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Human Liver Dehydroepiandrosterone Sulfotransferase: Molecular Cloning and Expression of cDNA

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SUMMARY

Sulfation is an important pathway in the metabolism of many hormones and drugs. Human liver contains at least three well characterized sulfotransferase (ST) enzymes, i.e., dehydroepiandrosterone (DHEA) ST and two forms of phenol sulfotransferase (PST). Our goal was to purify, to obtain partial amino acid sequence for, and to clone and express cDNA for human liver DHEA ST. Polymerase chain reaction primers were designed on the basis of homology among rat liver hydroxysteroid ST, rat liver PST, and bovine estrogen ST. These primers amplified a unique sequence from human liver cDNA, and this polymerase chain reaction product was used to screen a human liver cDNA library. Two clones were isolated that contained identical open reading frames, of 855 nucleotides, that encoded a protein of 285 amino acids. The deduced amino acid sequence of the encoded protein included two separate 27- and 23-amino acid sequences that were identical to those obtained by microsequencing of proteolytic fragments from purified human liver DHEA ST. Translation, in a rabbit reticulocyte lysate system, of mRNA transcribed *in vitro* from the two cDNA clones resulted in a 35-kDa translation product that comigrated with purified human liver DHEA ST during sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This translation product also catalyzed the sulfation of DHEA but not the sulfation of model substrates for the two forms of PST found in human liver. The two cDNA clones were also used to create expression constructs with the eukaryotic expression vector P91023(B), and these constructs were used to transfect COS-1 cells. The transfected cells expressed a high level of DHEA ST activity, and this enzyme activity displayed a pattern of inhibition by the ST inhibitor 2,6-dichloro-4-nitrophenol identical to that of human liver DHEA ST. Cloning of cDNA for this important human sulfate-conjugating enzyme will enhance understanding of the relationship between DHEA ST and other human liver STs, as well as ST enzymes in other species.

Sulfate conjugation is an important pathway in the biotransformation of many hormones, neurotransmitters, drugs, and xenobiotic compounds (1–3). The liver is a major organ in which hormone and drug metabolism occurs, and human hepatic preparations catalyze a series of ST reactions, all of which utilize PAPS as a sulfate donor (4–6). At least three separate, well defined, cytoplasmic ST enzymes are present in human liver (7, 8). One of these enzymes is DHEA ST (8). DHEA is quantitatively among the most important of the circulating steroid hormones in humans (9). DHEA and its sulfate conjugate play a critical role in the hormonal regulation of pregnancy (10–12), and they may also be involved in the pathophysiology of cardiovascular disease (13).

It is important to differentiate DHEA ST from the other well characterized ST enzymes in the human liver, the two PSTs. These three enzymes differ in their substrate specificities, inhibitor sensitivities, physical properties, and regulation among individuals (7, 8, 14–18). However, all three enzymes are dimers with monomeric molecular masses that vary from 32 to 35 kDa (7, 8, 19, 20). One of the two forms of PST is relatively TS, is sensitive to inhibition by DCNP, and preferentially catalyzes the sulfation of simple phenols such as *p*-nitrophenol (7, 14–17). The other form of PST is relatively TL, is resistant to DCNP inhibition, and preferentially catalyzes the sulfation of monoamines such as dopamine. DHEA ST has a DCNP inhibition profile similar to that of TL PST and thermal stability intermediate between that of the two forms of PST (18). Levels of activity of DHEA ST and the two forms of PST are regulated independently, but the activities of both forms of PST in human tissue are under genetic control (21–25). The genetic regulation of TS PST in the liver is related to

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ABBREVIATIONS: ST, sulfotransferase; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; DHEA, dehydroepiandrosterone; DCNP, 2,6-dichloro-4-nitrophenol; PST, phenol sulfotransferase; TS, thermostable; TL, thermolabile; FCS, fetal calf serum; DMEM, Dulbecco's modified Eagle's medium; PVDF, polyvinylidene difluoride; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction.

the existence of two isozymes of that form of the enzyme that can be separated by ion exchange chromatography (7, 25).

The cytoplasmic ST enzymes in human tissue, like many hormone- and drug-conjugating activities, have overlapping substrate specificities. For example, at millimolar concentrations the monoamine neurotransmitter dopamine can serve as a substrate for TS PST, and *p*-nitrophenol at millimolar concentrations can serve as a substrate for TL PST (7, 17). Although DHEA ST is the major steroid hormone ST in human liver (8), TS PST can also catalyze the sulfation of steroid hormones such as estrone and 17β-estradiol (18). However, DHEA ST is unable to catalyze the sulfate conjugation of either *p*-nitrophenol or dopamine (8). Elucidation of the relationship among these enzymes, determination of the total number of ST enzymes present in human tissue, and understanding of their regulation will be made possible by the molecular cloning of their cDNAs. In the present experiments, we purified human liver DHEA ST, obtained partial amino acid sequence information for the enzyme after limited proteolysis, used the PCR to obtain a probe that was used to isolate several cDNAs from a human liver cDNA library, and expressed functional DHEA ST from the cloned cDNAs in both a rabbit reticulocyte lysate system and COS-1 cells.

Materials and Methods

Tissue acquisition. Pathologically normal hepatic tissue was obtained from patients who underwent clinically indicated partial hepatectomies for the removal of either primary or metastatic hepatic tumors. All tissue was obtained under guidelines approved by the Mayo Clinic Institutional Review Board. The tissue was stored at −80°, and hepatic tissue from three to 10 individuals was pooled for the purification of DHEA ST and TS PST.

DHEA ST and TS PST purification. DHEA ST and TS PST were separated from TL PST and were partially purified as described by Otterness *et al.* (19). The purification procedure included, in sequence, DEAE-Sepharose CL-6B ion exchange, Affi-Gel Blue, and heparin-Sepharose CL-6B chromatography (Fig. 1).

ST assays. ST activities were assayed by the method of Foldes and Meek (26), as modified by Watson *et al.* (27) for the measurement of DHEA ST activity and by Campbell *et al.* (7) for the measurement of TS and TL PST activities. The assay conditions used were optimal for the measurement of all three activities in human liver. The sulfate acceptor substrates were 5 μM DHEA, 4 μM *p*-nitrophenol, and 60 μM dopamine for DHEA ST, TS PST, and TL PST, respectively. All three activities were assayed in the presence of 0.4 μM [^{35}S]PAPS.

Protein assay. Protein concentrations were measured by the method of Bradford (28), with bovine serum albumin as a standard.

Photoaffinity labeling and two-dimensional gel electrophoresis. Photoaffinity labeling of enzyme preparations with [^{35}S]PAPS was performed as described by Otterness *et al.* (29). After photoaffinity labeling, enzyme preparations were subjected to two-dimensional gel electrophoresis (30). Ten to twenty micrograms of partially purified DHEA ST/TS PST were applied to isoelectric focusing gels over a pH range from 3 to 10. The second dimension was a 12.5% polyacrylamide-SDS slab gel.

Limited proteolysis. Proteins that had been separated by two-dimensional gel electrophoresis were subjected to limited proteolysis by the method of Cleveland *et al.* (31). Specifically, 10–20 μg of partially purified ST were subjected to two-dimensional gel electrophoresis, proteins were visualized with Coomassie Blue, and individual proteins were excised from the gel and placed in the wells of a 15% polyacrylamide-SDS slab gel. Proteases used included *Staphylococcus aureus* V8 protease (0.033 μg/well), papain (0.1 μg/well), and α-chymotrypsin (0.1 μg/well). Proteolysis times were 30 min for *S. aureus* V8 protease, 2.5 hr for papain, and 2 hr for α-chymotrypsin. After electrophoresis, peptide fragments were transferred to PVDF membranes for 3 hr at 70 V, in 192 mM glycine, 25 mM Tris, pH 8.3, 15% methanol, by the method of Towbin *et al.* (32). The PVDF membranes were stained in 40% methanol, 0.5% Coomassie Blue, followed by destaining with 50% methanol.

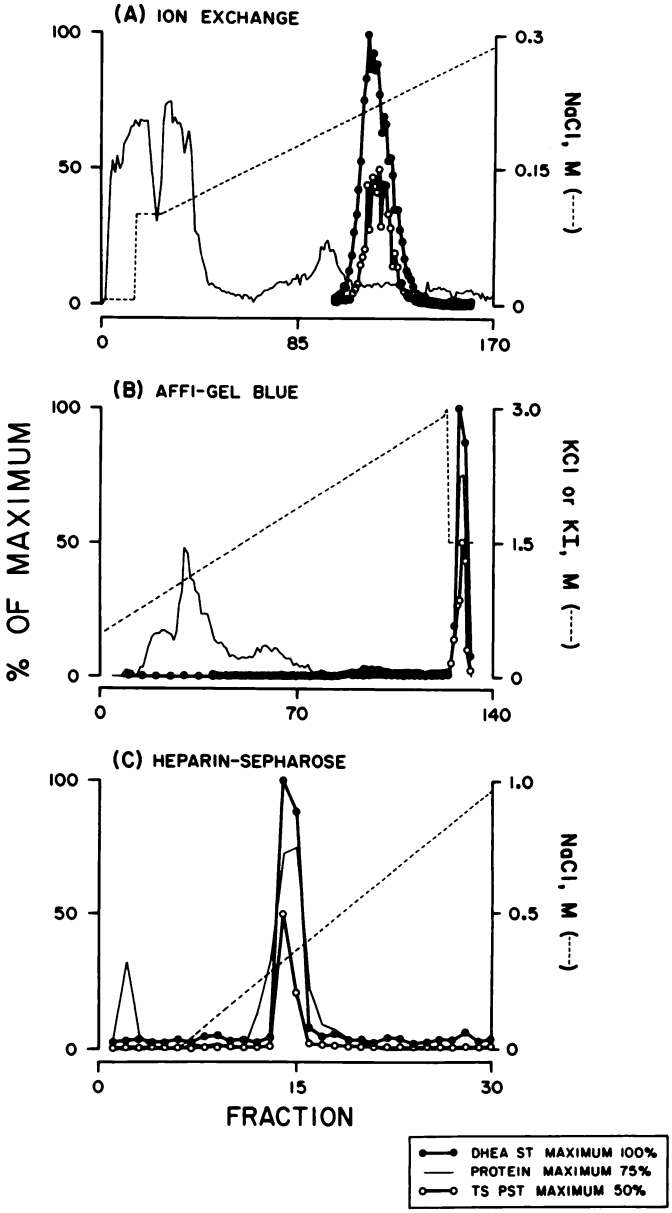

Fig. 1. Purification of human liver DHEA ST (●) and TS PST (○) by sequential DEAE-Sepharose CL-6B ion exchange (A), Affi-Gel Blue (B), and heparin-Sepharose CL-6B (C) chromatography. Elution of protein (–) is also shown. Different relative scales were used to display peak heights (see *box at lower right*), to prevent superimposition of peaks.

Amino acid sequencing. Proteins or proteolytic fragments isolated by two-dimensional gel electrophoresis and transferred to PVDF membranes were sequenced by a gas-phase method with an ABI 470A protein sequencer, using an ABI 120A on-line phenylthiohydantoin analyzer (Applied Biosystems Inc., Foster City, IA). Data collection and analysis were performed with an IBM PS/2 50Z and with Beckman System Gold high performance liquid chromatography software (Beckman Instruments, Inc., San Ramon, CA).

Preparation of RNA and PCR. Total RNA was isolated from a

frozen surgical biopsy sample of human liver by extraction with guanidine HCl, followed by centrifugation through a cushion of CsCl. First-strand cDNA synthesis was performed using an oligo(dT) primer and murine reverse transcriptase. The first-strand cDNA was used as template for PCR. Oligonucleotide primers for PCR were designed on the basis of homology among rat liver hydroxysteroid ST, rat liver PST, and bovine placental estrogen ST (33–35). The conserved amino acid sequences used to design the primers were YPKSGT [5′-TA(T or C)CC(C or A)AA(G or A)TC(A or T)GG(A or T)AC-3′] and GDWKN [anti-sense, 5′-ATT(C or T)TTCCAGTC(C or T)CC-3′]. Only codons utilized in rat liver hydroxysteroid ST, rat liver PST, and bovine placenta estrogen ST for those two amino acid sequences were used to generate the PCR primers. The PCR reaction was performed in a 100-μl reaction volume (10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 50 μM each of the four deoxynucleoside triphosphates, and 1 unit of *Thermus aquaticus* polymerase), in a Perkin Elmer Cetus DNA thermal cycler (Emeryville, CA). The conditions used, after initial denaturation for 10 min at 95°, were 35 cycles of 1 min at 94°, 2 min at 47°, and 3 min at 72°, followed by a final 10-min incubation at 72°. The PCR product was isolated on a 1% agarose gel and was removed from the gel with the GeneClean kit (BIO 101, Inc., La Jolla, CA).

Cloning of PCR product and screening of cDNA library. The ends of the PCR product were filled in by use of the Klenow fragment of DNA polymerase. The product was then ligated into the *Sma*I site of pBluescript (Stratagene, La Jolla, CA) and transformed into *Escherichia coli* DH5α made competent by the method of Hanahan (36). Eleven positive clones were isolated and partially sequenced by the dideoxy method of Sanger *et al.* (37), with the ^{35}S-sequencing protocol of the Sequenase kit version 2.0 (United States Biochemical Corp., Cleveland, OH). The insert from one clone was excised and used to screen a human liver cDNA library constructed in the Uni-Zap XR vector (Stratagene) and grown in XL-1 Blue cells. Cloned PCR product that had been nick-translated with [α-^{32}P]dATP was used to screen 10^6 plaque-forming units. Plaques were transferred to nitrocellulose filters and were hybridized by the method of Thomas (38). The 13 positive clones were excised *in vivo* from Uni-Zap XR, and clones were partially sequenced with the Sequenase kit version 2.0. Both strands for two of the clones, A and G, were completely sequenced by use of a combination of synthetic sequencing primers and *Sau*3AI subclones.

***In vitro* transcription and translation.** cDNA clones A and G were linearized with *Kpn*I, and T_3 RNA transcripts were synthesized with the mCAP mRNA capping kit (Stratagene). *In vitro* translation of these RNA samples was performed in a rabbit reticulocyte lysate system (39). Translated proteins were analyzed by SDS-PAGE (40) and were also assayed for the presence of ST enzymatic activity.

Expression of cDNAs in COS-1 cells. pBluescript clones A and G were digested with *Xba*I and *Kpn*I to excise inserts, and the cDNAs were ligated into pBluescribe to create a second *Eco*RI site at the 3′ ends of the inserts. The pBluescribe clones were digested with *Eco*RI, and the cDNA inserts were cloned into the *Eco*RI site of the expression vector P91023(B) (41, 42). Positive P91023(B) clones were isolated by colony screening performed with the nick-translated cloned PCR product (43), and the orientation of the inserts was determined by restriction mapping. The expression constructs were designated 11A and 17G for clones A and G, respectively, whereas antisense orientation constructs were designated 3A and 15G. COS-1 cells were plated in 100-mm dishes at a density of 1.2×10^6 cells/dish, in DMEM with 10% FCS, and were grown overnight. The purified plasmid (0.5 μg) was mixed with DEAE-dextran and DMEM (44, 45) and was added to the cell culture dishes. Mock-transfected cells were treated with no DNA, with expression vector alone, or with constructs that contained antisense orientations of clones A and G. After 4 hr, the DNA-DEAE-dextran solution was replaced by DMEM with 10% dimethylsulfoxide for 2 min, followed by 0.1 mM chloroquine in DMEM for 2 hr (46). Cells were then grown in DMEM with 10% FCS for 40–42 hr. The cells were harvested, cell pellets were washed in 5 ml of PBS, and the pellets were homogenized for 30 sec in 2 ml of 5 mM potassium phosphate buffer, pH 6.5. Homogenates were centrifuged at 4° for 15 min, and supernatants were then centrifuged at 100,000 × *g* for 1 hr at 4°. Fifty-microliter aliquots of the 100,000 × *g* supernatants were assayed for DHEA ST, TS PST, and TL PST enzymatic activities.

Data analysis. The University of Wisconsin Genetics Computer Group software package (47) was used to analyze sequence information and to make comparisons between the sequences of DHEA ST cDNA clones and the sequences of other cloned ST enzymes. The GenBank Genetics Sequence Data Bank and the EMBL Nucleotide Sequence Database were used to search for nucleotide homologies, whereas the Swiss-Prot Protein Sequence Database was used to search for protein structural homologies.

Materials. [^{35}S]PAPS (1.5–2.5 Ci/mmol and 270 Ci/mmol) was purchased from New England Nuclear (Boston, MA). [α-^{32}P]dATP (>3000 Ci/mmol) was obtained from ICN Biomedicals, Inc. (Costa Mesa, CA). α-^{35}S-dATP (>1000 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). DNA polymerase, T_4 DNA ligase, murine reverse transcriptase, DMEM, and FCS were purchased from GIBCO BRL (Gaithersburg, MD). Restriction enzymes were obtained from GIBCO BRL and Boehringer Mannheim Corp. (Indianapolis, IN). DEAE-Sepharose CL-6B, heparin-Sepharose CL-6B, and DEAE-dextran were obtained from Pharmacia LKB Biotechnology, Inc. (Piscataway, NJ). Affi-Gel Blue and SDS-PAGE reagents were purchased from Bio-Rad Laboratories (Richmond, CA). DHEA, dopamine HCl, *p*-nitrophenol, *S. aureus* V8 protease, α-chymotrypsin, and papain were obtained from Sigma Chemical Co. (St. Louis, MO). Ampholines were purchased from Serva Biochemicals (Westbury, NY), and Immobilon-PVDF membranes were obtained from Millipore Corporation (Bedford, MA).

Results

Protein purification, limited proteolysis, and partial amino acid sequence. Human liver DHEA ST and TS PST activities in 100,000 × *g* supernatant preparations copurified through three sequential chromatographic steps (Fig. 1). It had been reported previously that these two enzymes were difficult to separate chromatographically (8). However, as reported previously (7), TL PST was easily separated from DHEA ST and TS PST by DEAE-Sepharose CL-6B ion exchange chromatography, and, after the ion exchange step, the enzyme preparation contained virtually no TL PST activity. When a DHEA ST/TS PST enzyme preparation purified as shown in Fig. 1 was analyzed by two-dimensional gel electrophoresis, three separate proteins with molecular mass values of approximately 35 kDa were present (Fig. 2, *left*). DHEA ST and TS PST in human liver are dimeric proteins composed of monomeric subunits with molecular mass values of approximately 35 kDa (8, 19). All three of these proteins could be photoaffinity labeled with

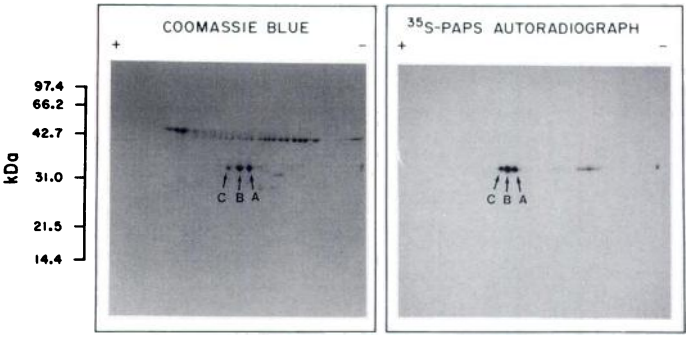

Fig. 2. Two-dimensional gel electrophoresis of human liver DHEA ST and TS PST purified by use of the chromatographic steps shown in Fig. 1. *Left*, Coomassie Blue stain; *right*, photoaffinity labeling with [^{35}S]PAPS, the sulfate donor for ST reactions.

[^{35}S]PAPS (Fig. 2, *right*), the sulfate donor for ST reactions (5, 29). Because human liver contains two genetically determined isozymes of TS PST (7, 25), it seemed likely that these three proteins might represent DHEA ST and the two TS PST isozymes present in human liver. However, because the identities of the three proteins were unknown, they were given the working designations of proteins A, B, and C (Fig. 2).

The amino terminus of each of the three 35-kDa proteins shown in Fig. 2 was blocked to Edman degradation. Therefore, limited proteolysis was performed to generate peptide fragments for use in amino acid sequencing. Three different proteases, *S. aureus* V8 protease, α-chymotrypsin, and papain, were used to generate peptide fragments. Each protease generated a different pattern of peptide fragments, but patterns of fragments obtained with a given protease were identical for each of the three proteins. SDS-PAGE of proteins A, B, and C after their removal from a two-dimensional gel is shown in Fig. 3. Fig. 3 also shows the pattern of peptide fragments generated from each of these three proteins by *S. aureus* V8 protease. The *arrows* in Fig. 3 identify proteolytic peptide fragments, with approximate molecular mass values of 25 and 7 kDa, that were used to obtain partial amino acid sequence information. Identical amino acid sequences were found for all three proteins with the 25-kDa peptide (Fig. 3), and the 7-kDa peptide generated by *S. aureus* V8 protease (Fig. 3) also gave identical amino acid sequence information for proteins A and B (Fig. 4). There was an inadequate quantity of the 7-kDa fragment of protein C for use in sequencing. Amino acid sequence information for peptide fragments generated by α-chymotrypsin and papain was obtained in a similar fashion. That information either confirmed or extended data obtained with the 25- and 7-kDa fragments generated by *S. aureus* V8 protease (Fig. 4). The amino acid sequences shown in Fig. 4 displayed a high degree of homology to sequences present in rat liver hydroxysteroid ST (34), rat liver PST (35), and bovine placental estrogen ST (33).

cDNA cloning. Information obtained during the limited proteolysis experiments was used in initial attempts to clone

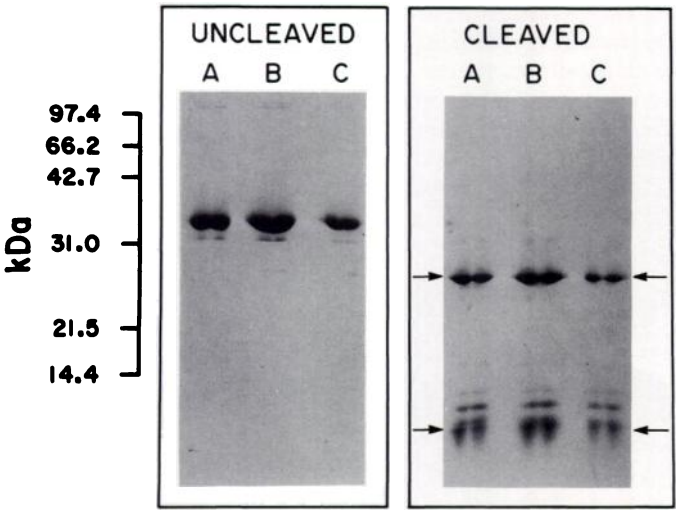

Fig. 3. Limited proteolysis by *S. aureus* V8 protease. *Left*, electrophoresis of uncleaved proteins A, B, and C (see Fig. 2), after these proteins were separated by two-dimensional gel electrophoresis; *right*, electrophoresis of these proteins after limited proteolysis with *S. aureus* V8 protease. *Arrows*, 25-kDa and 7-kDa fragments from which amino acid sequence information was obtained.

	7 kDa Fragment			25 kDa Fragment		
	A	B	C	A	B	C
					**	
1	D/E	D/E	N.D.	D/E	E	E D/E
2	K	K		I	I	I
3	N	N		G	G	G G
4	F	F		Y	Y	Y Y
5	L	L		T	T	T T
6	L	L		A	A	A A
7	L	L		L	L	L L
8	S	S *		S	S	S S
9	Y	Y Y		E	E	E E
10	E	E E		T	T	T T
11	E	E E		E	E	E E
12	L	L L		S	S	S
13	K	K K		P	P	P
14	Q	Q Q		R		
15	D	D D		L		
16	T	T T		F		
17		G G		S		
18		R R		S		
19		T T		H		
20		I		L		
21		E		P		
22		K		I		
23		I		Q		
24				L		
25				F		
26				P		
27				K		

Fig. 4. Amino acid sequences of fragments isolated during limited proteolysis of proteins A, B, and C with *S. aureus* V8 protease (see Figs. 2 and 3). Amino acid sequence information obtained from fragments isolated during limited proteolysis performed with α-chymotrypsin (*) and papain (**) is also shown. *N.D.*, not done.

human liver DHEA ST cDNA. Two oligonucleotides were designed, one that was 192-fold degenerate and one that was 256-fold degenerate. However, we were unsuccessful in an attempt to amplify a PCR product from human liver cDNA with these primers, perhaps because of the degree of degeneracy of the oligonucleotide primers. Therefore, two different oligonucleotide primers were designed on the basis of sequence homology among previously cloned ST cDNAs from non-human mammalian species. These oligonucleotides were used as PCR primers to amplify, from human liver cDNA, a segment that was approximately 650 base pairs in length, the approximate size of the PCR product anticipated on the basis of possible homology of human liver DHEA ST cDNA to rat and bovine ST cDNAs. This PCR product was cloned into pBluescript and was partially sequenced. The amino acid sequences encoded by the PCR product included all of the amino acids determined by sequencing of peptide fragments obtained from the human liver DHEA ST/TS PST preparation (Fig. 4).

The cloned PCR product was then used to screen a human liver cDNA library. Thirteen positive clones were isolated, and 10 were at least partially sequenced. One was found to be a cloning artifact, seven were truncated at the 5′ ends, and two clones, A and G (approximately 1750 and 1080 nucleotides in length, respectively), were sequenced in both directions and were found to contain identical open reading frames of 855 nucleotides that encoded a protein of 285 amino acids (Fig. 5). The amino acid sequences of the two peptide fragments gen-

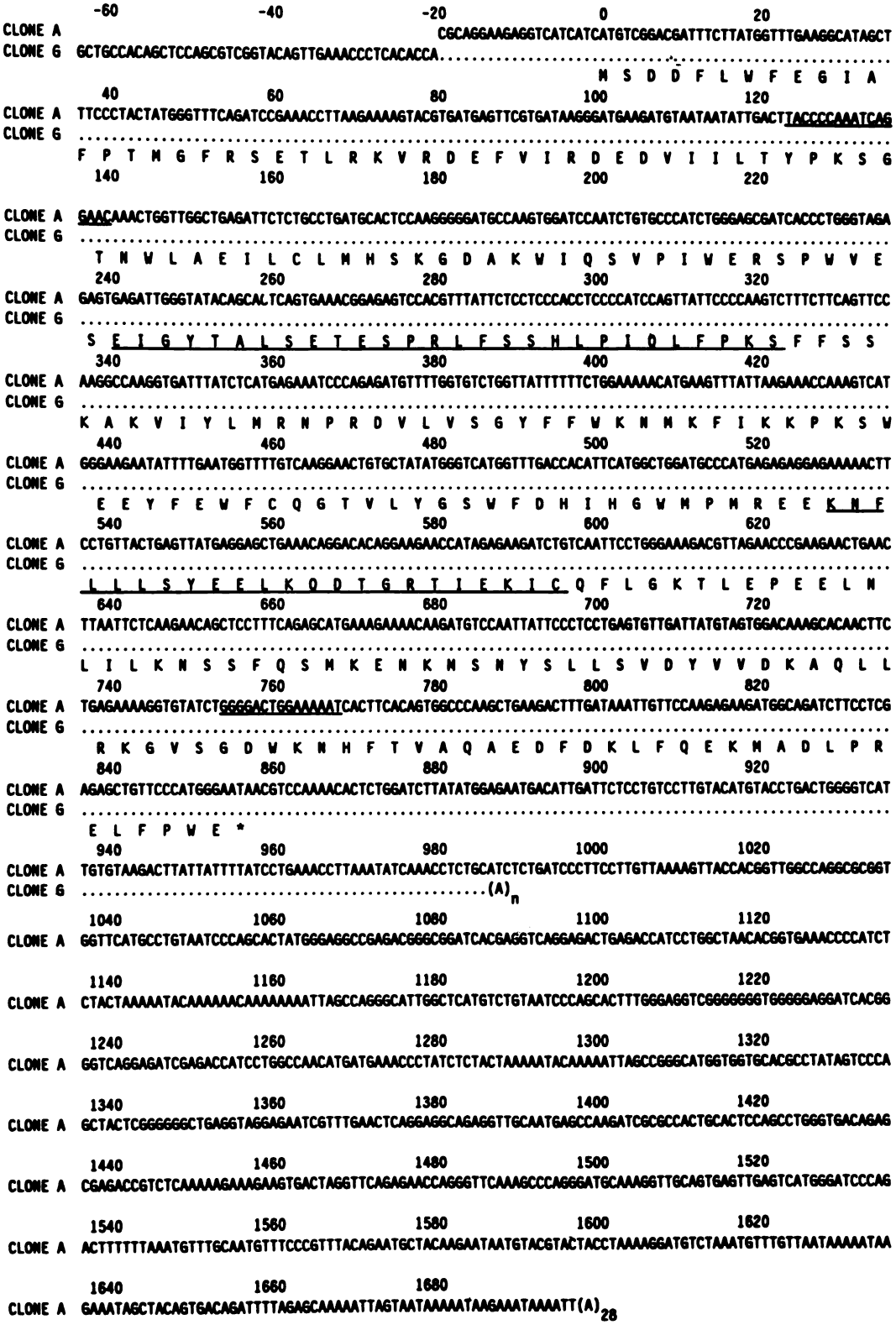

Fig. 5. Nucleotide sequences of human liver DHEA ST cDNA clones A and G. The *dotted line* represents nucleotides present in clone G that were identical to those in clone A. *Underlined areas in the deduced amino acid sequence*, areas identical to the amino acid sequences present in human liver DHEA ST, shown in Fig. 4. *Underlined areas in the nucleotide sequences*, those that were used to design the PCR primers. Nucleotides 1149, 1156, and 1166 were the locations of polyadenylation sites for other truncated clones, as was one site located eight nucleotides 3′ to the polyadenylation site for clone A.

erated by *S. aureus* V8 protease treatment of proteins A, B, and C in the DHEA ST/TS PST preparations (Figs. 2 and 3) were encoded within the open reading frame of clones A and G. The nucleotide sequence of clone G was 100% identical to that of clone A, except for the presence of additional nucleotides at the 5′ end of clone G (Fig. 5). Clone A had a much longer 3′ untranslated region (approximately 840 nucleotides) than did clone G (approximately 130 nucleotides) (Fig. 5). However, both clones terminated with poly(dA) tails. The predicted molecular mass of the protein encoded by both clones was 33.78 kDa. Of the truncated clones that were isolated, two had poly(A) tails that originated at nucleotide 1149, two had poly(A) tails that originated at nucleotide 1156, two had poly(A) tails that originated at nucleotide 1166, and one had a poly(A) tail

that originated eight nucleotides beyond the polyadenylation site of clone A.

Expression of DHEA ST activity. Clones A and G were transcribed *in vitro*, and the mRNAs obtained were translated with a rabbit reticulocyte lysate system. The translation products for both mRNAs were 35-kDa proteins (Fig. 6). Furthermore, when these preparations were assayed for DHEA ST, TS PST, and TL PST enzyme activities with DHEA, *p*-nitrophenol, and dopamine, model substrates for each of the three enzymes, respectively, translation products for both clones were able to catalyze the sulfation of DHEA but not that of either *p*-nitrophenol or dopamine.

Clones A and G were then subcloned, in both directions, into the eukaryotic expression vector P91023(B). Vectors with clones A and G present in both orientations were transfected into COS-1 cells. After transient expression, DHEA ST, TS PST, and TL PST activities were measured in homogenates of these cells, with model substrates for each of the three enzymes, under optimal conditions for the measurement of the enzyme activities in human liver preparations (7, 27). Both clones A and G were capable of catalyzing the sulfation of DHEA when placed in the proper orientation in the expression vector (designated 11A and 17G, respectively), whereas, when transfection was performed with P91023(B) alone or with clones A and G in antisense orientations (designated 3A and 15G, respectively), no DHEA ST enzymatic activity was present (Table 1). No TS PST or TL PST activity could be detected after transfection above the very low basal activity levels present in the COS-1 cells. The sensitivity of the DHEA ST activity expressed in COS-1 cells to inhibition by DCNP was also examined. When the DCNP inhibition profile of the DHEA ST activity expressed in the cells was compared with those of human liver DHEA ST, TS PST, and TL PST, the inhibition profile of the expressed ST activity was virtually identical to that of human liver DHEA ST (Fig. 7).

Human liver DHEA ST sequence: comparison with other STs. The predicted amino acid sequence of human liver DHEA ST (Fig. 5) was compared with those reported previously

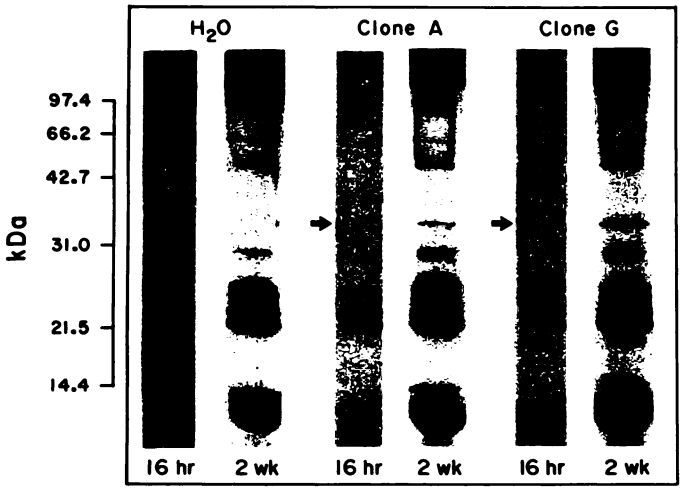

Fig. 6. Translation of clones A and G by a rabbit reticulocyte lysate system. SDS-PAGE of protein products after the translation of cDNA clones A and G by a rabbit reticulocyte lysate system is shown. Both clones encoded a protein with an approximate molecular mass of 35 kDa (see *arrows*). Two different exposure times for autoradiograms are shown. Results obtained with a control sample, in which only water was added to the system, are also shown. See text for details.

TABLE 1

Expression of DHEA ST enzymatic activity after transfection of COS-1 cells with clones A and G in either sense (11A and 17G, respectively) or antisense (3A and 15G, respectively) orientation

The results of two separate experiments are shown. The COS-1 cells had no detectable basal DHEA ST activity. Average basal TS PST activity in the COS-1 cell preparations ranged from 411 to 1015 cpm, and average basal TL PST activity ranged from 869 to 941 cpm in the two experiments.

	DHEA ST enzymatic activity	
	Experiment 1	Experiment 2
	cpm	
No DNA control	0	11
No DNA control	0	0
P91023(B) control	28	0
3A (antisense)	0	16
11A (sense)	58,882	29,864
15G (antisense)	0	106
17G (sense)	59,435	28,805

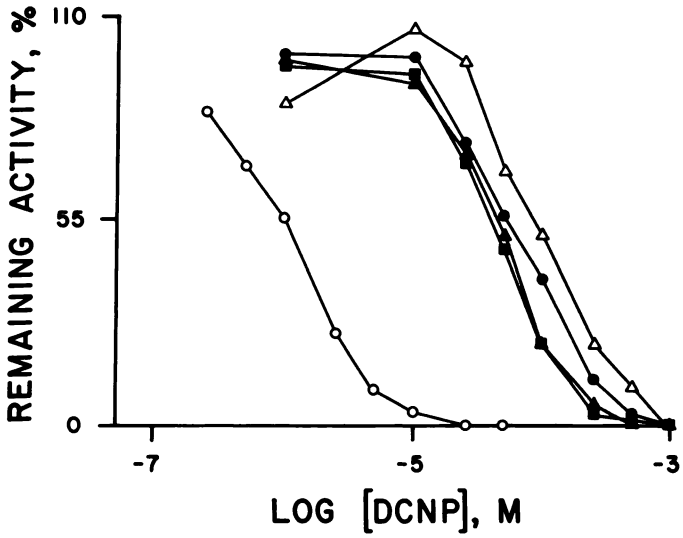

Fig. 7. DCNP inhibition of human liver DHEA ST (●), TS PST (○), and TL PST (△), as well as inhibition of DHEA ST activity expressed in COS-1 cells by cDNA clones A (11A) (▲) and G (17G) (■).

for ST enzymes from other species (33–35). The predicted amino acid sequence of DHEA ST was 63% identical to that of rat hydroxysteroid ST, 39% identical to that of rat PST, and 39% identical to that of bovine estrogen ST. The predicted amino acid sequence of DHEA ST also displayed 61% identity to that of rat senescence marker protein (48), a protein that has been postulated to be an isozyme of rat hydroxysteroid ST (34). Comparisons of the nucleotide sequence of human liver DHEA ST cDNA with those of previously cloned ST enzymes showed a higher degree of identity than did the amino acid sequence comparisons. The nucleotide sequence of DHEA ST within the open reading frame was 74% identical to that of rat hydroxysteroid ST, 59% identical to that of rat liver PST, and 54% identical to that of bovine placental estrogen ST.

Discussion

Sulfation is a major pathway in the metabolism of steroid hormones, bile acids, neurotransmitters, drugs, and xenobiotic compounds (1–3). DHEA ST is one of the major enzymes in human liver that catalyzes the sulfation of steroid hormones and of bile acids (8, 49). DHEA is quantitatively the most

important circulating steroid hormone in both male and female humans (9), and this steroid, as well as its sulfate conjugate, plays a critical role in the hormonal regulation of human pregnancy (10–12). However, human liver also contains other cytoplasmic ST enzymes, including TS and TL PST (7). Although these three ST enzymes differ with regard to substrate specificities, inhibitor sensitivities, physical properties, and regulation, they, like many other hormone- and drug-conjugating enzymes, show overlap in substrate specificity (7, 14–18). For example, even though DHEA ST appears to play the major role in the sulfate conjugation of estrone in human liver, TS PST is also capable of catalyzing that reaction (18). Conversely, TS PST appears to play the primary role in the sulfate conjugation of 17β-estradiol in human liver, but DHEA ST can also catalyze sulfation of that hormone (18). In the present experiments, we succeeded in cloning human liver DHEA ST cDNA and expressed the cloned cDNA as a first step toward enhancing our understanding of the molecular basis for the regulation of the sulfate conjugation of steroid hormones in humans.

The approach used began with the purification of human liver DHEA ST. Like other investigators (8), we found that DHEA ST and TS PST were difficult to separate using chromatographic purification procedures. However, the use of photoaffinity labeling with [^{35}S]PAPS, followed by two-dimensional gel electrophoresis, made it possible to separate three 35-kDa proteins present in the purified DHEA ST/TS PST preparations that could be photoaffinity labeled with the sulfate donor for ST enzymes (Fig. 2). Although all three of these proteins were blocked to Edman degradation, limited proteolysis made it possible to obtain partial amino acid sequence information for all three proteins. Amino acid sequences of peptide fragments obtained from all three proteins were identical within the areas sequenced, and these sequences showed a high degree of homology to deduced amino acid sequences in rat and bovine ST enzymes for which cDNAs had been cloned (33–35). A comparison of the degree of homology among these non-human ST enzyme cDNAs made it possible to design oligonucleotide primers that were used to amplify a PCR product from human liver cDNA. That PCR product contained nucleotide sequences that encoded the amino acid sequences present in the three 35-kDa proteins in the DHEA ST/TS PST preparations. When the PCR product was used to screen a human liver cDNA library, multiple clones, at least two of which contained an 855-nucleotide open reading frame, were isolated. Both of these clones were completely sequenced, and they both terminated in poly(A) sequences. Other clones isolated terminated in poly(A) sequences in slightly different locations (Fig. 5), raising the possibility of differential processing at the 3′ ends of DHEA ST transcripts. However, the absence of a consensus polyadenylation signal upstream from the poly(A) tail in most of the clones was inconsistent with this interpretation. Obviously, additional studies will be required to address the issue of possible variability in processing of the 3′ ends of these transcripts.

Expression of clones A and G in a rabbit reticulocyte lysate system yielded a 35-kDa protein that catalyzed the sulfation of DHEA but not that of *p*-nitrophenol or dopamine, model substrates for human liver TS and TL PST, respectively (7). Expression of these two clones in COS-1 cells demonstrated that the cDNAs encoded a protein capable of catalyzing the sulfate conjugation of DHEA but not the sulfation of *p*-nitrophenol or dopamine (Table 1). Furthermore, the sensitivity of the expressed enzyme activity to DCNP inhibition was virtually identical to that of human liver DHEA ST, but it was quite different from the DCNP inhibition profile for TS PST (Fig. 7). Human liver DHEA ST cDNA showed a high degree of homology with the cDNAs of STs cloned previously from the rat liver and the bovine placenta (33–35). In addition, our partial amino acid sequencing of the three proteins in the human liver DHEA ST/TS PST enzyme preparation suggested that these two enzymes exhibited significant homology. Isolation and expression of human liver DHEA ST cDNA should make it possible to characterize the biochemical and molecular relationships among the ST enzymes present in human liver, as well as the relationship between these STs and those found in other species. It also represents a step toward understanding the regulation of the expression of the enzymes that catalyze this important reaction in the biotransformation of many steroid hormones, neurotransmitters, drugs, and xenobiotic compounds.

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