Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 8695–8699, September 1995
Biochemistry

Human fatty acid synthase: Properties and molecular cloning

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ABSTRACT **Fatty acid synthase (FAS; EC 2.3.1.85) was purified to near homogeneity from a human hepatoma cell line, HepG2. The HepG2 FAS has a specific activity of 600 nmol of NADPH oxidized per min per mg, which is about half that of chicken liver FAS. All the partial activities of human FAS are comparable to those of other animal FASs, except for the β-ketoacyl synthase, whose significantly lower activity is attributable to the low 4′-phosphopantetheine content of HepG2 FAS. We cloned the human brain FAS cDNA. The cDNA sequence has an open reading frame of 7512 bp that encodes 2504 amino acids (M_r, 272,516). The amino acid sequence of the human FAS has 79% and 63% identity, respectively, with the sequences of the rat and chicken enzymes. Northern analysis revealed that human FAS mRNA was about 9.3 kb in size and that its level varied among human tissues, with brain, lung, and liver tissues showing prominent expression. The nucleotide sequence of a segment of the HepG2 FAS cDNA (bases 2327–3964) was identical to that of the cDNA from normal human liver and brain tissues, except for a 53-bp sequence (bases 3892–3944) that does not alter the reading frame. This altered sequence is also present in HepG2 genomic DNA. The origin and significance of this sequence variance in the HepG2 FAS gene are unclear, but the variance apparently does not contribute to the lower activity of HepG2 FAS.**

The synthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA, and NADPH is a complex process catalyzed by the fatty acid synthase (FAS). In animal tissues, the active synthase is a homodimer of a multifunctional protein that is organized in a head-to-tail fashion, generating two active catalytic centers (1). The seven partial activities and the site for the prosthetic group, 4′-phosphopantetheine (acyl carrier protein), are arranged on the multifunctional protein subunit from the amino to carboxyl termini in the following order: β-ketoacyl synthase, acetyl-CoA and malonyl-CoA transacylases, dehydratase, enoyl reductase, ketoacyl reductase, acyl carrier protein, and thioesterase (1).

Most information about the synthase is derived from nonhuman animal studies, so little is known about the human synthase. Investigators who isolated the enzyme from human biopsy tissues (2–5) or cell lines (6) have shown that, except for the enzyme from the SKBR3 cell line (6), all the human FAS preparations had lower activity than the FASs of other animals. This lower activity of the human FAS is comparable to the activities of related human enzymes that are involved in lipogenesis (e.g., pyruvate dehydrogenase, citrate lyase, and glucose-6-phosphate dehydrogenase), which are lower (by a factor of 4–7) in human tissues than in other animal tissues, implying that lipogenesis in humans is highly repressed (7–9). Here we describe the purification and catalytic properties of human FAS from HepG2 human hepatoma cells and report how the relatively low specific activity of the enzyme results from the low 4′-phosphopantetheine content of the protein. We also report the cloning and sequence analyses of the human FAS cDNA.§

MATERIALS AND METHODS

Materials. All the reagents and chemicals were purchased from Sigma, except for [1-^{14}C]acetyl-CoA and [2-^{14}C]malonyl-CoA (Amersham), triacetic acid lactone (4-hydroxy-6-methyl-2-pyrone; Aldrich) *N*-acetyl-*S*-acetoacetylcysteamine (Research Plus, Bayonne, NJ), DNA-modifying enzymes (New England Biolabs), dideoxy sequencing kits (United States Biochemical), and radiochemicals from (DuPont/NEN). Chromatography columns (FPLC, Superose-6 HR 10/30) and all other chromatographic equipment were purchased from Pharmacia. Human brain and liver poly(A)$^+$ RNA and a multiple human tissue Northern blot were obtained from Clontech.

Assays of FAS and Its Partial Activities. The synthase activity was assayed by measuring the rate of oxidation of NADPH or the incorporation of radiolabeled acetyl-CoA or malonyl-CoA into palmitate (10). The partial activities of FAS were assayed as described earlier: the acetyl/malonyl transacylases (11), dehydratase (12), β-ketoacyl synthase (12), β-ketoacyl reductase (13), β-hydroxyacyl enoyl reductase (12), and thioesterase (14). The β-ketoacyl synthase activity was also determined by measuring the increase in absorbance at 280 nm due to formation of triacetic acid lactone (15).

4′-Phosphopantetheine Content. To determine the 4′-phosphopantetheine content of FAS, we developed a simple and sensitive method based on spectrophotometric measurement of the phenylthiocarbamoyl (PTC) derivative of taurine released after performic acid oxidation and hydrolysis of the protein. PTC-taurine can be separated from other PTC-amino acids by using reverse-phase high-performance liquid chromatography (HPLC) and following the absorbance at 254 nm. In this system, the PTC derivatives of cysteic acid and taurine are eluted close to each other. The molar ratio of cysteic acid to taurine was determined and used to normalize the peak heights based on the known amino acid compositions of the chicken and human FASs. In a typical analysis, 10–20 μg of human or chicken FAS, after being dialyzed against distilled water and lyophilized, was treated with performic acid (0.2 ml) at room temperature for 2 hr. The oxidized FAS was lyophilized and hydrolyzed in 200 μl of 6 M HCl at 110°C for 24 hr. The acid was removed by repeated lyophilization, and the amino acid hydrolysate was treated with 20 μl of a phenyl isothiocyanate solution (ethanol/water/triethylamine/phenyl isothiocyanate, 7:1:2:1, vol/vol). After incubation at 37°C for 1 hr, the PTC products were dried in vacuum, and the residue was dissolved in the isocratic elution buffer (aqueous 0.1%

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Abbreviation: FAS, fatty acid synthase.

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§The sequence reported in this paper has been deposited in the GenBank database (accession no. U26644).

formic acid/0.4% acetic acid) and analyzed on a Rainin Microsorb C_{18} column (0.46 cm × 10 cm; particle size, 3 μm; pore size, 100 Å). A standard mixture of taurine and cysteic acid (1:40, based on their relative presence in the FAS subunit) was treated with phenyl isothiocyanate as described above and separated by reverse-phase HPLC under the same experimental conditions. The values obtained were used to normalize the recovery of 4′-phosphopantetheine.

Cloning of Human FAS cDNA. The human brain FAS cDNA clone EST01325 (16) was obtained from the American Type Culture Collection. Sequence analysis of this cDNA showed that it contained the FAS sequence from base 3576 to base 7245 and was missing 267 bp of the 3′ coding sequence. Using this cDNA as a probe, we screened a human brain cDNA library in λZAPII vector (Stratagene) and isolated clone B27-2, which contained the missing 3′ sequence. By using primers based on the sequence of EST01325 [bases 3946–3964 (17)] and the rat FAS sequence [bases 2417–2436 (18)], we performed reverse transcriptase-initiated polymerase chain reaction (PCR) on brain poly(A)$^+$ RNA (19, 20) and isolated clone C-30. By using primers based on the sequences of Try 3-27 [bases 559–576 (17)] and C-30 (bases 2434–2451), we isolated clone C-27B. We also used primers based on a genomic clone, clone A (data not shown), which contained the first three exons of human FAS and a reverse primer from C-27B (bases 1198–1212), and obtained clone C-10. The PCR products were isolated, made blunt ended, and cloned into the *Sma* I site of Bluescript vector (21).

Miscellaneous Procedures. The FASs of chicken (22) and rat (23) liver were isolated as described. Previously described procedures were used for protein determination (24), SDS/PAGE analysis (25), and DNA sequencing, Northern blotting, random primer labeling, and isolation of plasmid DNA (21). The fatty acids were analyzed as methyl esters on a Varian 3500 gas/liquid chromatograph equipped with a DB-225 capillary column (0.25 mm × 30 m).

RESULTS AND DISCUSSION

Purification and Properties of Human FAS. The human hepatoma cell line HepG2 was grown to confluency in fifty 2-liter roller bottles containing Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cells were collected, harvested, and washed three times with 500 ml of cold phosphate-buffered saline (50 mM potassium phosphate, pH 7.4/150 mM NaCl). The cell pellet (100 g) was suspended in 300 ml of buffer A [50 mM Tris·HCl, pH 8.0/1 mM EDTA/5 mM dithiothreitol/10% (vol/vol) glycerol containing the protease inhibitors leupeptin, antitrypsin, and aprotinin, each at 5 μg/ml] and was disrupted in a Potter–Elvehjem homogenizer with a tight-fitting pestle. The cell lysate was centrifuged for 30 min at 30,000 × *g*, and the supernatant was fractionated with ammonium sulfate. The protein fraction that precipitated between 25% and 40% saturation was collected, dialyzed against buffer B (50 mM potassium phosphate, pH 7.4/1 mM EDTA/5 mM dithiothreitol/5% glycerol), and fractionated on a DEAE-Bio-Gel column (Bio-Rad; bed volume, 100 ml) by first washing the column with 300 ml of buffer B and by following with a gradient of 0–0.2 M NaCl in 300 ml of buffer B. The active protein was isolated after precipitation with ammonium sulfate at 40% saturation, dissolved in 10 ml of buffer B, dialyzed against buffer B, and loaded onto a Sephacryl S-300 column (Pharmacia; bed volume, 150 ml; size, 2 cm × 80 cm). The synthase was eluted with buffer B, precipitated with ammonium sulfate, dissolved in buffer A, and fractionated on a Superose 6 column. The active enzyme was eluted with buffer A, precipitated with ammonium sulfate, redissolved in buffer A, and stored at −80°C. This procedure yielded 1.5 mg of highly purified FAS (600 nmol of NADPH oxidized per min per mg of protein) with an overall yield of 31%. Although a similar procedure was followed to prepare the FAS from HeLa cells, the yield and the specific activity (300 nmol of NADPH oxidized per min per mg of protein) were very low.

The FAS preparations were homogeneous as judged by SDS/PAGE, with an estimated subunit molecular weight of 265,000, which is comparable to that of the rat and chicken enzymes (Fig. 1). Human FAS had an optimal pH value of 6.5 to 6.7 for fatty acid synthesis, and the apparent K_m values were 8 μM for acetyl-CoA, 20 μM for malonyl-CoA, and 25 μM for NADPH. The specificity of human FAS for the acyl primer is similar to that of rat liver FAS (26) but differs from that of chicken liver FAS in that it prefers butyryl-CoA (K_m = 4 μM) over acetyl-CoA. In addition, the fatty acids synthesized by the human FAS, like those of the rat synthase, are primarily palmitate (90%) with small amounts of myristate (4%) and stearate (6%), whereas the products of synthesis by the chicken FAS (26) are palmitate (60–70%) with larger amounts of stearate (15–20%) and myristate (5%). Acetoacetyl-CoA can substitute for acetyl-CoA as a primer of fatty acid synthesis catalyzed by human FAS without a lag in NADPH oxidation (data not shown), suggesting that the conversion of the acetoacetyl group into a butyryl group by the β-ketoacyl reductase, β-hydroxyacyl dehydratase, and enoyl reductase partial activities in FAS proceeds efficiently to provide a primer for fatty acid synthesis without intermission. The ability of human FAS to use acetoacetyl-CoA as a primer in fatty acid synthesis may be physiologically significant since it provides a way to consume acetoacetyl-CoA and shorter chain fatty acids in the synthesis of long-chain fatty acids.

The overall human FAS activity, whether measured by NADPH oxidation or by incorporation of radiolabeled acetyl-CoA or malonyl-CoA into fatty acids, was about one-third that of the chicken enzyme (Table 1). However, most partial activities were nearly equal to those of the chicken enzyme, except for the β-hydroxyacyl dehydratase and enoyl reductase, which were 3.2 and 20.8 times higher in the human synthase than in the chicken FAS (Table 1). On the other hand, the partial activity of the β-ketoacyl synthase was about one-third that of the chicken enzyme. To verify the values obtained for the activity of the β-ketoacyl synthase, the partial activity was assayed by two independent methods: (*i*) the incorporation of [^{14}C]HCO_3^- into *N*-acetyl-*S*-acetoacetylcysteamine by reversing the β-ketoacyl synthase (12) and (*ii*) the formation of

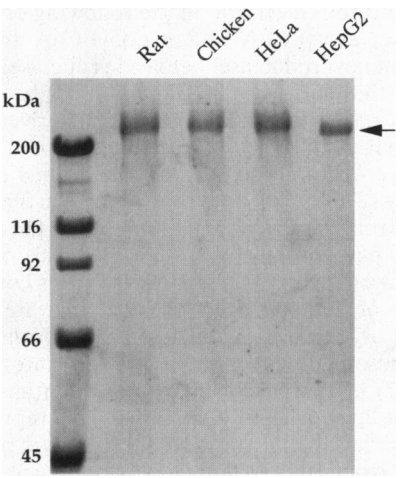

FIG. 1. SDS/PAGE analysis of human fatty acid synthase. Purified samples, 2 μg each, of rat, chicken, and human (HeLa and HepG2) FASs were subjected to SDS/6% PAGE, and the gel was stained with Coomassie blue. The left lane contained proteins of the indicated molecular mass as markers.

Table 1. Comparison of human FAS and chicken FAS activities

Functional assays	Activity, nmol/min per mg of protein		Ratio, human/chicken
	Human FAS	Chicken FAS	
FAS activities			
NADPH oxidation	462.0	1478.0	0.3
[1-^{14}C]acetyl-CoA	29.6	76.9	0.4
[2-^{14}C]malonyl-CoA	220.6	652.7	0.3
FAS partial activities			
Acetyl transacylase	1336.0	1192.0	1.1
Malonyl transacylase	558.0	494.0	1.1
β-Ketoacyl synthase	0.2*	0.7*	0.3
	89.3†	292.7†	0.3
β-Ketoacyl reductase	2514.0	3450.0	0.7
β-Hydroxyacyl dehydratase	56.7	17.3	3.3
Enoyl reductase	89.3	4.3	20.8
Thioesterase	54.8	33.5	1.6

The assays to measure the overall activity and partial activities of FAS were performed as described under *Materials and Methods.* The ratio represents the value of the specific activity of human FAS divided by that of the chicken enzyme in each assay under the same experimental conditions.
*Values obtained from the incorporation of [^{14}C]HCO_3^- into *N*-acetyl-*S*-acetoacetylcysteine.
†Values obtained from the formation of triacetic acid lactone.

triacetic acid lactone (15) in the absence of NADPH. Both assays gave the same relative values (Table 1).

To further investigate the cause of low β-ketoacyl synthase partial activity, we determined the content of the prosthetic group, 4′-phosphopantetheine. Since the amount of human FAS available was very limited, we developed a sensitive method that was based on formation and analysis of PTC-taurine (see *Materials and Methods*). The results of such analysis showed that the HepG2 FAS contained 0.42 mol of pantetheine per mol of synthase subunit as compared to 0.96 mol per mol of chicken synthase. A comparison of these values with the fatty acid synthesis activity of the two enzymes suggested that the lower catalytic activity of the human FAS could be largely due to its low pantetheine content. Although the regulation of FAS activity by means of transcription and mRNA stabilization (27) has been reported, little is known about how FAS activity is regulated posttranslationally. Since the prosthetic group is indispensable for fatty acid synthesis and its turnover rate is more rapid than that of FAS protein, at least in rat tissues (28–30), Roncari (3) has proposed that the turnover of 4′-phosphopantetheine is an efficient mode for regulating FAS activity. The loss of 4′-phosphopantetheine from HepG2 FAS suggests that such a mechanism may be operative in the HepG2 cell line.

Characterization of Human FAS cDNA. As described under *Materials and Methods*, we have isolated four overlapping cDNA clones: C-10, C-27B, C-30, and B27-2. These clones and the cDNA clone EST01325 (16) cover the entire coding region of the human brain FAS (Fig. 2*A*). The sequence of the rat FAS primer used in the PCR cloning of C-30 was determined by the sequence of the overlapping clone C-27B (Fig. 2). The sequence of the primer used from the HepG2 cDNA clone, Try3-27 (17), was found to be the same in the brain tissue, on the basis of the sequence of clone C-10. The cDNA sequence was partially confirmed by sequencing subclones of the human FAS genomic DNA clone 43D6 (17). The restriction map of this genomic DNA clone identified several restriction sites in common with the cDNA sequence. Using these restriction sites, we generated subclones GHFAS22, GHFAS24, GHFAS8, GHFAS2, and GHFAS1 (Fig. 2*B*) and determined the sequence from both ends of these fragments. The exonic sequences of the genomic DNA clones had 100% identity with the corresponding cDNA sequences. Sequencing of clone GHFAS22 revealed that it contained only the 5′ flanking sequence and not the FAS coding sequence. We also located the translation initiation codon (ATG) and the termination codon (TAG) in the sequences of genomic DNA clones GHFAS24 and GHFAS1, respectively. Based on the locations of the start and stop codons in the restriction map (Fig. 2*B*), we calculated that a genomic DNA sequence of ≈19 kb contains the 7512-bp coding sequence of human FAS. Schweizer *et al.* (31) reported that rat FAS is encoded by 15–16 kb of genomic DNA. In contrast, the entire goose FAS gene spans ≈50 kb (32).

Analysis of the Human FAS Sequence. The nucleotide sequence of human brain FAS cDNA has an open reading frame of 7512 nt coding for 2504 amino acids (Fig. 3*A*). The molecular weight of the protein is calculated to be 272,516, which is close to the estimated molecular weight of 265,000 for HepG2 FAS (Fig. 1). The amino acid sequence shows about 79% and 63% identity, respectively, with the amino acid sequences of the rat and chicken FASs. We readily identified the highly conserved active sites of the acetyl/malonyl transacylases, the β-ketoacyl synthase, and the thioesterase

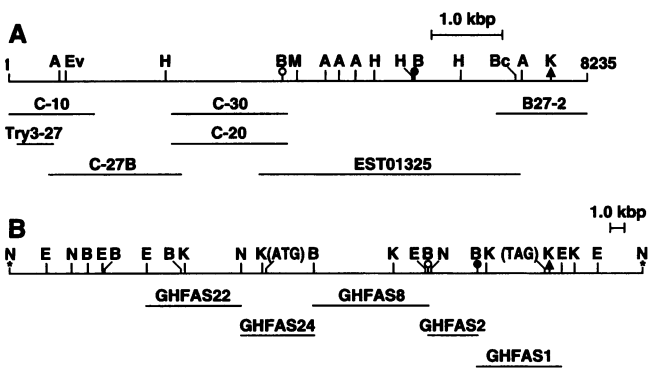

FIG. 2. Partial restriction maps of human FAS cDNA and genomic clones. (*A*) Restriction map of human FAS cDNA obtained from the sequence of the overlapping brain cDNA clones C-10, C-27B, C-30, EST01325, and B27-2. HepG2 FAS cDNA clones Try3-27 and C-20 are also shown. (*B*) Restriction map of the human FAS cosmid clone 43D6 (17). The locations of the ATG translational start codon and the TAG translational stop codon are shown in parentheses. The genomic DNA subclones GHFAS22, GHFAS24, GHFAS8, GHFAS2, and GHFAS1, which were partially sequenced, are shown. The *Bam*HI and *Kpn* I restriction sites that are common to both the cDNA and genomic clones are indicated as open and solid circles for *Bam*HI and as solid triangles for *Kpn* I on both restriction maps. Restriction sites: A, *Acc* I; B, *Bam*HI; Bc, *Bcl* I; E, *Eco*RI; Ev, *Eco*RV; H, *Hin*cII; K, *Kpn* I; M, *Mlu* I; N, *Not* I.

A

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MEEVVIAGMFGKLPESENLQ EFWDNLIGGVDMVTDDDRRW KAGLYGLPRRSGKLKDLSRF DASFFGVHPKQAHTMDPQLR      80
LLLEATYEAIVDGGINPDSL RGTHTGVWVGVSGSETSEAL SRDPETLVGYSMVGCQRAMM ANRLSFFFDFRGPSIALDTA     160
CSSSLMALQNAYQAIHSGQC PAAIVGGINVLLKPNTSVQF LRLGMLSPEGTCKAFDTAGN GYCRSEGVVAVLLTKKSLAR     240
KVYTTILNKGTNTDGFKEQG VTFPQDIQEQPIRSLYQSAG VAPESFEYIEAHGPGTKVGD PQERNGITRALCATRQEPLL     320
IGSTKSNMGHPEPASGLDAL AKVLLSLEHGLWAPNLHFHS PNPEIPALLDGRLQVVDQPL PVRGGNVGINSFGFGGSNMH     400
IILRPNTQSAPAPAPHATLP RLLRASGRTPEAVQKLLEQG LRHSQGLAFLSMLNDIAAVP ATAMPFRGYAVLGGETRWPR     480
VQQVPAGERPLWFICSGMGT QWRGMGLSLMRLDRFRDSIL RSDEAVNRFGLKVSQLLLST DESTFDDIVHSFVSLTAIQI     560
GLIDLLSCMGPEADGIVGHS LGEWLSVRDGCLSQEEAVLA AYWRGQCIKEAPLPAGAMAA VGLSWEECKQRCPPAVVPAC     640
HNSKDTVTISGPQAPVFEFV EQLRKEGVFAKEVRTGGMAF HSYFMEAIAPPLLQELKKVI REPKPRSARWLSTSIPEAQW     720
HSSLARTSSAEYNVNNLVSP VLFQEALWHVPEHAVVLEIA PTPCPQAVLKRVRKPSCTII PRMKKDHRDNLEFFLAGIGR     800
LHLSGIDANPNALFPPVESP APRGTPLISPLIKWDHSLAW DAPAAEDFPNGSGSPSATIY TCTPSSESPDRYLVDHTIDG     880
RVLFPATGYLSIVWKTLARA WAGLEQLPVVFEDVVQHQAT ILPKTGTVSLEVRLLEATGA FEVSENGNLVVSGKVYQWDD     960
PDPRLFDHPESPHPNSPRSP LFLAQAEVYKELRLRGYDYG PHFQGILEASLEGDSGRLLW KDNWVSFMDTMLQMSILGSA    1040
KHGLYLPTRVTAIHIDPATH RQKLYTLQDKAQVADVVVSR WPRVTVAGGVHISGLHTESA PRRHEEQQVPILEKFCFTPH    1120
TEEGCLSEHAALEEELQLCK GLVEALETKVTQQGLKMVVP DWTGPRSPRDPSQQELPRLL SAACRLQLNGNLQLELAQVL    1200
AQERPKLPEDPLLSGLLDSP ALKACLDTAVENMPSLKMKV VEVLAGHGHLYSRIPGLLSP HPLLQLSYTATDRHPQALEA    1280
AQAELQQHDVAQGQWDPADP APSALGSADLLVCNCAVAAL GDPASALSNMVAALREGGFL LLHTLLRGHPRDIVAFLTST    1360
EPQYGQGILSQDAWESLFSR VSLRLVGLKKSFYGATLFLC RRPTPQDSPIFLPVDDTSFR WVESLKGILADEDSSRPVWL    1440
KAINCATSGVVGLVNCLRRE PGGTVRCVLLSNLSSTSHVP EVDPGSAELQKVLQGDLVMN VYRDGAWGVFRHFLLEDKPE    1520
EPTAHAFVSTLTRGDLSSIR WVCSSLRHAQPTCPGAQLCT VYYASLNFRDIMLATGKLSP DAIPGKWTSQDSLLGMEFSG    1600
RDASGKRVMGLVPAKGLATS VLLSPDFLWDVPSNWTLEEA ASVPVVYSTAYYALVVRGRV RPGETLLIHSGSGGVGQAAI    1680
AIALSLGCRVFTTVGSAEKR AYLQARFPQLDSTSFANSRD TSFEQHVLWHTGGKGVDLVL NSLAEEKLQASVRCFGTHGR    1760
FLEIGKFDLSQNHPLGMAIF LKNVTFHGVLLDAFFNESSA DWREVWALVEAAIRDGVVRP LKCTVFHGAQVEDAFRYMAQ    1840
GKHIGKVVVQVLAEEPAVLK GAKPKLMSAISKTFCPAHKS YIIAGGLGGFGLELAQWLIQ RGVQKLVLTSRSGIRTGYQA    1920
KQVRRWRRQGLQVQVSTSNI SSLEGARGLIAEAAQLGPVG GVFNLAVVLRDGLLENQTPE FFQDVCKPKYSGTLNLDRVT    2000
REACPELDYFVVFSSVSCGR GNAGQSNYGFANSAMERICE KRRHEGLPGLAVQWGAIGTV GILVETMSTNDTIVSGTLPT    2080
RIGVLGLEVLDLFLNQPHMV LSSFVLAEKAAAYRDRDSQR DLVEAVAHILGIRDLAAVNL GGSLADLGLDSLMSAPVRQT    2160
LERELNLVLSVREVRQLTLR KLQELSSKADEASELACPTP KEDGLAQQQTQLNLRSLLVK PEGPTLMRLNSVQSSERPLF    2240
LVHPIEATTVFHSLGPGLSI PTYGLQCTPAAPLDSIHSLA AYYIDCIRQVQPEGPYRVAG YSYGACVAFEMCSQLQAQQS    2320
PAPTHNSLFLFDGSPTYVLA YTQSYRAKLTPGCKAEAETE AICFFVQQFTDMEHNRVLEA LLPLKGLEERVAAAVDLIIK    2400
SHQGLDRQELSFAARSFYYR LRAADQYTPKAKYSGNVMLL RAKTGGRYGEDLGADYNLSQ VCDGKVSVHIIEGDHRTLLE    2480
GSGLESIISIIHSSLAEPRV SREG*                                                            2504
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B

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HepG2:  3892  ACGCCCACCCAAGACGCCAGTAGTTTGTCCTCACTGAGTTGGCAACAAGTGGCT   3945
        1298  ThrProThrGlnAspAlaSerSerLeuSerSerLeuSerTyrGlnGlnValAla  1315
Brain:  3892  GCAGACCCTGCCCCCAGCGCCCTGGGCAGCGCGGACCTCCTGGTGTGCAACTGT   3945
        1298  AlaAspProAlaProSerAlaLeuGlySerAlaAspLeuLeuValCysAsnCys  1315
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FIG. 3. (*A*) Deduced protein sequence of human brain FAS cDNA. The putative active sites of the condensing enzyme (Cys^{161}), the acetyl/malonyl transacylases (Ser^{580}), the β-hydroxyacyl dehydratase (His^{876}), and the thioesterase (Ser^{2302}, Asp^{2474}, and His^{2475}), the point of attachment of the 4′-phosphopantetheine (Ser^{2151}), the two nucleotide binding sites (Gly^{1671}, Gly^{1673}, Gly^{1676} and Gly^{1886}, Gly^{1888}, Gly^{1891}), and the pyridoxal phosphate binding site of the enoyl reductase (Lys^{1699}) are indicated in boldface letters and the surrounding sequences are underscored. Boldface amino acid sequences at positions 753–758 and 1285–1297 indicate peptide sequences that have been reported by Kuhajda *et al.* (33). (*B*) The 53-bp nucleotide sequence and the deduced 18-amino acid sequence of a HepG2 FAS cDNA clone (C-20) are shown with the corresponding brain sequences (positions 3892–3945).

and the attachment site of 4′-phosphopantetheine in the deduced amino acid sequence (boldface type in Fig. 3*A*). The catalytically important sequence motif at the active sites of the various partial activities are conserved (Fig. 3*A*).

Previous work has shown that the catalytic mechanism of the thioesterase may be similar to that of serine proteases and lipases, which employ a serine–histidine–aspartic acid catalytic triad as part of their catalytic mechanism (14, 34), and that the His^{274} of the chicken FAS thioesterase domain is essential for its activity (34). In addition, mutating the aspartic residue next to His^{237} caused partial loss (40%) of rat mammary gland thioesterase II (14). In agreement with these findings, the human brain FAS cDNA sequence also has these histidine (His^{2475}) and aspartic (Asp^{2474}) residues (Fig. 3*A*) in the corresponding positions in the sequences.

A consensus sequence for the active site of the β-hydroxyacyl dehydratase was reported as His^{878} . . . Gly^{882} . . . Pro^{887} (35). Mutagenesis of His^{878} to Ala resulted in the loss of β-hydroxyacyl dehydratase partial activity. In human FAS (Fig. 3*A*), we have identified a corresponding sequence, His^{876} . . . Gly^{880} . . . Pro^{885}, that may represent the active center of the dehydratase. However, the Cys^{879} in rat FAS is replaced by Thr^{877}. Interestingly, the β-hydroxyacyl dehydratase activity in human FAS is greater than that of chicken FAS. We do not know whether this elevated activity is related to the substitution of threonine for cysteine.

Kuhajda *et al.* (33) reported two peptide sequences (HAVVLE and LQQHDVAQEQWXP) of human FAS purified from the human breast carcinoma cell line ZR-75-1. These sequences were found in our human brain FAS amino acid sequences (residues 753–758 and 1285–1297, respectively). In our sequence, however, the E and X in the second peptide were replaced with G and D, respectively (Fig. 3*A*). Sequence analysis of the genomic clone GHFAS8 also confirmed the presence of glycine at this position. The discrepancy between our results and the published peptide sequence may be due to an error in peptide sequencing or to the characteristics of ZR-75-1 cells (see below).

Initially, we cloned C-20, a human FAS cDNA, by using HepG2 total RNA (Fig. 2*A*) and primers based on the rat FAS cDNA sequence (nt 2417–2436) and human brain cDNA EST01325 (nt 3946–3964). Beginning with nt 3576 in Fig. 3, the nucleotide sequence of C-20 is colinear with that of brain FAS, except for a 53-bp sequence (nt 3892–3944). The deduced amino acid sequence (positions 1297–1315) also differs in this region (Fig. 3*B*). Using PCR techniques and human FAS primers, we cloned a 1-kb HepG2 genomic DNA (GHepG21) that spans two exons and an intron. Sequencing of this clone confirmed the presence of the variant 53-bp sequence in HepG2 genomic DNA. On the other hand, a cDNA clone that was isolated from normal liver poly(A)$^+$ (Clontech) by reverse transcription–PCR and spans this region, the normal human genomic clone GHFAS2 (Fig. 2*B*), and the human breast carcinoma cell line T47-D all contained a sequence the same as that of brain cDNA clone EST01235. Together, these results showed that this 53-bp sequence, which is not found in normal

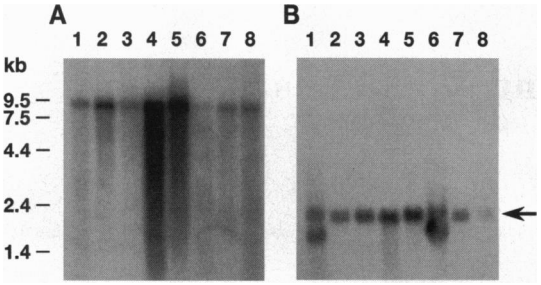

FIG. 4. Expression of human FAS in various tissues. Northern blot analysis of FAS mRNA was performed on various human tissues (Clontech). Each lane contained 2 μg of poly(A)$^+$ RNA. (*A*) The blot was probed with ^{32}P-labeled overlapping FAS cDNAs (C-10, C-27B, C-30, EST01325, and B27-2). Lanes: 1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas. (*B*) The same blot was stripped and reprobed with a ^{32}P-labeled β-actin cDNA.

genomic cDNA clones or in brain and liver cDNA clones, is unique to HepG2. Since this discrepancy occurs within an exon, this variation is not a result of alternative splicing. Whether the differences represent true polymorphism is yet to be determined.

There is only 15% sequence homology between HepG2 and brain sequences in the region coding for the interdomain of FAS domains I and II (Fig. 3*A*). Apparently, the variant 53-bp sequence lies in the interdomain region, which can accommodate mutations and may not be functionally important. Hence, the lower activity of HepG2 FAS is unlikely to be related to this sequence variation. In addition, the FAS activities from various tissue samples (2–5) and other cultured cells (33) already indicate that human FAS activity is lower than the activities of the rat and chicken FASs. Whether the lower FAS activity in the human enzyme is due to regulation of pantethenylation is yet to be established.

Finally, in humans there is only one species of FAS mRNA (9.3 kb in size) in the various tissues we tested (Fig. 4). In addition, our results demonstrate that FAS expression is high in brain, pulmonary, and hepatic tissues, which is consistent with the idea that these organs are the major sites of *de novo* fatty acid biosynthesis.

We thank Drs. Cheng-Chi Lee for providing the human cDNA library in λZAPII vector, James Kelly for providing the HepG2 cells, Richard Cook for performing the amino acid analysis of the human FAS, and Robert E. Anderson for performing the fatty acid analysis and Ms. Pamela Paradis Powell for editing the manuscript. This work was supported by grants from the National Institutes of Health (GM19091) and the Clayton Foundation.

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