Cloning, expression, and chromosomal localization of mouse liver bile acid CoA:amino acid N-acyltransferase

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Abstract A mouse liver λZap XR cDNA library was screened using the coding region of human bile acid CoA:amino acid N-acyltransferase (BAT) cDNA as a probe. Ten positive clones were isolated and purified, two of which apparently possessed complete open reading frames for BAT based on sequence analysis of the ends of the cDNAs. One clone (mBAT#9) was selected for sequence analysis and characterization. mBAT#9 is 1869 basepairs in length and the full-length cDNA possesses a 189 basepair 5′-nontranslated region, an open-reading frame of 1260 basepairs, and a 404 basepair 3′-nontranslated region followed by a poly(A) tail. The open-reading frame codes for a 420 amino acid protein with a calculated molecular mass of 46,525 daltons. The structural gene for mBAT was mapped to mouse Chromosome 4. The amino acid sequence of mBAT is 69% identical and 84% similar to that of hBAT, and 86% identical and 95% similar to that of *kan*-1, a putative rat liver BAT. Enzymatically active mBAT was expressed in *E. coli* using the bacterial expression vector pKK233-2. Immunoblot analysis of expressed mBAT with rabbit anti-human BAT polyclonal antibodies detected a single protein with a molecular mass of approximately 45,000 daltons. Cytosol from cells transformed with mBAT#9/pKK233-2 possessed significant amounts of BAT-catalyzed conjugating activity with taurine as substrate but the expressed enzyme did not use glycine or fluoro-β-alanine as substrates. The K_m value for taurine was 1.9 mM ± 0.1 mM in reactions with cholyl CoA as a cosubstrate. The specificity of mBAT for taurine as a substrate was confirmed by the demonstration, using HPLC-electrospray ionization mass spectrometry, that mouse gallbladder bile contained only taurine conjugates of bile acids. The identification of the types of amino acid conjugates of bile acids present in mouse bile had not been previously reported. These results indicate that a taurine-specific form of BAT has been cloned and expressed from mouse liver.—**Falany, C. N., H. Fortinberry, E. H. Leiter, and S. Barnes.** Cloning, expression, and chromosomal localization of mouse liver bile acid CoA: amino acid N-acyltransferase. *J. Lipid Res.* 1997. **38:** 1139–1148.

Supplementary key words bile acid • bile acid amidates • taurine • conjugation

Bile acids are the major solutes in bile. Greater than 99% of the bile acids secreted by the liver into the bile in mammals are conjugated with amino acids. Amino acid conjugation of bile acids to form N-acyl amidates increases the amphipathic nature of bile acids and hence their detergent properties (1). It also prevents their precipitation in the acidic milieu of the upper small intestine. In addition, amino acid conjugation decreases the formation of relatively insoluble complexes of bile acids with calcium (2). Bile acids form mixed micelles with phospholipids which serve to increase the aqueous solubility of cholesterol by a factor of 10^6. In the intestines, they are responsible for the solubilization and absorption of fats, vitamins, and fat- soluble compounds (1).

With the exception of the manatee (3), elephant and hyrax (4, 5) which secrete bile alcohol sulfates, mammals secrete bile acids conjugated with taurine and/or glycine. Some species such as dogs and cats synthesize only taurine conjugates, whereas other species such as rats and humans synthesize both taurine- and glycine-conjugated bile acids (4).

Amidation of bile acids in the liver requires the sequential action of two separate enzymes, bile acid CoA synthetase (BAS) and bile acid CoA:amino acid N-acyltransferase (BAT). In the first reaction, BAS catalyzes the formation of bile acid CoA thioesters. In the second reaction, BAT catalyzes the reaction between the bile acid CoA thioester and either taurine or glycine.

Our laboratory has recently reported the molecular cloning of human liver BAT (hBAT) and expression of the enzymatically active enzyme in *E. coli* (6). Expressed and purified hBAT utilized both taurine and glycine as substrates. The only other known substrate for hBAT is fluoro-β-alanine, a metabolite of the chemotherapeutic

Abbreviations: BAT, bile acid CoA:amino acid N- acyltransferase; BAS, bile acid CoA synthetase.

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agent 5-fluorouracil (7, 8). Substrate specificity studies have indicated that BAT is very selective in the amino acids with which it conjugates bile acids (9).

As part of a project to better understand the mechanism for the formation and physiological properties of bile acid conjugates and to develop a mouse knockout model of BAT, we have isolated and expressed the cDNA for mouse liver BAT in bacteria. This report describes the kinetic properties of the expressed mouse BAT (mBAT) as well as the chromosomal localization and sequence similarity to other mammalian BATs. Expressed mBAT uses taurine but not glycine as a substrate for the conjugation of bile acids and analysis of the composition of mouse bile detected only the presence of taurine conjugates of bile acids.

MATERIALS AND METHODS

Materials

The mouse liver λZap cDNA library was obtained from Stratagene (La Jolla, CA). Restriction enzymes and other DNA modifying enzymes were purchased from New England BioLabs (Beverly, MA) and Promega (Madison, WI). [α-^{35}S]dATP (3000 Ci/mmol) and [α-^{32}P]dCTP (800 Ci/mmol) were purchased from DuPont-NEN (Beverly, MA). pKK233-2 was obtained from Pharmacia Biotech Inc. (Piscataway, NJ). The PCR nucleotides and Taq DNA polymerase were purchased from Promega. Sequenase Version 2.0 sequencing kits were obtained from United States Biochemical Corporation (Cleveland, OH). Nitrocellulose membranes were purchased from Micron Separation Inc. (Westborough, MA). All other reagents were molecular biology grade.

Isolation of mBAT cDNA

A mouse liver λZap XR cDNA library was screened using the coding region of the human BAT cDNA as a probe (6). *E. coli* XL-1 Blue cells were infected with aliquots of the mouse liver cDNA library (approximately 30,000 pfu/150 mm petri plate) and incubated overnight at 37°C. To screen the plates, nitrocellulose filters were soaked in 1 M NaCl and then placed on the plates and marked for orientation. After 5 min, the filters were removed and placed in a denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 10 min. The filters were then neutralized in 0.5 M Tris-HCl, pH 7.0, with 1.5 M NaCl. The phage DNA was covalently bound to the filters by exposure to UV light using a Bio-Rad Gene Linker apparatus. The filters were pre-hybridized for 15 min in 6× SSC, 5× Denhardt's solution, 0.5% SDS, and 0.2 μg/ml sonicated salmon sperm DNA (10). hBAT cDNA was radiolabeled using the Prime-A-Gene procedure (Promega) and [^{32}P]dCTP (3000 Ci/mM) to a specific radioactivity of 10^9 cpm/μg DNA. Radiolabeled hBAT cDNA was then added to the filters in fresh hybridization solution at a concentration of 200,000 cpm/ml and allowed to incubate overnight at 65°C. The next day the filters were washed at 65°C two times with 2× SSC/0.1% SDS for 15 min and one time with 2× SSC for 15 min. The filters were then dried and exposed to autoradiography film with an intensifying screen overnight at −70°C. Positive plaques were purified by repeated cycles of dilution and rescreening until a single pfu could be isolated. This procedure resulted in the isolation of ten pure phage clones from the screening of approximately 300,000 pfu of the cDNA library. After isolation of the phage, the cDNAs were recovered in Bluescript phagemids by coinfection with helper phage (R408) as per the manufacturer's instructions (Stratagene).

Nucleotide sequence analysis of mBAT cDNAs

The mBAT cDNAs were subjected to double-stranded sequencing by the dideoxynucleotide chain termination method using Sequenase 2.0 and [α-^{35}S]dATP to label newly synthesized strands. The ^{35}S-labeled products were resolved on 6% polyacrylamide-urea gels using a buffer gradient of 0.5× to 2.5× TBE (TBE = 89 mM Tris-borate, 2 mM EDTA). The complete cDNA sequence of mBAT was obtained by subcloning restriction fragments into pBluescript for sequencing with T3 and T7 primers or by sequencing short fragments generated by Sau 3A or Hae III digestion and subcloned into pBluescript. Oligonucleotide primers synthesized to internal sequences of mBAT were also used to sequence specific regions. Sequence gels were read manually and analyzed using MacVector sequence analysis software (Kodak).

Generation of mBAT bacterial expression vector

In order to express enzymatically active mBAT in *E. coli*, the mBAT cDNA was subcloned into the Nco I–Pst I sites of the bacterial expression vector pKK233-2 (Clontech) in a three- step procedure. An internal 685 basepair Nco I-Pst I fragment was isolated from the BAT cDNA and subcloned into the Nco I and Pst I sites of pKK233-2. The 3′-780 basepair Pst I–Pst I fragment was then subcloned into the Pst I site of this plasmid and the orientation of the Pst I fragment was established by restriction mapping. In order to complete the BAT cDNA and insert the codon for the initial methionine into the Nco I site adjacent to the ribosome binding site, a 220 basepair Nco I fragment with the initial methionine codon incorporated into an Nco I site was synthesized. PCR was used to generate a Nco I site incorporating the initial methionine codon. A 19-nucleotide primer was synthesized to bases 181–200 in which the two adenosines prior to the ATG were changed to cyto-

sines (underlined), (5′-CTGCAAACCATGGCCAAGC-3′). The template for PCR was the mBAT#9/pBluescript vector. Twenty cycles of denaturation, 1 min at 95°C, annealing for 1 min at 59°C, and extension for 40 sec at 72°C were performed. Then, T3 primer was added and the reaction was subjected to 30 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 46°C, and extension for 40 sec at 72°C. PCR products were extracted with chloroform, ethanol precipitated, digested with Nco I, and purified after agarose gel electrophoresis using a PCR Cleanup kit (Promega). The 220 basepair Nco I–Nco I fragment was subcloned into the pKK233-2 vector containing the 3′-portion of the mBAT cDNA. The correct orientation of the Nco I fragment was determined by restriction mapping and sequence analysis.

Bacterial expression of mBAT

The pKK233-2/mBAT#9 expression vector was transformed into *E. coli* DH5α cells made competent using a $CaCl_2$ procedure (11). mBAT activity was induced and isolated as described previously for hBAT (6). The mBAT activity in the induced bacterial cytosol was partially purified by DEAE anion-exchange chromatography as previously described (12). The column fractions were assayed for BAT activity and by immunoblot analysis using the rabbit anti-human BAT antibody.

BAT assay

mBAT enzyme activity was determined using a radioassay as described previously using [^{3}H]taurine and [^{3}H]glycine as substrates (6, 8). Briefly, [^{3}H]taurine was used for conjugation to unlabeled cholyl CoA to form [^{3}H]taurine conjugates. The sensitivity of the assay will allow for the detection of 0.1 nmol cholyl CoA conjugated in 30 min. Cholyl CoA was chemically synthesized as described previously (8), using a modification of the method of Shah and Staple (13). Protein concentrations were determined using the Bio-Rad protein assay with gamma globulin as a standard. Assays utilizing fluoro-β-alanine as a substrate were performed as described previously except the reaction mixtures were analyzed by mass spectrometry using a PE-Sciex (Concord, Ontario, Canada) API III triple quadrupole instrument in the Mass Spectrometry Core Facility of the UAB Comprehensive Cancer Center.

Analysis of mouse and rat bile

Bile was recovered from the gallbladder of a normal AJ male mouse and from the common bile duct of a female Sprague-Dawley rat under ether anesthesia. The biles were diluted in 1 ml of water and passed over an activated C_{18} Sep-Pak cartridge. Retained bile acids were eluted from the cartridge with 2 ml of methanol. Aliquots were injected onto a 10 cm × 2.1 mm i.d. C_8 reversed-phase column using a linear gradient of 30–100% acetonitrile in 0.1% acetic acid. The flow rate was 0.2 ml/min; the column eluate was split 1:1, with 0.1 ml/min being passed into the IonSpray™ interface of the PE-Sciex API III triple quadrupole mass spectrometer operating in the negative ion mode, with an orifice potential of −60V. Spectra were recorded from 300–800 *m/z* at 1.5-sec intervals. The operation of the mass spectrometer and analysis of data were carried out using two Macintosh Quadra 950 computers interfaced with an Ethernet link.

Northern blot analysis of mBAT expression

The analysis of the expression of mBAT message in different tissues was carried out using a commercial mouse multiple tissue Northern blot (Clontech). The blot contained 2 μg of poly A+ RNA from eight different mouse tissues. The membrane was hybridized with the mBAT cDNA labeled with [^{32}P]dCTP by random-priming using a Pharmacia Oligolabeling Kit. Hybridization was carried out for 2 h at 65°C in Quickhyb containing 1.25×10^6 cpm/ml of [^{32}P]DNA. The blot was washed under high stringency conditions and autoradiography was then performed at −70°C with an intensifying screen.

Immunoblot analysis of mBAT

For immunoblot analysis of expressed mBAT, cytosolic proteins prepared from the *E. coli* expressing mBAT were resolved by SDS-PAGE in a 12.5% gel, electrophoretically transferred to nitrocellulose membranes, and analyzed for immunoreactivity using specific rabbit anti-hBAT polyclonal antibodies at a 1/1,500 dilution (6, 14). Immunoconjugates were visualized by chemiluminescence using the Lumiglo method (KPL).

Chromosomal localization of mBAT gene

The chromosomal localization of the mBAT gene was carried out using the backcross panel of DNAs from the Jackson Laboratory Backcross DNA Panel Mapping Resource (15). The probe for these studies was a 1533 basepair fragment of the mBAT cDNA containing the complete coding region of the cDNA. The differential presence of a 5 kb Pst I restriction fragment variant in the B6 versus a 6 kb restriction fragment in *M. spetus* was used to type the (C57B46JEi × SPRET/Ei)F1 × SPRET/Ei DNA panel (DNA from 94 first backcross segregants) by Southern blot analysis.

RESULTS

Molecular characterization of mBAT cDNAs

A mouse liver λZap XR cDNA library was screened under moderate stringency conditions using the human

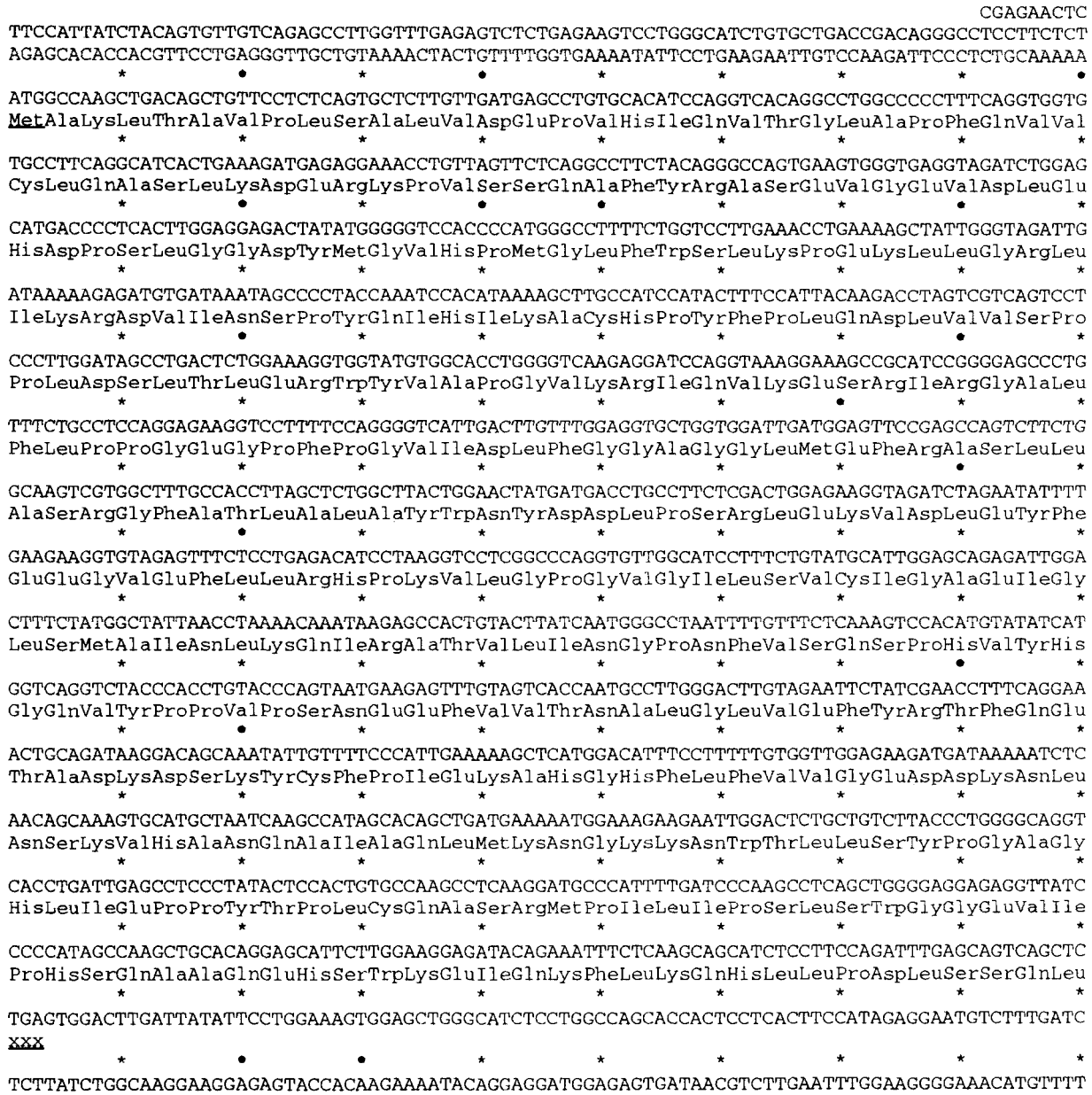

Fig. 1. Nucleotide sequence and translated amino acid sequence of the mBAT#9 cDNA. The amino acid sequence is numbered on the right. The asterisk denotes the stop codon.

BAT cDNA as a probe (6). Ten positive clones were isolated and purified to single plaque forming units by repeated cycles of dilution and rescreening. Two of these clones apparently possessed complete open reading frames for BAT based on sequence analysis of the ends of the cDNAs. One clone, mBAT#9, was selected for sequence analysis and characterization. mBAT #9 is 1869 basepairs in length. The full-length cDNA possessed a 189 basepair 5′-nontranslated region, an open-reading frame of 1260 basepairs and a 404 basepair 3′- nontranslated region followed by a poly(A) tail (**Fig. 1**). The open-reading frame codes for a 420 amino acid protein with a calculated molecular mass of 46,525 daltons.

Figure 2 shows the comparison of the amino acid sequence of mBAT with that of hBAT and *kan-1*, a cDNA isolated from rat liver that is the putative sequence of rat BAT (16). These are the only reported sequences of BAT enzymes. The amino acid sequence of mBAT is

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mBAT  MAKLTAVPLSALVDEPVHIQVTGLAPFQVVCLQASLKDER-KPVSSQAFYRASEVGEVDL  59
rBAT                       R    T            DKGNLFN                  60
hBAT   IQ   T V           RA   I   M SF    E  NGDMFY   H   N F        60

mBAT  EHDPSLGGDYMGVHPMGLFWSLKPEKLLGRLIKRDVINSPYQIHIKACHPYFPLQDLVVS 119
rBAT   R S                  M      T  V    M R HKV   L      VEGK I  120
hBAT  N AS                        T  L    M R F VQV LYDLELIVNNK A  120

mBAT  PPLDSLTLERWYVAPGVKRIQVKESRIRGALFLPPGEGPFPGVIDLFGGAGGLMEFRASL 179
rBAT  SS    I     M    T  H   G                                F      180
hBAT  A KA             T  K R G L           L          L   L       180

mBAT  LASRGFATLALAYWNYDDLPSRLEKVDLEYFEEGVEFLLRHPKVLGPGVGILSVCIGAEI 239
rBAT     H          G                                              240
hBAT          S     H  E   RKP VT       AAN        F S   VV   Q VQ  240

mBAT  GLSMAINLKQIRATVLINGPNFVSQSPHVYHGQVYPPVPSNEEFVVTNALGLVEFYRTFQ 299
rBAT             T            SN    R K FQ T CS    T             E 300
hBAT        Y   VT       T  PFGI Q     IHQ L HSAQLIS      L L    E 300

mBAT  ETADKDSKYCFPIEKAHGHFLFVVGEDDKNLNSKVHANQAIAQLMKNGKKNWTLLSYPGA 359
rBAT                                        K        S              360
hBAT  T QVGA Q L    E Q Q   I   G  TI   A  E   G  KRH  N             360

mBAT  GHLIEPPYTPLCQASRMPILIPSLSWGGEVIPHSQAAQEHSWKEIQKFLKQHLLPDLSSL 420
rBAT          S   S     FV   IN        -A                  N GFN   420
hBAT          S   C  TTHD R-- H        -A     A     R  RK   I  VT   418
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Fig. 2. Comparison of the translated amino acid sequences of mBAT, *kan-1* and hBAT cDNAs. The amino acid sequences were aligned using the MacVector Sequence Analysis programs. The differences in the sequences of *kan-1*, a putative form of rBAT (16) and hBAT (6) are shown as compared to the sequence of mBAT. Dashes indicate the presence of gaps inserted to optimize sequence alignments. The xxx denotes the stop codon.

69% identical and 84% similar to that of hBAT, and 86% identical and 95% similar to that of *kan-1*. The regions of dissimilarity between the three amino acid sequences are spread fairly uniformly along the lengths of the proteins. The most extensive region of dissimilarity was from residues 88–123.

Expression of mBAT

mBAT was expressed in *E. coli* using the bacterial expression vector pKK233-2, which had previously been used for the expression of hBAT (6). Expressed mBAT migrated with a molecular mass of approximately 45,000 daltons during SDS-polyacrylamide gel electrophoresis and reacted with the rabbit anti-hBAT polyclonal antibodies during immunoblot analysis. No immunoreactive protein was observed in cytosol prepared from bacteria transformed with the pKK233-2 vector alone. Cytosol prepared from *E. coli* DH5α cells transformed with the bacterial expression vector pKK233-2 alone did not possess detectable levels of BAT activity with cholyl-CoA and either glycine or taurine as co-substrates. **Figure 3** shows that cytosol from cells transformed with mBAT#9/pKK233-2 possessed significant amounts of cholyl- CoA conjugating activity with taurine and the BAT activity coeluted with immunoreactive mBAT during DEAE-Sepharose Cl-6B chromatography. These results demonstrate that mBAT#9 encodes an enzymatically active protein. No conjugating activity was detected with glycine as a co-substrate or with fluoro-β-alanine as a substrate.

For kinetics studies of mBAT activity, DEAE-Sepharose Cl-6B chromatography was carried out to resolve the mBAT activity from the cholyl-CoA hydrolyzing activity. The taurine conjugating activity of the partially purified mBAT was linear with respect to incubation time and protein concentration (data not shown). The apparent K_m value for taurine was 1.9 ± 0.1 mM. No activity was detected using either [^{3}H]glycine or fluoro-β-alanine as substrates. Addition of concentrations of glycine up to 5 mM to the taurine conjugation reactions did not cause any detectable inhibition of the rate of taurocholate formation.

Northern blot analysis of mouse tissues

Figure 4 shows that the mBAT cDNA hybridized to a single major RNA species with an approximate length of 1900 nucleotides in mouse liver poly A+ RNA. No reactive RNA bands were detected in RNA isolated from the other tissues tested. The length of the mBAT#9 cDNA is 1869 bp (Fig. 1) which correlates well with the size of the poly A+ RNA detected in mouse liver.

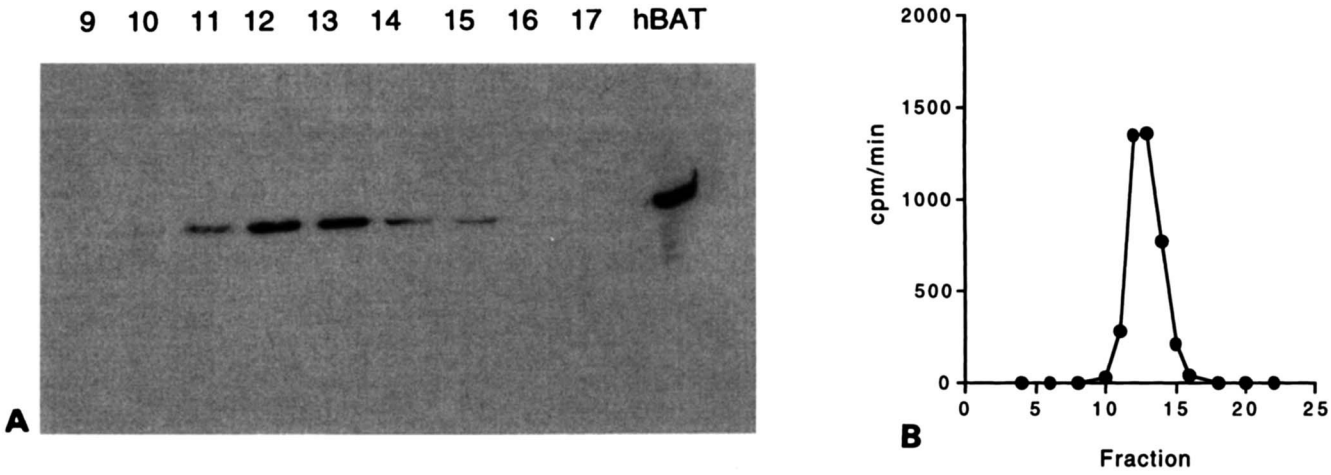

Fig. 3. Immunoblot analysis of mBAT expressed in *E. coli*. The mBAT cDNA was inserted into the bacterial expression vector pKK233-2 and expressed in *E. coli* DH5α cells as described in Methods. Cytosol was prepared from the *E. coli* DH5α cells and applied to a DEAE-Sepharose Cl-6B column equilibrated in 10 mM triethanolamine, pH 7.4, containing 1 mM dithiothreitol. The column was then eluted with a 0–300 mM NaCl gradient in the same buffer and the fractions were assayed for BAT activity. Panel A shows the immunoblot analysis of the column fractions using rabbit anti-hBAT IgG (14). Panel B shows the elution of BAT activity in the same fractions using cholyl CoA and taurine as substrates.

Chromosomal localization

The structural gene encoding mouse BAT has been assigned the symbol *Baat*. Using the Jadeson BSS backcross (15), the structural gene for mBAT was mapped to Chromosome 4. The strain distribution pattern revealed linkage to markers on proximal Chromosome 4 (**Fig. 5,** top), with 1/94 recombinants with *D4Bir12* (approximately 1 cM proximal to *Baat*) and 5/94 recombinants with *D4Bir14* (approximately 5.3 cM distal). The localization of *Baat* on Chromosome 4 is summarized in Fig. 5, bottom.

HPLC-MS of bile acid conjugates in mouse and rat bile

To investigate the forms of bile acid amidates present in mouse and rat bile, extracts of bile from both species were analyzed by HPLC-MS. Selected ion chromatograms prepared from the total ion current revealed that only taurine-conjugated bile acids were present in extracts of mouse bile (**Fig. 6**). Two major peaks (514 *m/z*) detected in the samples of mouse bile were tauro-β-muricholic acid and taurocholic acid. No peaks were detected for glycine-conjugated bile acids (*m/z* of 464 for trihydroxy bile acids, *m/z* 448 for dihydroxy bile acids). In contrast, HPLC-MS analysis of rat bile revealed that it contained both glycine and taurine dihydroxy and trihydroxy bile acids (**Fig. 7**).

DISCUSSION

A full-length cDNA encoding mouse BAT was isolated from a mouse liver cDNA library and the enzymatically active enzyme was expressed in *E. coli* DH5α cells. Analysis of the kinetic properties of expressed mBAT shows that the enzyme is a taurine-specific conjugating form of BAT. This was confirmed by the lack of glycine-conjugated bile acids in mouse gallbladder bile as demonstrated by HPLC-mass spectrometry. To the author's knowledge, this is the first report of the types of amino acid conjugates of bile acids formed in mice.

Different animal species are capable of conjugating the CoA derivatives of bile acids with taurine, or both taurine and glycine (4). The evolutionary pattern of taurine-specific conjugation of bile acids is generally associated with a carnivorous diet. Species such as cats and seals tend to be taurine-specific conjugators possibly because meat is a major source of taurine and is therefore readily available in their diets. Herbivores and omnivores are generally associated with an ability to conjugate bile acids with both taurine and glycine although these differences may only represent trends within different families and genera (4).

Cloned and expressed hBAT has been previously reported to conjugate cholyl-CoA with taurine, glycine, and fluoro-β-alanine (6). Expressed mBAT has been shown in the present study to utilize only taurine as a substrate. This observation was confirmed by analysis of mouse bile by HPLC-ESI-MS. Only taurine conjugates of bile acids were identified in mouse bile. Although the bile acids in the mouse have been studied by several other investigators, the nature of their conjugates has not been previously reported. The failure to detect glycine conjugates in mouse bile was not due to the electrospray ionization method as glycine conjugates form ions almost as efficiently as taurine conjugates (17). Rat bile collected by bile duct cannulation, in contrast to

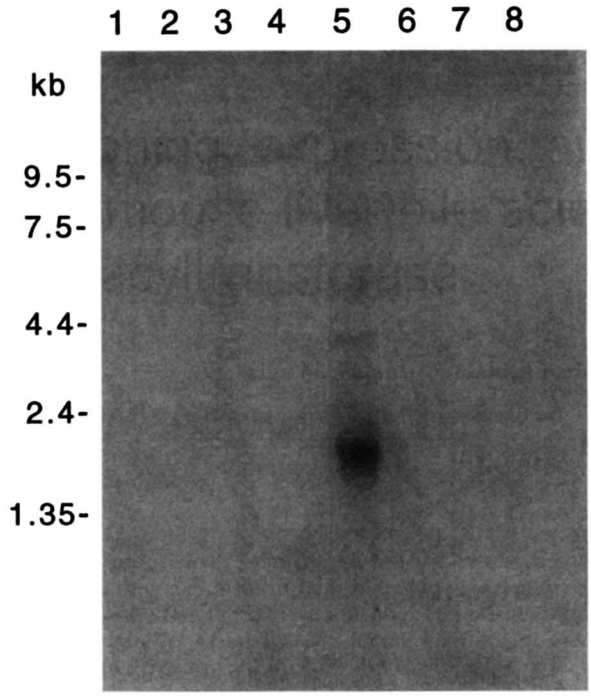

Fig. 4. Northern blot analysis of the expression of mBAT message in mouse tissues. A commercial multiple tissue Northern blot (Clontech) containing RNA isolated from 8 mouse tissues was probed with [^{32}P]mBAT cDNA. The lanes contained 2 μg poly A+ RNA isolated from (1) heart; (2) brain; (3) spleen; (4) lung; (5) liver; (6) skeletal muscle; (7) kidney; and (8) testis.

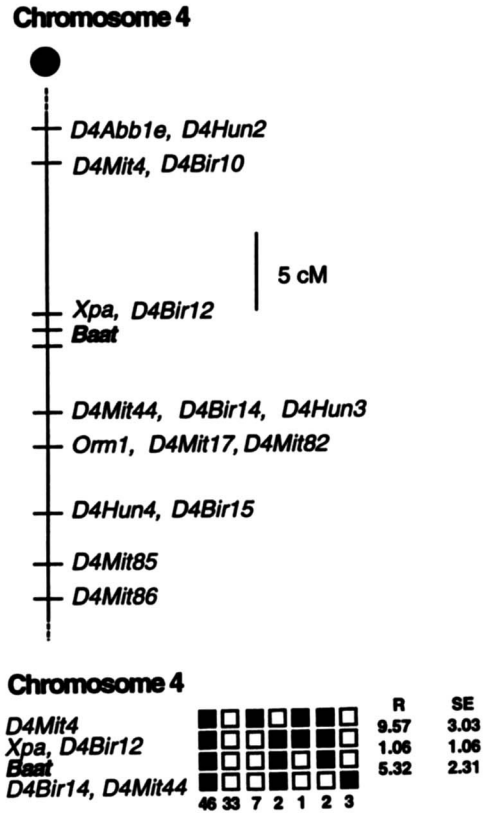

Fig. 5. Localization and linkage map of the mBAT structural gene (*Baat*) on mouse chromosome 4. The upper panel shows the linkage pattern of the *Baat* gene to markers on proximal Chromosome 4. The lower panel summarizes the strain distribution pattern of the linkage of *Baat* to the markers used to localize the *Baat* gene. The filled boxes denote the presence of B_6 alleles, while the open boxes denote the SPRET allele. The numbers at the bottom of each column denote the total number of individuals with the given haplotype. The recombination frequency (R) ± S.E.M. between the linked markers as shown.

mouse bile, contained both taurine and glycine bile acid conjugates in a ratio of under 2:1, as noted previously (18). Zhang, Barnes, and Diasio (18) have reported that glycine and taurine conjugates are formed at approximately the same rates in rat liver; however, the glycine conjugates are more rapidly deconjugated during enterohepatic circulation which gives rise to the ratio of taurine to glycine conjugates of about 8:1 observed in normal bile.

The similarity between the amino acid sequences of mBAT, hBAT and *kan-1* (rBAT) did not correlate with the ability of the enzymes to utilize taurine or taurine and glycine as substrates. The amino acid sequence of mBAT is 84% similar to the amino acid sequence of human BAT. *Kan-1,* a putative BAT sequence isolated from a rat liver cDNA library, was more closely related to mBAT to which it is 95% similar. The rabbit anti-hBAT polyclonal antibody is capable of recognizing both mBAT (6) and rBAT (19). Although the BAT sequences from mouse and rat are more similar in sequence than either sequence is to hBAT, rats and humans are capable of forming both taurine and glycine conjugates of bile acids whereas only taurine conjugates have been detected in the mouse. mBAT, which does not use glycine as a substrate, is also not inhibited by glycine, indicating that the amino acid differences in the proteins prevent glycine from binding at the active site. These observations suggest that the evolutionary changes in the sequence of the BAT enzyme that determine its substrate selectivity probably involve specific amino acids.

The catalytic mechanism of BAT is proposed to involve the transient formation of a bile acid thioester with a cysteine in the active site of BAT (20). The sequence of hBAT contains only three cysteine residues, one at position 235 and a doublet at 372/373. In contrast, the sequence of mBAT has five cysteines, at positions 31, 107, 234, 309, and 371, and the sequence of *kan-1* possesses six cysteines, at 31, 108, 235, 280, 310, and 372. The cysteines correspond to positions 235 and 372 in hBAT and are therefore conserved in the sequence of each of the BAT sequences. As one of the cysteines in the cysteine doublet in hBAT is not conserved in mBAT, this would suggest that the cysteine at position 235 in hBAT and at 234 in mBAT is involved in the formation of a bile acid thioester at the active

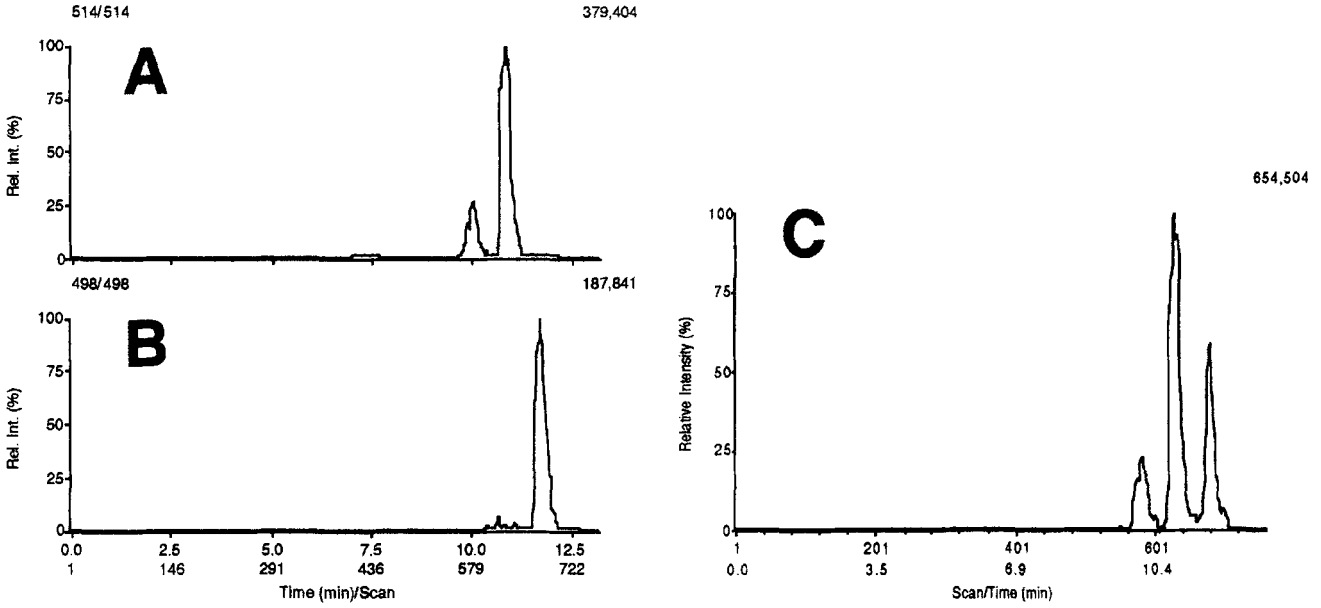

Fig. 6. Selected ion chromatograms from HPLC-ESI-MS analysis of mouse bile. The bile acids were separated using a O_8 reversed-phase column at a flow rate of 0.2 ml/min as described in Methods. Part of the column eluate (0.1 ml/min) was diverted to an electrospray ionization interface of the mass spectrometer. Negative ion spectra were recorded from 300–800 amu. Reconstructed ion chromatograms are presented for (A) taurine-conjugated trihydroxy bile acids (m/z 514) and (B) taurine-conjugated dihydroxy bile acids (m/z 498). No ions were detected for glycine-conjugated trihydroxy (m/z 464) or dihydroxy bile acids (m/z 448). An ion chromatogram (C) for the mass range m/z 400–550 indicates that only the taurine- conjugated bile acids could be detected in mouse bile.

site of BAT. This has been confirmed by recent experiments in which the CYS-235 in hBAT was mutated to an ALA and the mutant protein was expressed in *E. coli* using the bacterial expression vector pKK233-2. The ALA-235 hBAT mutant-did not express any BAT activity, even though the mutant protein was expressed normally (21).

Northern blot analysis of mBAT message in different mouse tissues only detected mBAT message in liver poly A+ RNA. No mBAT message was detected in poly A+ RNA isolated from heart, brain, spleen, lung, skeletal muscle, kidney, or testis tissue. Kwakye et al. (22, 23) have reported the presence of both BAT and BAS activity and immunoreactivity in rat kidney, suggesting that bile acid conjugation can occur in kidney tissue. However, the formation of bile acid conjugates by rat kidney tissues has not been demonstrated.

The structural gene for mBAT has been assigned the

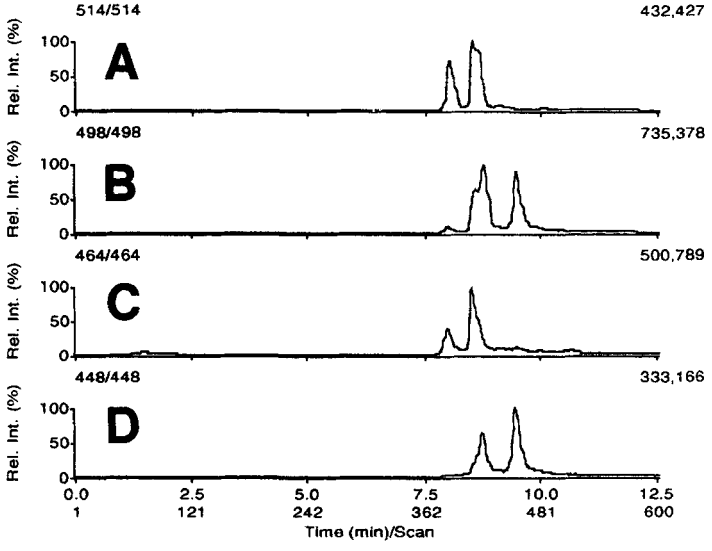

Fig. 7. Selected ion chromatograms from HPL-ESI-MS analysis of rat bile. HPLC conditions are the same as in Fig. 7. Rat bile contains (A) taurine-conjugated trihydroxy bile acids (m/z 514), (B) taurine-conjugated dihydroxy bile acids (m/z 498), (C) glycine-conjugated trihydroxy bile acids (m/z 464), and (D) glycine-conjugated dihydroxy bile acids (m/z 448).

symbol *Baat* and was mapped to the proximal arm of mouse Chromosome 4. These results predict that the human homologue of *Baat* would be found on Chromosome 9q22 as this region contains the xeroderma pigmentosum, complementation group A locus (XPAC). The mouse Xpa locus is approximately 1 cM proximal to *Baat* in a region exhibiting extensive homology to human Chromosome 9q (24, 25). Similarly, the rat homologue of the *Baat* gene would be expected to be on rat Chromosome 5 based on comparative mapping data (26). The molecular cloning studies also suggest that BAT in each of these species may be the product of a single gene.

Analysis of the cDNAs for BAT in humans, rats, and mice suggests that a single BAT gene encodes an enzyme in a given species capable of utilizing taurine or both taurine and glycine to conjugate bile acids. Characterization of BAT in more species is needed to confirm whether this is a consistent feature of bile acid metabolism. Minor changes in the amino acid sequence of these enzymes is apparently sufficient to generate the changes in the substrate selectivity. Comparison of the structure of BAT in different species will also aid in understanding the evolutionary trends towards the use of the different amino acids in bile acid conjugation in mammalian species.

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