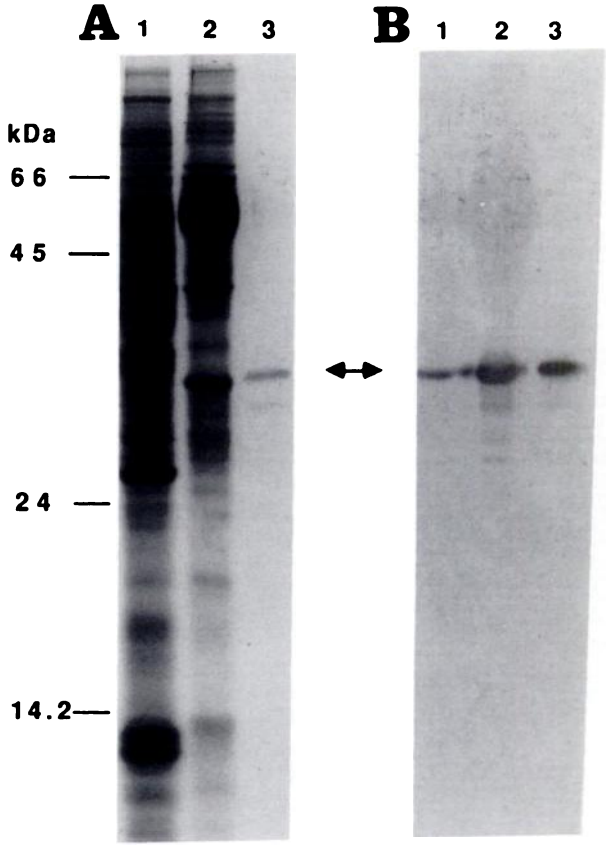

Fig. 2. SDS-PAGE (A) and the corresponding immunoblot (B) of the purification of DHEA-ST from human liver cytosol. A, Samples of DHEA-ST activity from each step in the purification were subjected to SDS-PAGE in a 12.5% polyacrylamide gel and stained with Coomassie Blue, as described in Materials and Methods. The lanes contain the following samples: *lane 1*, human liver cytosol, 18 μg; *lane 2*, DEAE-Sepharose fraction, 75 μg; *lane 3*, PAP-agarose affinity fraction, 2.5 μg. B, Rabbit anti-human liver DHEA-ST was used to detect DHEA-ST in an immunoblot of an identical 12.5% polyacrylamide gel as shown in A. Each lane was probed with a 1/10,000 dilution of rabbit anti-human liver DHEA-ST IgG, as described in Materials and Methods.

cytosol. P-PST activity eluted from the anion exchange column at 112 mM NaCl and was followed by a single peak of DHEA-ST activity, which eluted at 130 mM NaCl. The elution of human adrenal P-PST and DHEA-ST activities was identical to the elution pattern of these activities during DEAE-Sepharose chromatography of human liver cytosol. Two forms of human liver P-PST, P_I-PST and P_{II}-PST, have been identified, based on their elution during anion exchange chromatography, and may represent different allelic forms of P-PST (22, 23). The elution pattern of P-PST activity for all of the adrenals tested was similar to that observed during the chromatography of human liver cytosol containing only the P_I-PST form of PST. A total of 18 whole adrenals from different donors have also been examined, and no evidence of size or charge heterogeneity was observed during the anion exchange chromatography of DHEA-ST activity in these samples.

Fractions obtained from the DEAE-Sepharose CL-6B procedure that contained DHEA-ST activity were combined for further purification by PAP-agarose affinity chromatography (Fig. 5). Due to the relatively low levels of ST activity in adrenal cytosol, P-PST activity was not completely resolved from DHEA-ST activity, in order to increase the recovery of DHEA-ST. Table 1 shows a summary of the purification pro-

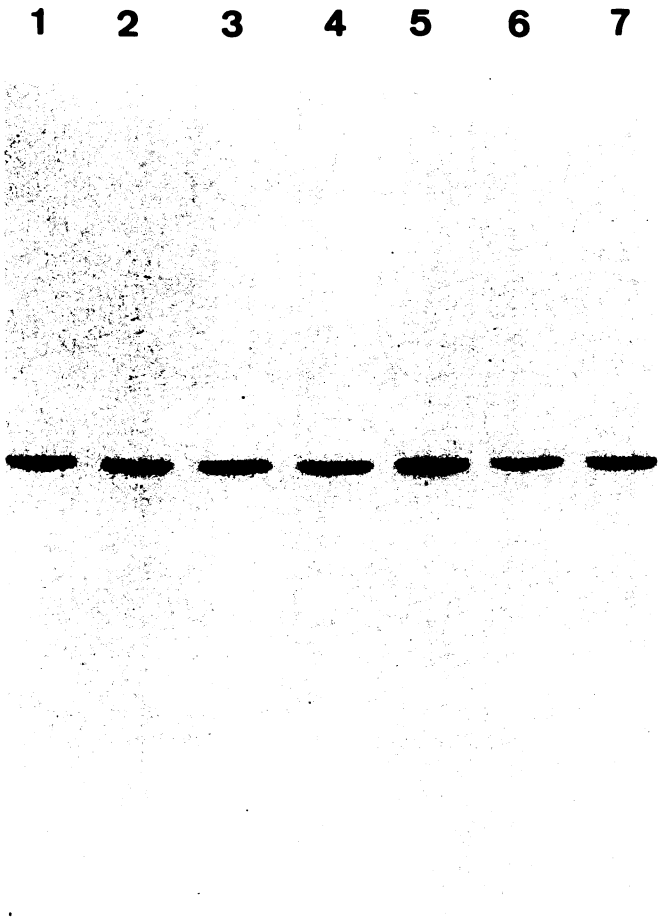

Fig. 3. Immunoblot of human liver cytosol from seven different individuals. Each lane contains approximately 25 μg of cytosol from HL2 to HL8, resolved by SDS-PAGE on a 10% polyacrylamide gel. The protein was electrophoretically transferred to nitrocellulose paper by the method of Towbin *et al.* (19) and was probed with a 1/5000 dilution of rabbit anti-DHEA-ST antiserum, as described in Materials and Methods. *Lane 1*, HL2; *lane 2*, HL3; *lane 3*, HL4; *lane 4*, HL5; *lane 5*, HL6; *lane 6*, HL7; *lane 7*, HL8.

cedure for DHEA-ST activity. Human adrenal DHEA-ST activity was purified 320-fold, compared with the activity in human adrenal cytosol, and approximately 6% of the initial DHEA-ST activity was recovered after this purification procedure.

Fig. 6 shows the comparison of the molecular masses of purified DHEA-ST and P-PST from human liver and adrenal cytosol. Both the liver and adrenal DHEA-ST and P-PST activities coeluted with proteins of 35-kDa and 32-kDa molecular masses, respectively. Immunoblot analysis of adrenal cytosol and purified DHEA-ST from adrenal and liver with rabbit anti-human liver DHEA-ST IgG detected a single 35-kDa protein in each preparation. In an identical immunoblot, only the 32-kDa protein reacted with the anti-human PST antibody (24), supporting its identification as P-PST (data not shown).

Human liver DHEA-ST has been shown to sulfate a variety of different steroid and bile acid substrates. Therefore, the ability of human adrenal DHEA-ST to sulfate several different steroid substrates was tested. Adrenal DHEA-ST was the most reactive towards DHEA (Fig. 7). Epiandrosterone, which also

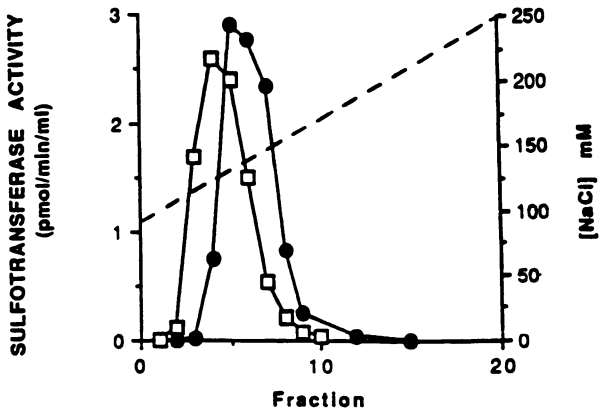

Fig. 4. DEAE-Sepharose chromatography of DHEA-ST and P-PST activities in human adrenal cytosol. Human adrenal cytosol (300 mg) was applied to a DEAE-Sepharose CL-6B column (1.5 × 15 cm) that had been equilibrated in TEA buffer. The column was washed with 30 ml of TEA buffer, followed by 65 ml of TEA buffer containing 100 mM NaCl. DHEA-ST activity was eluted with a 300-ml linear NaCl gradient (100–250 mM) in TEA buffer. Fractions were assayed for DHEA-ST activity (●) and P-PST activity (□) using 3 μM DHEA and 4 μM *p*-nitrophenol, respectively, as described in Materials and Methods.

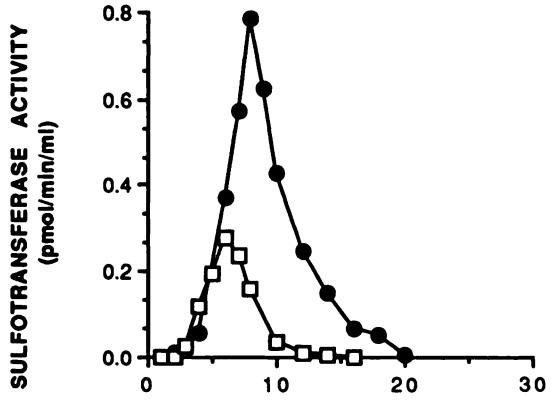

Fig. 5. Elution of human adrenal DHEA-ST and P-PST activities during PAP-agarose affinity chromatography. DHEA-ST activity obtained from the DEAE-Sepharose CL-6B procedure was concentrated by ultrafiltration and applied to a PAP-agarose affinity column (1.0 × 7 cm). The column was washed with 25 ml of TEA buffer containing 50 mM NaCl. DHEA-ST activity was eluted with a 30-ml linear gradient of PAPS (0–20 μM). DHEA-ST activity (●) and P-PST activity (□) were assayed as described in Materials and Methods.

TABLE 1

Purification of DHEA-ST from human adrenal cytosol

Cytosol was prepared from whole human adrenal glands and the purification was performed as described in Materials and Methods. One unit of activity represents 1 nmol of substrate conjugated/min.

	Total protein	Specific activity	Total activity	Purification	Recovery
	mg	*units/mg of protein*	*units*	*fold*	*%*
Cytosol	280	0.01	27	1.0	100
DEAE-Sepharose	31	0.04	12	4.0	45
PAP-agarose	0.05	31	1.7	320	6.2

possesses a 3β-hydroxyl group, was sulfated at approximately 45% of the rate of DHEA; however, androsterone, which possesses a 3-hydroxyl group, was sulfated at only 32% of the rate of DHEA. Adrenal DHEA-ST was also capable of sulfating

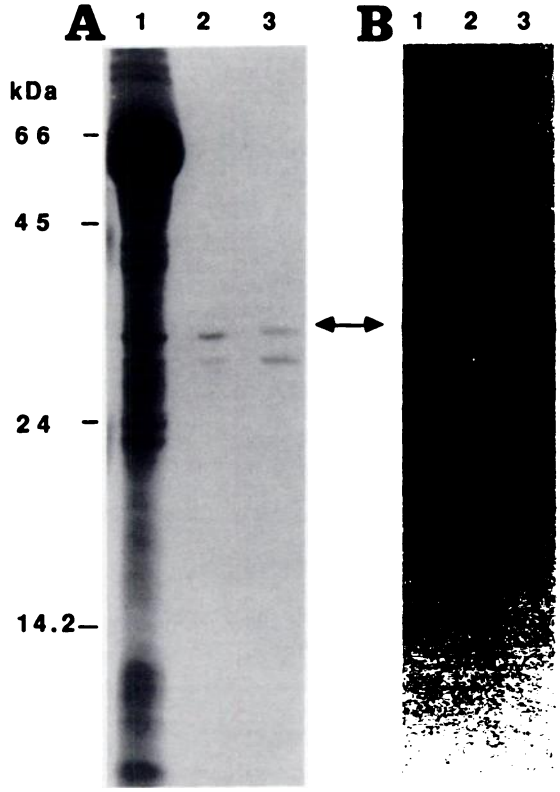

Fig. 6. SDS-PAGE (A) and immunoblot (B) analysis of human adrenal cytosol and purified human adrenal and liver STs. A, Human adrenal cytosol and purified adrenal and liver STs were resolved by SDS-PAGE in a 12.5% polyacrylamide gel and were stained with Coomassie Blue. *Lane 1*, human adrenal cytosol, 30 μg; *lane 2*, purified human adrenal STs, 3.5 μg; *lane 3*, purified human liver STs, 4.0 μg. B, Rabbit anti-human liver DHEA-ST was used to probe an immunoblot of an identical 12.5% polyacrylamide gel as shown in A. A 1/10,000 dilution of rabbit anti-human liver DHEA-ST IgG was used, as described in Materials and Methods.

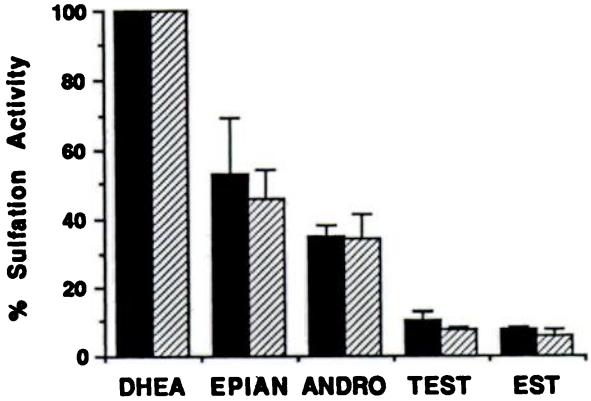

Fig. 7. Ratio of human adrenal cytosolic DHEA-ST activity to purified adrenal DHEA-ST activity. Cytosolic human adrenal DHEA-ST activity (■) and affinity-purified DHEA-ST activity (▩) toward DHEA, epiandrosterone (*EPIAN*), androsterone (*ANDRO*), β-estradiol (*EST*), and testosterone (*TEST*) were compared. Results are plotted as means ± standard deviations (three experiments), with 3 μM steroid used for each assay, as described in Materials and Methods.

estrone, β-estradiol, and testosterone at lower rates. The ratio of sulfation between the cytosolic and purified adrenal DHEA-ST was approximately the same for each of the steroids tested, suggesting that other forms of ST capable of sulfating DHEA, epiandrosterone, and testosterone are not present in adrenal cytosol.

The kinetic properties of the adrenal DHEA-ST are very similar to those of human liver DHEA-ST. As observed with the liver enzyme, adrenal DHEA-ST activity had a very similar pattern of substrate inhibition at DHEA concentrations above 3 μM (13). Substrate inhibition was not observed with increasing PAPS concentrations. The apparent K_m values for DHEA and PAPS obtained with the adrenal enzyme were 1.0 and 1.6 μM, respectively, as estimated by the method of Cleland (25) for enzymes demonstrating substrate inhibition. Cortisol, 11β,17α,21-trihydroxypregn-4-ene-3,20-dione, was also tested as a substrate for the liver and adrenal DHEA-STs. No evidence for cortisol sulfation was detected with either enzyme; however, the presence of 10 μM cortisol in DHEA assays inhibited DHEA sulfation by 30%.

Discussion

Sulfation is an important reaction in the metabolism of steroids in human liver and adrenal tissue. In the liver, sulfation decreases the biological activity and facilitates the excretion of steroid compounds. Sulfation also functions as a detoxification pathway for toxic hydrophobic bile acids, such as lithocholic acid, by decreasing their ability to be reabsorbed into the bile. Multiple forms of steroid STs have been identified and purified from rodent liver (11), and recent reports indicate that this heterogeneity is also present at the molecular level (26, 27). In contrast to the multiplicity observed in rodents, only a single steroid ST, DHEA-ST, which is capable of sulfating the 3-hydroxy-position of a variety of steroids, has been isolated and characterized from human liver cytosol (13). This enzyme is also responsible for most, if not all, of the bile acid ST activity in human liver cytosol (14). This report shows that specific polyclonal antibodies raised to human liver DHEA-ST detected a single protein of the same molecular weight in cytosol prepared from a number of individuals. The presence of a single immunoreactive protein in human liver cytosol supports the conclusion that there is a single major ST in the liver responsible for the sulfation of steroids and bile acids.

Previous characterization of polyclonal antibodies specific for the human PSTs revealed that these antibodies did not react with DHEA-ST in human liver cytosol (24). Similarly, anti-human liver DHEA-ST antibodies did not cross-react with either form of PST present in human liver or adrenal cytosol. Also, levels of DHEA-ST and P-PST activity in human liver cytosol are apparently independently regulated, indicating that these activities represent two distinct forms of ST (13, 14, 23). From these results, it can be concluded that DHEA-ST is structurally and biochemically different from the PSTs, although they may belong to the same family of enzymes.

Presently, only three distinct forms of cytosolic ST, DHEA-ST, P-PST, and M-PST, have been identified in human liver cytosol. The small number of human liver STs differs from the typical pattern of multiple forms of drug-metabolizing enzymes that possess overlapping substrate specificities. At least eight different steroid STs have been isolated and characterized from rat liver cytosol (11, 28–30). The absence of detectable cortisol sulfation or a separate estrogen ST activity distinct from these enzymes in both human adrenal and liver tissue also differs from the results observed in rats. A recent preliminary report indicates that human liver P-PST is capable of sulfating the

phenolic estrogens but not the alcoholic 3-hydroxy-position of steroids (31).

Multiple forms of the steroid and bile acid ST in rat liver have been demonstrated to be involved in the metabolism of both cortisol and estrogens (28, 30). These different forms of ST possess overlapping substrate specificities; however, several of the forms are relatively specific for cortisol or estrogen sulfation (32). If DHEA-ST is the major steroid/bile acid ST present in human liver, the function of estrogen and cortisol ST activities may have been replaced by the PSTs or by a different conjugating system, such as glucuronidation.

In contrast to the liver, sulfation of steroids in the adrenal is the last step in the biosynthesis and secretion of several steroids. Humans secrete large quantities of steroid sulfates from the adrenal, with the major steroid being DHEAS (5). DHEAS is second in abundance only to cortisol in the amount of steroid secreted per day from the adrenals. Besides DHEAS, several other steroids are also secreted as sulfates, including progesterone and to a lesser degree testosterone and the estrogens (6). The characterization of a human adrenal steroid ST has been previously reported (33). This enzyme possessed physical properties very similar to the ST described in this report. However, the steroid ST had "wave-like kinetics," which differ from the pattern of substrate inhibition observed with DHEA-ST purified from the liver and adrenal (13). Also, this steroid ST was capable of sulfating deoxycorticosterone, as well as cortisol, and was more reactive toward epiandrosterone and androst-5-ene-3β,17-diol than DHEA (34). The molecular characterization and expression of human adrenal DHEA-ST will aid in understanding the kinetic properties of the enzyme and its role in the sulfation of steroids in adrenal tissue.

The results of this investigation show that the adrenal form of DHEA-ST is very similar if not identical to the liver form. The report that human PST from adrenal pheochromcytoma tissue is biochemically similar to the platelet form of PST (35) agrees with our findings that the human adrenal and liver forms of DHEA-ST are very similar. Preliminary studies of the immunolocalization of DHEA-ST in human adrenal tissues indicate that DHEA-ST is localized to the zona reticularis of the adrenal cortex.[1] This is the zone of the adult adrenal cortex that is primarily responsible for the biosynthesis and secretion of steroids (6).

Although DHEA-ST is responsible for the majority of steroid and bile acid sulfation present in human adrenal and liver tissue, it is also possible that other minor forms of ST are present in human tissues. Estrogen ST activity has been partially purified from human placental and breast tumor tissue and shown to sulfate estrone, estriol, DHEA, and androsterone (36, 37). Also, it has been reported that a 21-hydroxysteroid ST activity in fetal tissue is responsible for the high levels of deoxycorticosterone sulfate present in the plasma of pregnant women and their fetuses (38). However, the relationship of these activities to DHEA-ST or the PSTs is unclear at this time. It is possible that multiple allelic forms of DHEA-ST exist, as is the case with the human PSTs (22). Further characterization of human liver DHEA-ST at the molecular and genomic level is necessary to determine its relationship to the human PSTs as well as the heterogeneity of human steroid STs.

[1] Unpublished observations.

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