Vol. 187, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1992 Pages 448-454

MOLECULAR CLONING OF THE ALCOHOL/HYDROXYSTEROID FORM (hST_a) OF SULFOTRANSFERASE FROM HUMAN LIVER#

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Received July 9, 1992

SUMMARY. A cDNA encoding the human alcohol/hydroxysteroid sulfotransferase (h-ST-a), which catalyzes the sulfo-conjugation of many drugs and hormones, was isolated from a human liver cDNA library using a rat ST_a (rST_a) cDNA probe. The cDNA, designated as hST_a, consists of 1069 base pairs (bp) and contains an 855-nucleotide open reading frame beginning at nucleotide 65, which encodes a 285 amino acid polypeptide of 33.76 kDa. A second cDNA clone (1563 bp) was truncated 5' at nucleotide 231 (lacking the first 15 amino acids) with identical coding region, however, it had a much longer 3' untranslated region (UTR). Both clones contained a short segment of poly(A)$^+$ tail. Northern blot analysis of an adult human liver showed that there are at least 2 mature mRNA with sizes ranging from approximately 1.1 kb to 1.7 kb, verifying the authenticity of the obtained cDNA clones. From the sequence alignment, the hST_a shares 62%/74%, 39%/59%, 35%/48%, 36%/54% identity with rST_a, rST_p (phenol), rST_e (estrogen), and bovine ST_e (bST_e) at the deduced amino acid and DNA levels, respectively, indicating that there are at least three subfamilies (alcohol, phenol and estrogen) of genes that encode for sulfotransferases in mammals.

Sulfotransferases (STs)◊ are Phase II metabolizing enzymes that sulfo-conjugate a variety of endogenous and exogenous compounds, such as biogenic amines (e.g., catecholamines), steroid hormones (i.e., androgens), bile acids (e.g., hyodeoxycholic acid), drugs (e.g., propranolol and acetaminophen), and carcinogens (e.g., N-hydroxy-2-acetylaminofluorene and hydroxylated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) (1,2). STs also play an important role in the biosynthesis of proteoglycans, conferring important recognition properties to cell surfaces influencing cell adhesion and migration, and to the binding of growth factors and enzyme inhibitors (3).

#During the preparation of this manuscript, a report on the cloning of human liver DHEA sulfotransferase was published by Otterness et al. (20). The nucleotide sequence of its cDNA has 100% identity when compared to that of hST_a cDNA.

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◊ST(s) is used to denote sulfotransferase protein(s) and italic *ST* with subscript, e.g., ST_a, indicates the gene that encodes the protein.

0006-291X/92 $4.00

STs belong to a multigene family which comprises at least five genes in the rat (4-8), and one of bovine origin (9). These conjugating enzymes catalyze the coupling of sulfate ($SO_3^=$) from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to molecules possessing phenols, enols, alcohols or amines forming mono-esters of sulfuric acid or sulfate esters (10). Conjugation with sulfate confers greater polarity and water solubility on the parent agents, thereby facilitating biliary and/or urinary excretion and detoxification.

ST-a catalyzes O-sulfation of carcinogenic and non-carcinogenic exogenous alcohols, as well as endogenous steroidal alcohols, such as dehydroepiandrosterone (DHEA) (1). Regulation of the metabolism of the naturally occurring steroid DHEA has been implicated in a variety of disease processes, such as aging, diabetes, autoimmune diseases, cancer, obesity and atherosclerosis (11). Unlike primates, rodents do not synthesize DHEA from cholesterol, and thus its effects can be investigated by administering as a supplement to the diet. Such studies have shown DHEA to have remarkable chemopreventive effects on the above disorders. DHEA also found to affect the expression of cellular P450s, thereby influencing the balance of metabolic activities associated with the initiation phase of chemical carcinogenesis and/or toxicity. (11). Hence, examining the regulation of ST-a, one of the major enzymes that metabolize DHEA, should provide important insights towards our understanding of the molecular basis for the regulation of sulfate conjugation of steroid hormones in humans and in rodents. The molecular cloning of *hST$_a$* cDNA would provide the first step towards these goals.

MATERIALS AND METHODS

PCR Cloning of rat *ST$_a$* Two oligonucleotides were designed from published sequences for *rST$_a$* (5) and used for PCR amplification of the cDNA. These are oligo S1 (5'-TTTATAATGCCAGACTATACTTGGTTT-3'), which is located at the 5' most end of the *rST$_a$* cDNA, i.e., it covers the translation start site methionine (ATG), which is underlined, and oligo S2 (5'-ATTTTAATCCCATGGGAACAT-3'), which presides at the 3' most end of the cDNA, i.e., it includes the stop codon TAA and TTA in the antisense strand (underlined). These primers were used to amplify a female Sprague-Dawley rat liver (Clontech, Palo Alto, CA) single-stranded cDNA template obtained by reverse transcription of total mRNA using cDNA Synthesis Kit (Boehringer Mannheim, Indianapolis, IN) with both oligo-dT and random primers according to protocols provided by the manufacturer. PCR amplification started when both the forward (S1) and reverse (S2) primers together with the cDNA template were present, and a product of 872 bp was observed, which specified the full-length cDNA of *rST$_a$*. The PCR product was cut with *Bam*HI/*Nco*I to yield a 655 bp fragment, and blunted with Klenow DNA polymerase I (U.S. Biochemical Corp. (USB), Cleveland, OH) and ligated into *Eco*RV-digested, phosphatase-treated plasmid pBluescript SK+ vector (Stratagene, La Jolla, CA). To determine the authenticity of the PCR product as indeed being the cDNA of *rST$_a$*, sequence analysis from both ends was carried out on double stranded DNA by the dideoxynucleotide chain termination method of Sanger (12), modified as in Sequenase Version 2.0 protocols (USB). The nucleotides sequence of the PCR product was identical to that published by Ogura et al. (5), indicating that the correct cDNA had been obtained.

Screening of cDNA library A 49 years old normal male Caucasian liver cDNA library in phage UNI-ZAP XR Vector and the host cells (*E. coli* strain XL-1 Blue) was purchased from Stratagene. 600,000 plaques at a density of ~30,000 per 150-mm plate were screened using a 655 bp *Bam*HI/*Nco*I fragment of the rat *ST$_a$* PCR product obtained above, according to methods described in Ausubel et al. (13). The probes were prepared by random hexamer primed synthesis

(Prime-a-Gene Labeling System; Promega, Madison, WI) using [α-^{32}P]dCTP (3000 Ci/mmol, NEN Dupont; 1 Ci = 37 GBq) to generate a specific activity of 1-3 x 10^9 cpm/μg DNA (14). Positive plaques were replated and rescreened twice until six isolated positive plaques were selected for *in vivo* excision of pBluescript SK vector with the cDNA inserts using R408 helper phage, according to protocols provided by the manufacturer (Stratagene). The clones were purified and sequenced on double-stranded DNA as described below.

DNA Sequence Analysis The sequence of the inserts were determined on double stranded DNA by the dideoxynucleotide chain termination method of Sanger (12), modified as in Sequenase Version 2.0 protocols (USB).

RESULTS AND DISCUSSION

Plaque screening of the human liver UNI-ZAP cDNA library (6 x 10^5 independent clones) with the radiolabeled rat ST_a cDNA probes identified six ST_a cDNA clones. All six clones shows positive hybridization on Southern blotting (data not shown) with insert sizes ranging from 1 to 1.5 kilobases (kb). Limited sequencing from both ends of all the clones indicated that there are two sets of cDNA clones with inserts of 1.1 kb and 1.5 kb. One of each of the clones, (1.1 kb designated clone I and 1.5 kb clone II), were further characterized by sequencing their entire cDNA. Figure 1 shows the nucleotide sequence of both clones together with the deduced amino acid sequence. Clone II has 1563 base pairs (bp) nucleotides and is a partial cDNA, which lacks the first 15 amino acid residues (as indicated by the star at nucleotide 231). However, it has a much longer 3' untranslated region (UTR) than clone I as is shown in Figure 1. Clone I has 1069 bp nucleotides and contains an 855-nucleotide open reading frame (OPR) beginning at nucleotide 65 and encoding a 285 amino acid polypeptide. There is no signal peptide insertion sequence or putative transmembrane hydrophobic domain, indicative of a cytosolic protein. For clone I, the translation stop codon, TAA, is located 5' of a short segment of poly(A)$^+$ tail, and there is no consensus sequences for polyadenylation (***AATAAA***), but instead there is *CTTAAA* at -20 bp from the poly(A)$^+$ tail. In contrast, clone II has three polyadenylation signals and a short segment of poly(A)$^+$ tail as shown in Figure 1.

To investigate whether these cDNA clones are physiological, Northern blot analysis was performed on an adult human liver total mRNA (provided by Drs. Ida S. Owens and Joseph K. Ritter, NIH), and hybridized to a 898 bp *Eco*RI/*Xho*I fragment of clone I. The results are shown in Figure 2. There are at least two prominent transcripts of sizes 1.1 and 1.7 kb, indicating that the cloned cDNAs are real and not artifacts of cloning. Several genes [mouse dihydrofolate reductase (DHFR) (15), rat α2μ-globulin (16), chicken vimentin (17), and human N-*ras* (18)] had been reported to utilize alternative sites of polyadenylation to generate multiple mRNAs, which encode identical proteins. For DHFR, as many as seven mRNAs were observed, each having different lengths of 3' noncoding sequences; the usage of these different poly(A)$^+$ sites appears to be regulated during growth (19). For human ST_a, Otterness et al. (20) reported at least there are six polyadenylation sites. These alternative flanking noncoding sequences might have important function in the regulation of gene expression, such as RNA transport or stability (21,22). The physiologic significance of polyadenylations of human ST_a remains to be elucidated.

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1                    CTGCCACAGCCTCCAGCGGTGGCTACAGTTGAAACCCTCACACCACGCAGGAAGAGGTCATCATC

65   ATG TCG GAC GAT TTC TTA TGG TTT GAA GGC ATA GCT TTC CCT ACT ATG GGT TTC AGA TCC
1    Met Ser Asp Asp Phe Leu Trp Phe Glu Gly Ile Ala Phe Pro Thr Met Gly Phe Arg Ser

125  GAA ACC TTA AGA AAA GTA CGT GAT GAG TTC GTG ATA AGG GAT GAA GAT GTA ATA ATA TTG
21   Glu Thr Leu Arg Lys Val Arg Asp Glu Phe Val Ile Arg Asp Glu Asp Val Ile Ile Leu
                                                                *
185  ACT TAC CCC AAA TCA GGA ACA AAC TGG TTG GCT GAG ATT CTC TGC CTG ATG CAC TCC AAG
41   Thr Tyr Pro Lys Ser Gly Thr Asn Trp Leu Ala Glu Ile Leu Cys Leu Met His Ser Lys

245  GGG GAT GCC AAG TGG ATC CAA TCT GTG CCC ATC TGG GAG CGA TCA CCC TGG GTA GAG AGT
61   Gly Asp Ala Lys Trp Ile Gln Ser Val Pro Ile Trp Glu Arg Ser Pro Trp Val Glu Ser

305  GAG ATT GGG TAT ACA GCA CTC AGT GAA ACG GAG AGT CCA CGT TTA TTC TCC TCC CAC CTC
81   Glu Ile Gly Tyr Thr Ala Leu Ser Glu Thr Glu Ser Pro Arg Leu Phe Ser Ser His Leu

365  CCC ATC CAG TTA TTC CCC AAG TCT TTC TTC AGT TCC AAG GCC AAG GTG ATT TAT CTC ATG
101  Pro Ile Gln Leu Phe Pro Lys Ser Phe Phe Ser Ser Lys Ala Lys Val Ile Tyr Leu Met

425  AGA AAT CCC AGA GAT GTT TTG GTG TCT GGT TAT TTT TTC TGG AAA AAC ATG AAG TTT ATT
121  Arg Asn Pro Arg Asp Val Leu Val Ser Gly Tyr Phe Phe Trp Lys Asn Met Lys Phe Ile

485  AAG AAA CCA AAG TCA TGG GAA GAA TAT TTT GAA TGG TTT TGT CAA GGA ACT GTC GTA TAT
141  Lys Lys pro Lys Ser Trp Glu Glu Tyr Phe Glu Trp Phe Cys Gln Gly Thr Val Val Tyr

545  GGG TCA TGG TTT GAC CAC ATT CAT GGC TGG ATG CCC ATG AGA GAG GAG AAA AAC TTC CTG
161  Gly Ser Trp Phe Asp His Ile His Gly Trp Met Pro Met Arg Glu Glu Lys Asn Phe Leu

605  TTA CTG AGT TAT GAG GAG CTG AAA CAG GAC ACA GGA AGA ACC ATA GAG AAG ATC TGT CAA
181  Leu Leu Ser Tyr Glu Glu Leu Lys Gln Asp Thr Gly Arg Thr Ile Glu Lys Ile Cys Gln

665  TTC CTG GGA AAG ACG TTA GAA CCC GAA GAA CTG AAC TTA ATT CTC AAG AAC AGC TCC TTT
201  Phe Leu Gly Lys Thr Leu Glu Pro Glu Glu Leu Asn Leu Ile Leu Lys Asn Ser Ser Phe

725  CAG AGC ATG AAA GAA AAC AAG ATG TCC AAT TAT TCC CTC CTG AGT GTT GAT TAT GTA GTG
221  Gln Thr Met lys Glu Asn Lys Met Ser Asn Tyr Ser Leu Leu Ser Val Asp Tyr Val Val

785  GAC AAA GCA CAA CTT CTG AGA AAA GGT GTA TCT GGG GAC TGG AAA AAT CAC TTC ACA GTG
241  Asp Lys Ala Gln Leu Leu Arg Lys Gly Val Ser Gly Asp Trp Lys Asn His Phe Thr Val

845  GCC CAA GCT GAA GAC TTT GAT AAA TTG TTC CAA GAG AAG ATG GCA GAT CTT CCT CGA GAG
261  Ala Gln Ala Glu Asp Phe Asp Lys Leu Phe Gln Glu Lys Met Ala Asp Leu Pro Arg Glu

905  CTG TTC CCA TGG GAA TAA CGTCCAAAACACTCTGGATCTTATATGGAGAATGACATTGATTCTCCTGTCCTTG
281  Leu Phe Pro Trp Glu ***

979  TACATGTACCTGACTGGGGTCATTGTGTAAGACTTATTATTTTATCCTGAAACCTTAAATATCAAACCTCTGC(A)21I
1053 ATCTCTGATCCCTTCCTTGTTAAAAGTTACCACGGTTGGCCAGGCGCGGTGGTTCATGCCTGTAATCCCAGCACTATGG
1132 GAGGCCGAGACGGGCGGATCACGAGGTCAGGAGACTGAGACCATCCTGGCTAACACGGTGAAACCCCATCTCTACTAAA
1211 AATACAAAAAAACAAAAAAAATTAGCCAGGGCATTGGCTCATGTCTGTAATCCCAGCACTTTGGGAGGTCGGGGGGGTGG
1290 GGGAGGATCACGGGGTCAGGAGATCGAGACCATCCTGGCCAACATGATGAAACCCTATCTCTACTAAAAATACAAAAAT
1369 TAGCCGGGCATGGTGGTGCACGCCTATAGTCCCAGCTACTCGGGGGGCTGAGGTAGGAGAATCGTTTGAACTCAGGAGG
1448 CAGAGGTTGCAATGAGCCAAGATCGCGCCACTGCACTCCAGCCTGGGTGACAGAGCGAGACCGTCTCAAAAAGAAAGAA
1527 GTGACTAGGTTCAGAGAACCAGGGTTCAAAGCCCAGGGATGCAAAGGTTGCAGTGAGTTGAGTCATGGGATCCCAGACT
1606 TTTTTAAATGTTTGCAATGTTTCCCGTTTACAGAATGCTACAAGAATAATGTACGTACTACCTAAAAGGATGTCTAAAT
1685 GTTTGTT***AATAAA***AATAAGAAATAGCTACAGTGACAGATTTTAGAGCAAAAATTAGT***AATAAA***AATAAGA***AATAAA***ATT
1764 ACAGGAGCAATT(A)18
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Figure 1. Nucleotide and deduced amino acid sequences of human ST_a cDNA. Clone I consists of nucleotides 1 to 1050 and 21 poly $(A)^+$ tail, whereas clone II begins at nucleotide 231, as indicated by an asterisk (*), and extends to nucleotide 1794. Stop codon is designated by ***. Consensus polyadenylation signal is denoted by bold italic.

Figure 3 shows the alignment of the deduced amino acid sequences for the family of STs obtained from different species. The conserved amino acids are shown on the top lines. The human liver alcohol/hydroxysteroid ST (h-ST-a) shares extensive amino acid sequence homology

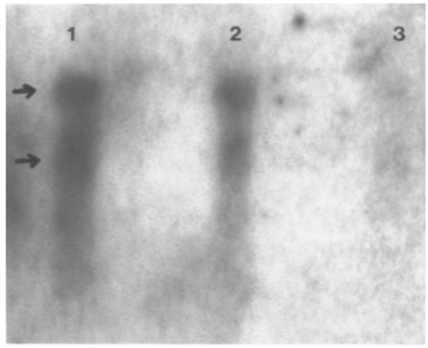

Figure 2. Northern Blot analysis of an adult human liver total mRNA (provided by Drs. Ida S. Owens and Joseph K. Ritter, NIH) with a 898 bp *Eco*RI/*Xho*I fragment of clone I of hST_a. Lanes 1, 2 and 3 indicate 20, 10 and 5 µg of total mRNA, respectively. The two major transcripts, 1.1 kb and 1.7 kb are indicated by the arrows.

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                                                              Domain I
Conserved          S P F      GIP                          F ARPDDL I TYPKSGTTWL
h-ST-a      1   MSDDFLWFEGIAFPTMGFR-----SETLRKV---RDEFVIRDEDVIILTYPKSGTNWL
r-ST-a      1   MP-DYTWFEGIPFPAFGIP-----KETLQNV---CNKFVVKEEDLILLTYPKSGTNWL
r-ST-p      1   MEFSRPP----LVHVKGIP----LIKYPAETIGPLQNFTAWPDDLLISTYPKSGTTWM
r-ST-e      1   METSMPEYYDVFGDFHGFLMDKRFTKYWED----VETFLARPDDLLIVTYPKSGSTWI
b-ST-e      1   MSSSKPSFSDYFGKLGGIPMYKKFIEQFHN----VEEFEARPDDLVIVTYPKSGTTWL

                                                                Domain II
                SEI CMIY  GD EKC   I  R P LE          G   L E  SPRL KSHL
           51   AEILCLMHSKGDAKWIQSVPIWERSPWVE---SEI--GYTALSETESPRLFSSHL
           50   IEIVCLIQTKGDPKWIQSVTIWDRSPWIE---TDL--GYDMLIKKKGPRLITSHL
           51   SEILDMIYQGGKLEKCGRAPIYARVPFLEFKCPGVPSGLETLEETPAPRLLKTHL
           55   SEIVDMIYKEGDVEKCKEDALFNRIPDLECRNEDLINGIKQLKEKESPRIVKTHL
           55   SEIICMIYNNGDVEKCKEDVIFNRVPYLECSTEHVMKGVKQLNEMASPRIVKSHL

                P  LLP SF   K KVIYL RNAKDVVVSYY F     K  P P S   FV
          101   PIQLFPKSFFSSKAKVIYLMRNPRDVLVSGYFFWKNMKFIKKPKSWEEYF
          100   PMHLFSKSLFSSKAKVIYLIRNPRDVLVSGYYFWGKTTLAKKPDSLGTYV
          106   PLSLLPQSLLDQKVKVIYIARNAKDVVVSYYNFYNMAKLHPDPGTWDSFL
          110   PAKLLPASFWEKNCKIIYLCRNAKDVVVSYYYFFLIMKSYPNPKSFSEFV
          110   PVKLLPVSFWEKNCKIIYLSRNAKDVVVSYYFLILMVTAIPDPDSFQDFV

                Domain III
                E FM G VPYGSWF H K WWE R    VL LFYEDMKED  REI KI E
          151   EWFCQGTVVYGSWFDHIHGWMPMREEKNFLLLSYEELKQDTGRTIEKICQ
          150   EWFLKGYVPYGSWFEHIRAWLSMRELDNFLLLYYEDMKKDTMGTIKKICD
          156   ENFMDGEVSYGSWYQHVKEWWELRHTHPVLYLFYEDIKENPKREIKKILE
          160   EKFMEGQVPYGSWYDHVKSWWEKSKNSRVLFMFYEDMKEDIRREVVKLIE
          160   EKFMDGEVPYGSWFEHTKSWWEKSKNPQVLFLFYEDMKENIRKEVMKLLE

                                                           Domain IV
                FLGR L  E VD I KHTSFQ MKEN NTNY  LP E    KVSPFMRKG
          201   FLGKTLEPEELNLILKNSSFQTMKENKMSNYSLLSVDYVVDKA-QLLRKGV
          200   FLGKKLEPDELDLVLKYSSFQVMKENNMSNYNLMEKELILPGF-TFMRNGT
          206   FLGRSLPEETVDSIVHHTSFKKMKENCMTNYTTIPTEIMDHNVSPFMRKGT
          210   FLERDPSAELVDRIIQHTSFQEMKNNPCTNYSMLPETMIDLKVSPFMRKGI
          210   FLGRKASDELVDKIIKHTSFQEMKNNPSTNYTTLPDEVMNQKVSPFMRKGD

                 GDWKNHFTVAQ E FD HYQ  M D P KFR
          251   SGDWKNHFTVAQAEDFDKLFQEKMADLPRELFPWE
          250   TGDWKNHFTVAQAEAFDKVFQEKMAGFPPGMFPWD
          257   TGDWKNTFTVAQNERFDAHYAKTMTDCDFKFRCEL
          261   VGDWKNHFPEALRERFEEHYQQQMKDCPVKFRAEL
          261   VGDWKNHFTVALNEKFDMHYEQQMKGSTLKFRTKI
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Figure 3. Alignment of the deduced amino acid sequences of human ST_a, rat ST_a (5), rat ST_p (7), rat ST_e (8), and bovine ST_e (9). Conserved amino acids are indicated on the top lines. The conserved domains from the amino- to the carboxy-terminal are underlined and represented as Domains I, II, III and IV.

with the other ST-a subfamily members from other species, indicative of evolutionary conservation of important biological functions. The hST_a shows the greatest homology with rat liver alcohol/hydroxysteroid ST (rST_a; Fig. 3), with 62% and 74% identities at the nucleotide and amino acid levels (5). Furthermore, the rST_a shares very strong homology with the senescence marker protein SMP-2 (6), and rST_{20} (4), suggesting that there are several genes encoding for this subfamily of sulfotransferases in the rat. Whether similar findings will be obtained in other species, such as man and mouse, remains to be seen. Compared to the other subfamilies of sulfotransferases, such as phenol and estrogen, hST_a shares 39%/59%, 35%/48%, and 36%/54% homologies with the rST_p (7), rST_e (8), and bST_e (9) at the DNA and amino acid levels, respectively.

The alcohol/hydroxysteroid form of sulfotransferases had been implicated to play a critical role in the activation of certain carcinogens through O-sulfonations, e.g., 7-hydroxymethyl-12-methyl-benz[*a*]anthrancene (23) and 5-hydroxymethylchrysene (24), in rats. Whether similar activations occur in other species, such as man, as well as which ST-a isoenzyme(s) catalyze such reactions, can now be addressed *via* the expression of the individual cloned cDNAs in tissue culture. To this end, we are in the process of establishing a stable cell line expressing the hST_a.

ACKNOWLEDGMENTS

The authors thank Drs. Ida S. Owens and Joseph K. Ritter (HGB, NICHD, NIH) for providing the human liver mRNA, and Dr. Joe B. Harford (CBMB, NICHD, NIH) for insightful discussions.

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