The bile acid-inducible *baiF* gene from *Eubacterium sp.* strain VPI 12708 encodes a bile acid-coenzyme A hydrolase

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Abstract **The human intestinal *Eubacterium sp.* strain VPI 12708 has been shown to have a multistep biochemical pathway for bile acid 7α-dehydroxylation. A bile acid-inducible operon encoding 9 open reading frames has been cloned and sequenced from this organism. Several of the genes in this operon have been shown to catalyze specific reactions in the 7α-dehydroxylation pathway. The *baiF* gene from this operon was cloned, expressed in *Escherichia coli*, and found to encode a novel bile acid-coenzyme A (CoA) hydrolase. The subunit molecular mass of the purified bile acid-CoA hydrolase was calculated to be 47,466 daltons and the native enzyme had a relative molecular weight of 72,000. The K_m and V_{max} for cholyl-coenzyme A (CoA) hydrolysis was approximately 175 μM and 374 μmol/min per mg protein, respectively. The enzyme used cholyl-CoA, 3-dehydrocholyl-CoA, and chenodeoxycholyl-CoA as substrates. No hydrolytic activity was detected using acetyl-CoA, isovaleryl-CoA, palmitoyl-CoA, or phenylacetyl-CoA as substrates. Amino acid sequence database searches showed no significant similarity of bile acid-CoA hydrolase to other thioesterases, but significant amino acid sequence identity was found with *Escherichia coli* carnitine dehydratase. The characteristic thioesterase active site Gly-X-Ser-X-Gly motif was not found in the amino acid sequence of this enzyme. Bile acid-CoA hydrolase from *Eubacterium sp.* strain VPI 12708 may represent a new family of thioesterases.—**Ye, H-Q., D. H. Mallonee, J. E. Wells, I. Björkem, and P. B. Hylemon. **The bile acid-inducible *baiF* gene from *Eubacterium sp.* strain VPI 12708 encodes a bile acid-coenzyme A hydrolase. *J. Lipid Res.* 1999.** 40: **17–23.**

Supplementary key words 7α-dehydroxylation • secondary bile acid

Bile acids are synthesized from cholesterol in the liver. The two primary bile acids synthesized in humans are cholic acid and chenodeoxycholic acid and they are conjugated to either glycine or taurine prior to their active secretion from the liver (1). They undergo a recycling process between the liver and intestine called enterohepatic circulation. During their enterohepatic circulation several hundred milligrams are lost into the large intestines each day. In the large bowel, a small population of colonic bacteria catalyze 7α-dehydroxylation of cholic acid and chenodeoxycholic acid yielding deoxycholic and lithocholic acid, respectively (2, 3). Deoxycholic acid is absorbed from the colon and becomes part of the bile acid pool in humans. The amount of deoxycholic acid in the bile acid pool can vary from 0 to over 40% of the total pool (4).

Eubacterium sp. strain VPI 12708 is an anaerobic bacterium originally isolated from a human fecal sample and has been demonstrated to have a bile acid-inducible 7α-dehydroxylation pathway (5) (**Fig. 1**). This pathway has been proposed to serve as an ancillary electron acceptor for the fermentative metabolism of this organism. The oxidative part of this pathway is believed to begin by active transport of the primary bile acid into the bacterial cell (6), followed by synthesis of the coenzyme A (CoA) conjugate (7), oxidation of the 3α-hydroxy group (8), and insertion of a double bond between C-4 and C-5 (5). The next step in this pathway involves 7α-dehydration (9), yielding a 3-oxo-$\Delta^{4,6}$- intermediate that is sequentially reduced in three steps to the 7α-dehydroxylated bile acid and released from the cell (Fig. 1). It is not known when the coenzyme A is cleaved from the bile acid. However, the 3α-hydroxysteroid dehydrogenase, an enzyme in the oxidative arm of the pathway, has been shown to strongly prefer bile acid-CoA conjugates as substrates (8).

A large (~12 kb) bile acid-inducible operon has been cloned and sequenced from *Eubacterium sp.* strain VPI 12708 (10). This operon has been shown to encode nine open reading frames (*baiA* to *baiI*). The function of several of the gene products from this operon in the bile acid 7α-dehydroxylation pathway has been elucidated (**Fig. 2**).

Abbreviations: bai, bile acid inducible; CoA, coenzyme A; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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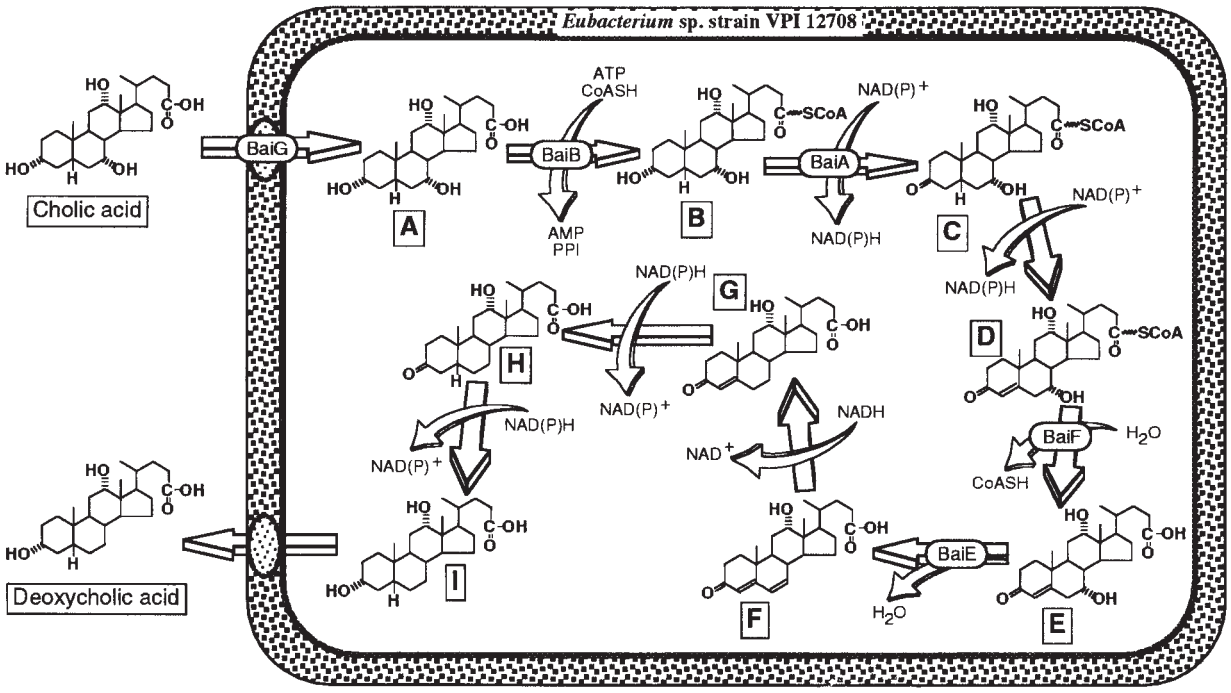

Fig. 1. Proposed cholic acid 7α-dehydroxylation pathway in *Eubacterium sp.* strain VPI 12708. Bile acid intermediates include: 3α,7α,12α-trihydroxy-5β-cholanoic acid, **A**; 3α,7α,12α-trihydroxy-5β-cholyl-CoA, **B**; 7α,12α-dihydroxy-3-oxo-5β-cholyl-CoA, **C;** 7α,12α-dihydroxy-3-oxo-4-cholenyl-CoA, **D**; 7α,12α-dihydoxy-3-oxo-4-cholenoic acid, **E**; 12α-hydroxy-3-oxo-4,6-choldienoic acid, **F**; 12α-hydroxy-3-oxo-4-cholenoic acid, **G**; 12α-hydroxy-3-oxo-5β-cholanoic acid, **H**; and 3α,12α-dihydroxy-5β-cholanoic acid, **I**. The proposed function of the *bai* gene products are indicated.

The *baiB* gene has been shown to encode a bile acid-CoA ligase (7), but how CoA is removed from the bile acid has not been elucidated. We initially hypothesized that the *baiF* gene may encode a bile acid-CoA hydrolase based on the observations with *Escherichia coli* extracts. When cell extracts containing the *baiF* gene product were added to *Eubacterium sp.* strain VPI 12708 extracts, there was a marked decrease in [^{14}C]cholic acid water-soluble (CoA-conjugated) counts. In this communication, we report the discovery and characterization of a novel bile acid-CoA hydrolase encoded by the *baiF* gene in the *bai* operon.

MATERIALS AND METHODS

Chemicals, enzymes, and bile acid-CoA hydrolase substrates

Radiolabeled [24-^{14}C]cholic acid and [24-^{14}C]chenodeoxycholic acid were purchased from DuPont NEN (Boston, MA). [24-^{14}C]cholyl-CoA was prepared enzymatically using bile acid-CoA ligase expressed in *E. coli* essentially as described previously (7). [24-^{14}C]-3-dehydrocholyl-CoA was synthesized enzymatically from [24-^{14}C]cholyl-CoA using 3α-hydroxysteroid dehydrogenase obtained from Sigma Chemical Co. (St. Louis, MO). Acetyl-CoA, isovaleryl-CoA, palmitoyl-CoA, phenylacetyl-CoA were also purchased from Sigma Chemical Co. Cholic acid and chenodeoxycholic acid were obtained from Calbiochem (San Diego, CA).

Chemical synthesis of unlabeled cholyl-CoA

The cholyl-CoA ester was synthesized by the mixed anhydride procedure essentially as described by Shah and Staple (11). Coupling of 75 μmol cholic acid with 63 μmol CoASH gave 15 μmol product that showed the expected UV-absorption at 232 nm. The purified material was found to contain less than 2% free cholic acid and was stable for several weeks when stored at 4°C in aqueous sodium acetate solution 0.01 m, pH 6.0.

Bacterial strains

E. coli DH5α was obtained from Gibco BRL (Gaithersburg, MD) and used as the host strain for expression of recombinant plasmids.

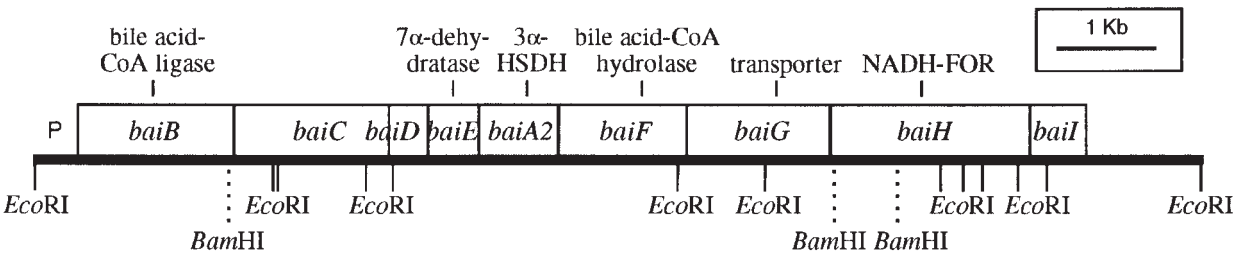

Fig. 2. The *bai* operon from *Eubacterium sp.* strain VPI 12708. The function of various *bai* gene products are indicted. Abb. NADH:FOR, NADH:flavin oxidoreductase, 3α-HSDH; 3α-hydroxysteroid dehydrogenase; transporter, bile acid transporter.

Expression of the *baiF* gene in *Escherichia coli*

Most of the *baiF* gene from *Eubacterium sp.* strain VPI 12708 is contained in a 2.9 kb *Eco*RI fragment that was initially cloned in *E. coli* by using a λgt11 vector, and then subcloned into a pUC8 vector (12). The sequence at the 3′ end of the *baiF* gene was obtained through direct sequencing of DNA obtained by inverse PCR (10). A 6 kb fragment, which contained the entire *baiF* gene, was obtained by *Bam*H1 digestion of chromosomal DNA from *Eubacterium sp.* strain VPI 12708. This fragment was used as the template for PCR amplification of the *baiF* gene using the synthetic oligonucleotide primers 5′-CCA**GTCGAC**TTACGGC TACTACGGTCAGCGTAGA-3′ and 5′-CCA**GGATCC**GGTGCT CATACTCTTACTCCTCTT-3′, which added *Sal*I and *Bam*HI sites, respectively (in bold). The DNA generated from this reaction was run on an agarose gel and the fragment corresponding to the amplified *baiF* gene was cut out and purified with a GeneClean kit (Bio 101 Inc., La Jolla, Calif). The purified DNA was digested with *Sal*I and *Bam*HI, ligated to pUC19 vector DNA, and transformed into *E. coli* DH5α. Isolated plasmids with the correct *baiF* insert were designated pUC19-47K. The *baiF* insert in pUC19-47K was completely sequenced to verify polymerase fidelity.

Expression of the *baiF* gene in *E. coli* was confirmed by a Western blot using rabbit polyclonal antibody which recognizes the *baiF* gene product essentially as described previously (12).

Bile acid-CoA hydrolase assay

Bile acid-CoA hydrolase activity was assayed by measuring the rate of conversion of [24-^{14}C]cholyl-CoA to free [24-^{14}C]cholic acid and CoA. The standard reaction mixture contained in final concentrations: 20 mm sodium phosphate buffer (pH 7.0); 5,000 dpm [24-^{14}C]cholyl-CoA, 100 μm unlabeled cholyl-CoA, and enzyme preparation in a total volume of 100 μl. The reaction was initiated by the addition of enzyme and carried out at 37°C. The reaction was stopped by the addition of 10 μl of 1 m HC1. Unconjugated cholic acid was extracted with ethyl-acetate phase and radioactivity in both the organic and aqueous phases was quantitated by liquid scintillation spectrometry. The reaction rate was linear for 2 min and over a protein concentration range of 0.01 to 0.12 μg/reaction mixture, using purified enzyme. A control reaction without enzyme was run and the background counts were subtracted. Substrate saturation kinetics were performed in two independent determinations and an average K_m and V_{max} were determined using a Hanes plot (13).

Reversed-phase HPLC of reaction products

A reversed phase C18 column (4.6 mm by 25 cm) (10-μm silica gel) was used for analysis of bile acid-CoA hydrolase reaction products. Reaction mixtures (20 μl) were loaded onto the column and eluted with 5–40% isopropanol in 20 mm ammonium bicarbonate buffer. Gradients were run over a 40-min time period at a flow rate of 0.8 ml/min. Free CoA and cholyl-CoA conjugates were monitored at 260 nm.

TLC of bile acid reaction products

Analysis of the bile acid component of cholyl-CoA was carried out on silica gel 1B thin-layer chromatography (TLC) plates (J.T. Baker, Inc., Phillipsburg, NJ). The cholyl-CoA hydrolase reaction was performed with standard reaction mixtures for 10 min at 37°C before being stopped with 1 m HC1. The reaction mixture was extracted with ethyl-acetate, spotted on TLC plates, and chromatographed with solvent system S1 (14). The plates were dried and exposed to Kodak X-Omat AR film (Eastman Kodak Co., Rochester, NY) for 24 h at room temperature before development. Known bile acid standards were run as controls.

Purification of bile acid-CoA hydrolase

Escherichia coli DH5α containing the pUC19-47K plasmid was used to inoculate two liters of Luria Broth (LB) containing ampicillin. This strain was grown aerobically at 37°C to a reading of 80 Klett units (red filter) before induction with 0.5 mm IPTG. After 2 h of additional growth, the induced cells were harvested by centrifugation, resuspended in 50 mm sodium phosphate buffer (pH 7.0) containing 20 mm 2-mercaptoethanol (2 ME) and 100 μg DNase, and broken by passing twice through a French Pressure Cell at 20,000 lb/in^2. The cell lysate was centrifuged at 105,000 *g* for 2 h at 4°C, and the supernatant fluid was collected and concentrated using a Centriprep-10 concentrator (Amicon Corp., Danvers, MA). The concentrated supernatant was diluted in 50% glycerol and stored at −20°C for further use. The soluble cell extract (360 mg) was applied to a Waters AP-2 DEAE high performance liquid chromatography (HPLC) column (Millipore Corp., Burlington, MA) equilibrated with 20 mm sodium phosphate buffer (pH 6.5) containing 20 mm 2 ME. Protein was eluted with a 0 to 500 mm NaCl gradient at a flow rate of 3.5 ml/min. Fractions containing high bile acid-CoA hydrolase activity were pooled, concentrated using a Centriprep-10 concentrator, and stored overnight in 10% glycerol at −20°C. These fractions were then applied to a Mono-Q ion-exchange column (Pharmacia LKB Biotechnology, Piscataway, NJ) equilibrated with 50 mm sodium phosphate buffer (pH 7.0) containing 20 mm 2-ME and 5% glycerol. Bile acid-CoA hydrolase was eluted with a 0 to 500 mm sodium chloride gradient at a flow rate of 1 ml/min using the equilibration buffer. Fractions containing activity were concentrated with a Centricon-10 microconcentrator, brought to 50% glycerol, and stored at −20°C.

Analysis of amino acid sequence data

Amino acid sequence analysis and data base searching was performed with the Wisconsin Package (Genetics Computer Group, Madison, WI).

RESULTS

Expression of the *baiF* gene in *E. coli*

Transformation of the pUC19-47K plasmid into *E. coli* resulted in excellent expression and accumulation of the *baiF* gene product (**Fig. 3**, compare lanes 1 and 2). Cell extracts of *E. coli* DH5α containing the pUC19-47K plasmid yielded ~330 mg of soluble protein, with *baiF* protein representing ~15% of the total protein. The expression and identity of the *baiF* gene product was confirmed by Western blotting using antibody to the 45 kilodalton protein (**Fig. 4**).

Purification of bile acid-CoA hydrolase

Eubacterium sp. strain VPI 12708 bile acid-CoA hydrolase was purified from *E. coli* (pUC19-47K) using HPLC. A 13-fold purification with a 38% yield was obtained from a typical two-step purification protocol (**Table 1**). Samples of each purification step were analyzed by SDS-PAGE (Fig. 3). The final enzyme preparation was greater than 90% pure (Fig. 3, lane 4). The purified enzyme had a subunit molecular mass of ~47,500 daltons as judged by SDS-PAGE. The native relative molecular weight was 72,000 using gel filtration chromatography. The bile acid-CoA hydrolase activity was stable for several months when

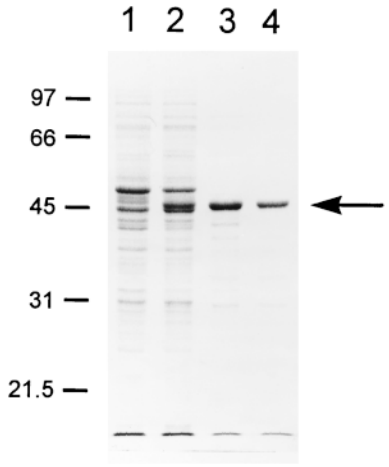

Fig. 3. Purification of bile acid-CoA hydrolase from *E. coli* expressing the *baiF* gene. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of crude and pooled fractions from various purification steps stained with Coomassie blue. Molecular weight standards are indicated on the left; 1. *E. coli* DH5α without plasmid construct (25 μg); 2. *E. coli* DH5α containing pUC19-47K plasmid (25 μg); 3. pooled DEAE fractions (8 μg); 4. Pooled Mono-Q fraction (2.5 μg).

stored at −20°C in 50% glycerol. *E. coli* DH5α (without plasmid) had some endogenous (~7.5% of total activity as compared with pUC19-47K plasmid) bile acid-CoA hydrolase (thioesterase) activity. The endogenous activity was fractionated on DEAE chromatography using identical conditions as described for the purification of bile acid-CoA hydrolase activity encoded by the *baiF* gene product. The endogenous activity eluted at a higher salt concentration on DEAE chromatography than the bile acid CoA hydrolase activity encoded by the *baiF* gene (data not shown).

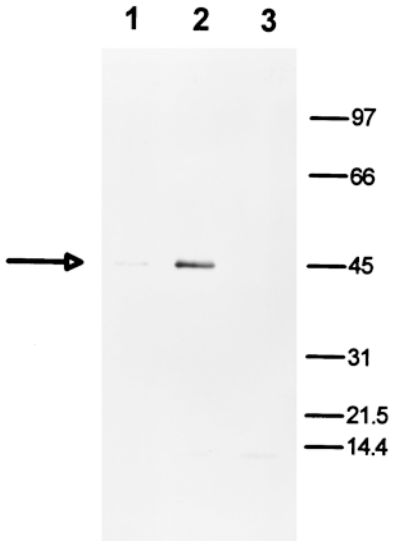

Fig. 4. Western immunoblot of *baiF* protein. Two μg of total soluble protein was used per lane for *Eubacterium sp.* strain VPI 12708 (1), *E. coli* DH5α pUC19-47K (2), and *E. coli* DH5α control (3).

TABLE 1. Purification of bile acid-CoA hydrolase from *E. coli*

Purification Step	Total Protein	Activity[a]	Sp. Act.	Yield	Purification
	mg	*U*	*U/mg*	*%*	*-fold*
Soluble cell extract	360	1732	4.81	100	
DEAE	32	1126	35	65	7.3
Mono-Q	11	659	61	38	12.68

[a]One unit of activity is defined as the amount of enzyme required to hydrolyze 1 μmol cholyl-CoA per min under standard assay conditions (see Materials and Methods).

Optimization of bile acid-CoA hydrolase activity

Bile acid-CoA hydrolase activity was measured by determining the rate of formation of ethyl-acetate-soluble (free cholic acid) radioactivity extracted from reaction mixtures containing [24-^{14}C]cholyl-CoA. Using standard reaction mixtures, the catalysis rate was linear for 2 min and over a protein concentration of 0.01 to 0.12 μg protein. Enzymatic activity was optimal between pH 6.0 and 7.0. No activity was detected below pH 4.0 or above pH 9.0.

Determination of apparent kinetic constants

K_m and V_{vax} values were obtained using initial velocity conditions. The approximate K_m and V_{max} values for cholyl-CoA hydrolysis were 175 μm and 374 μmol/min per mg protein, respectively. Kinetic constants were determined using a Hanes Plot (13).

Bile acid-CoA hydrolase substrate specificity and product identification

Products from the bile acid-CoA hydrolase reaction mixture were analyzed by reversed-phase HPLC and TLC. The elution times for CoA (HSCoA) and cholyl-CoA were determined by monitoring eluant at 260 nm. When purified bile acid-CoA hydrolase was added to standard reaction mixtures containing cholyl-CoA, a rapid accumulation of a product co-migrating with HSCoA was observed (**Fig. 5**, time 2 and 10 min). When the ethyl-acetate-soluble fractions from these reaction mixtures were analyzed by TLC, a single product co-migrating with cholic acid was observed (data not shown). Using either reversed-phase HPLC or TLC to identify enzymatic products, cholyl-CoA, 3-dehydrocholyl-CoA, and chenodeoxycholyl-CoA were shown to be used as substrates by bile acid CoA hydrolase. However, no hydrolytic activity was detected when acetyl-CoA, isovaleryl-CoA, palmitoyl-CoA, or phenylacetyl-CoA were used as substrates.

Amino acid sequence analysis of bile acid-CoA hydrolase

FASTA and BLAST searches were performed for amino acid sequence homology to bile acid CoA hydrolase. Sequences identified by these homology searches were then aligned with the GAP program. The best overall homology (38.2% identity and 60.1% similarity) was found with carnitine dehydratase from *E. coli* (15) and **Fig. 6**. However, the *baiF* gene product had no bile acid 7α-dehydratase activity using 7α,12α-dihydroxy-3-oxo-4-cholenoic acid as substrate (data not shown). No sequence homology was found with any reported 3-ketoacyl-CoA thiolases, acyl-

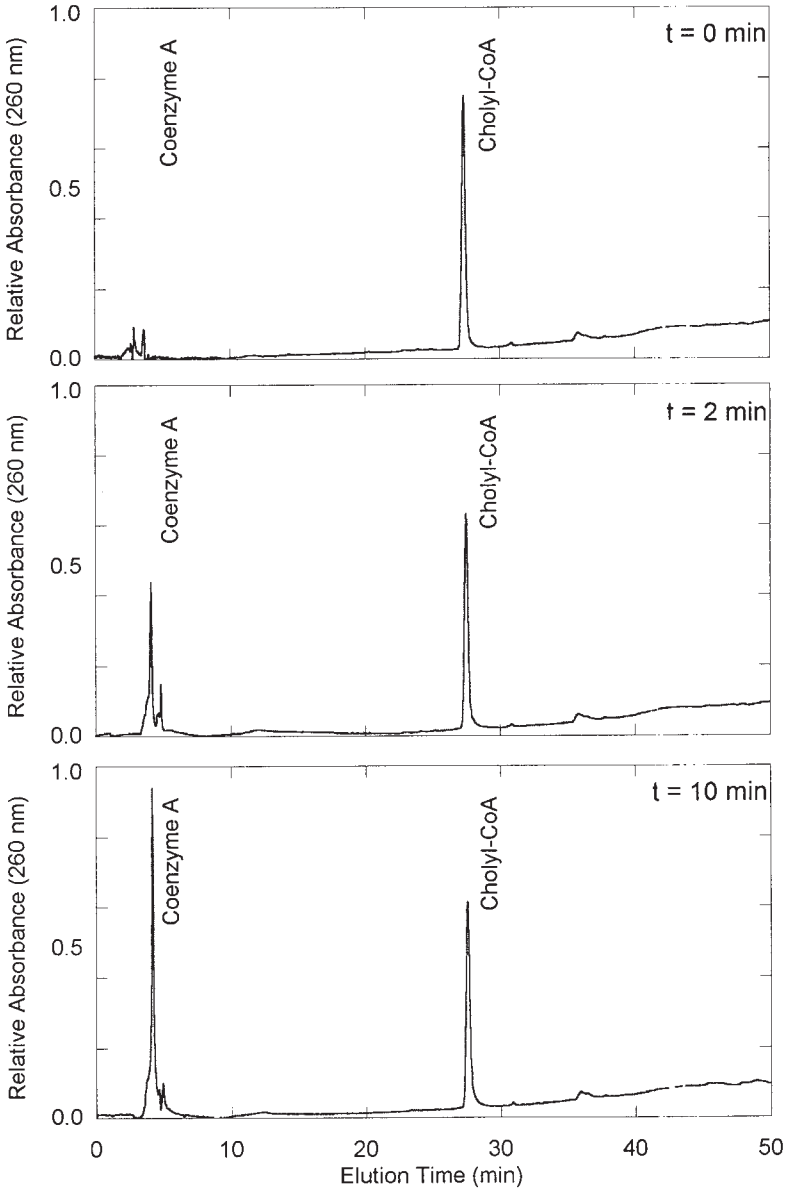

Fig. 5. Elution profiles of bile acid-CoA hydrolase product from C-18 reversed-phase HPLC using a 5–40% isopropanol gradient in 20 mm ammonium bicarbonate buffer (described in Materials and Methods). Shown are elution profiles of bile acid-CoA hydrolase products at 0, 2, and 10 min after enzyme addition to reaction mixtures. The elution times from coenzyme A and cholyl-CoA are indicated.

CoA hydrolases, or thiotransferases. In addition, consensus active site amino acid sequences for serine esterases (16–19), CoA transferases/acyl-CoA hydrolases (20), and 3-ketoacyl-CoA thiolases (21) could not be found in bile acid coenzyme A hydrolase.

DISCUSSION

Acyl-CoA hydrolase (thioesterase) activities have been reported in various mammalian tissues and in microorganisms. These enzymes are involved in a variety of biochemical processes including: regulation of intracellular fatty acid and acyl-CoA concentrations (22); hydrolysis of fatty acids from acyl-carrier protein (23); dehalogenation of chlorinated aromatic compounds in bacteria (24); bioluminescence (25); biosynthesis of polyketides (26); and cleavage of the acyl group from palmitoyl-protein including H-Ras and certain GTP binding proteins (27). As a group, these enzymes vary greatly in molecular weight, cellular location, and substrate specificity.

Most thioesterases from animal sources and some bacteria are sensitive to serine-directed inhibitors. A number of mammalian thioesterases have been shown to have a conserved active site motif Gly-X-Ser-X-Gly (16–19, 25). The active site serine is believed to act as a nucleophile during thioester hydrolysis. An examination of the amino acid sequence of the bile acid-CoA hydrolase from *Eubacterium sp.* strain VPI 12708 found no conserved active site motifs typical of these mammalian thioesterases, but there are a number of cysteine and histidine residues in bile acid-CoA hydrolase. *Escherichia coli* thioesterase I and several other mammalian thioesterases have a conserved Gly-X-His motif near the carboxyl terminal end of these enzymes (28), but this motif was not found in bile acid-CoA hydrolase. Amino acid sequence data base searches show no significant similarity with other thioesterases. Surprisingly, significant amino acid sequence similarity over the entire

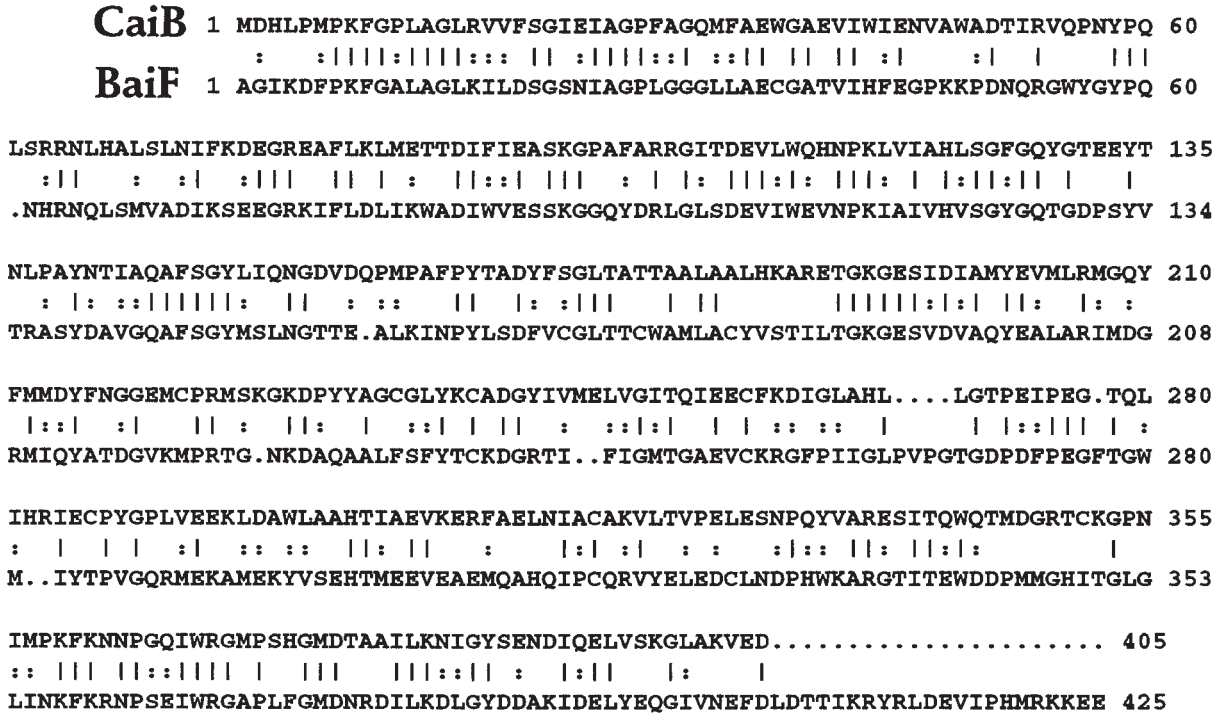

Fig. 6. Amino acid sequence alignment of bile acid-coenzyme A hydrolase from *Eubacterium sp.* strain VPI 12708 and carnitine dehydratase from *E. coli* using the GCG gap program. Vertical lines and colons indicate amino acid sequence identity and similarity, respectively.

protein was observed with *E. coli* carnitine dehydratase (Fig. 6). It is unknown whether carnitine dehydratase has thioesterase activity and to date there is no reported data that might indicate an evolutionary relationship between thioesterases and dehydratases. However, the genes encoding bile acid-CoA hydrolase and carnitine dehydratase may have been derived from a common ancestral gene. 2-Arylpropionyl-CoA epimerase, an important enzyme in ibuprofen metabolism (29), also has significant homology with bile acid CoA hydrolase. The normal physiological role of 2-arylpropionyl CoA epimerase in cell metabolism is unknown.

The precise role of bile acid-CoA hydrolase in bile acid 7α-dehydroxylation in *Eubacterium sp.* strain VPI 12708 is unknown. We have assayed for the ability of bile acid-CoA hydrolase to transfer the CoA moiety of cholyl-CoA to either acetate or cellular proteins, which would conserve the high energy thioester bond. These experiments yielded negative results, but we cannot totally exclude the possibility that this enzyme has CoA transferase activity. Our laboratory has shown that the *bai* operon of this bacterium encodes a bile acid-CoA ligase which is believed to be the first enzymatic step in the bile acid 7α-dehydroxylation pathway (7). It is unclear whether bile acids remain linked to CoA during all the various reactions of the 7α-dehydroxylation pathway. Nevertheless, CoA conjugates other than cholyl-CoA have been detected in cell extracts of this bacterium and 3α-hydroxysteroid dehydrogenase (*baiA*) appears to strongly prefer bile acid-CoA conjugates as substrates (8). The *baiE* gene has been reported to encode a bile acid 7α-dehydratase. This enzyme does not require bile acid-CoA conjugates as substrates (9). Therefore, the physiological substrate for the bile acid-CoA hydrolase may be 7α,12α-dihydroxy-3-oxo-4-cholenoyl-CoA. The high K_m (175 μm) of bile acid-CoA hydrolase for cholyl-CoA suggests that other bile acid intermediates may be the preferred substrates. This would prevent the possibility of a futile cycle between cholic acid-CoA ligase and cholyl-CoA hydrolase.

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REFERENCES

1. Vlahcevic, Z. R., D. M. Heuman, and P. B. Hylemon. 1996. Physiology and pathophysiology of enterohepatic circulation of bile acids. *In* Hepatology: a Textbook of Liver Diseases. 3rd ed. Vol. 1. D. Zakim and T. Boyer, editors. W. B. Saunders, Philadelphia. 376–417.
2. Ferrari, A., N. Pacini, E. Canzi, and R. Brano. 1980. Prevalence of O_2-intolerant microorganisms in primary bile acid dehydroxylating mouse intestinal microflora. *Curr. Microbiol.* **4:** 257–260.
3. Stellwag, E. J., and P. B. Hylemon. 1979. 7α-Dehydroxylation of cholic acid and chenodeoxycholic acid by *Clostridium leptum*. *J. Lipid Res.* **20:** 325–333.
4. Berr, F., G. Kulak-Ublick, G. Paumgartner, W. Münzing, and P. B. Hylemon. 1996. 7α-Dehydroxylating bacteria enhance deoxycholic acid input and cholesterol saturation of bile in patients with gallstones. *Gastroenterology.* **111:** 1611–1620.
5. Hylemon, P. B., P. D. Melone, C. V. Franklund, E. Lund, and I. Björkhem. 1991. Mechanism of intestinal 7α-dehydroxylation of

cholic acid: evidence that allo-deoxycholic acid is an inducible side-product. *J. Lipid Res.* **32:** 89–96.
6. Mallonee, D. H., and P. B. Hylemon. 1996. Sequencing and expression of a gene encoding a bile acid transporter from *Eubacterium sp.* strain VPI 12708. *J. Bacteriol.* **178:** 7053–7058.
7. Mallonee, D. H., J. L. Adams, and P. B. Hylemon. 1992. The bile acid-inducible *baiB* gene from *Eubacterium sp.* strain VPI 12708 encodes a bile acid-coenzyme A ligase. *J. Bacteriol.* **174:** 2065–2071.
8. Mallonee, D. H., M. A. Lijewski, and P. B. Hylemon. 1995. Expression in *Escherichia coli* and characterization of a bile acid-inducible 3α-hydroxysteroid dehydrogenase from *Eubacterium sp.* strain VPI 12708. *Curr. Microbiol.* **30:** 259–263.
9. Dawson, J. A., D. H. Mallonee, I. Björkhem, and P. B. Hylemon. 1996. Expression and characterization of a C_{24} bile acid 7α-dehydratase from *Eubacterium sp.* strain VPI 12708 in *Escherichia coli*. *J. Lipid Res.* **37:** 1258–1267.
10. Mallonee, D.H., W. B. White, and P. B. Hylemon. 1990. Cloning and sequencing of a bile acid-inducible operon from *Eubacterium sp.* strain VPI 12708. *J. Bacteriol.* **172:** 7011–7019.
11. Shah, P. P., and E. Staple. 1968. Synthesis of coenzyme A esters of some bile acids *Steroids.* **12:** 571–576.
12. White, W. B., J. P. Coleman, and P. B. Hylemon. 1988. Molecular cloning of a gene encoding a 45,000-dalton polypeptide associated with bile acid 7-dehydroxylation in *Eubacterium sp.* strain VPI 12708. *J. Bacteriol.* **170:** 611–616.
13. Hanes, C. S. 1932. CLXVII. Studies on plant amylases. I. The effect of starch concentration upon the velocity of hydrolysis by the amylase of germinated barley. *Biochem. J.* **26:** 1406–1421.
14. Eneroth, P. 1963. Thin-layer chromatography of bile acids. *J. Lipid Res.* **4:** 11–16.
15. Eichler, K., W-H. Schunck, H-P. Kleber, and M-A. Mandrand-Berthelot. 1994. Cloning, sequencing, and expression of the *Escherichia coli* gene encoding carnitine dehydratase. *J. Bacteriol.* **176:** 2970–2975.
16. Hardie, D. G., K. B. Dewart, A. Aitben, and A. D. McCarthy. 1985. Amino acid sequence around the reactive serine residue of the thioesterase domain of rabbit fatty acid synthase. *Biochim. Biophys. Acta.* **828:** 380–382.
17. Randhawa, Z. I., J. Naggert, R. W. Blacher, and S. Smith. 1987. Amino acid sequencing of the serine active-site region of the medium-chain S-acyl fatty acid synthetase thioester hydrolase from rat mammary gland. *Eur. J. Biochem.* **162:** 577–581.
18. Tai, M-H., S. S. Chirala, and S. J. Wakil. 1993. Roles of Ser101, Asp236, and His237 in catalysis of thioesterase II and the C-terminal region of the enzyme in its interaction with fatty acid synthase. *Proc. Natl. Acad. Sci. USA.* **90:** 1852–1836.
19. Witkowski, A., J. Naggert, H. E. Witkowska, Z. I. Randhawa, and S. Smith. 1992. Utilization of an active serine 101-cysteine mutant to demonstrate the proximity of the catalytic serine 101 and histidine 237 residues in thioesterase II. *J. Biol. Chem.* **267:** 18488–18492.
20. Mack, M., and W. Buckel. 1997. Conversion of glutaconate CoA-transferase from *Acidaminococcus fermentans* into an acyl-CoA hydrolase by site-directed mutagenesis. *FEBS Lett.* **405:** 209–212.
21. Bodnar, A. G., and R. A. Rachubinski. 1990. Cloning and sequence determination of cDNA encoding a second rat liver peroxisomal 3-keotacyl-CoA thiolase. *Gene.* **91:** 193–199.
22. Sanjanwala, M., G. Y. Sun, and R. A. MacQuarrie. 1987. Purification of long-chain acyl-CoA hydrolase from bovine heart microsomes and regulation of activity by lysophopholipids. *Arch Biochem. Biophys.* **258:** 299–306.
23. Lin, C. Y., and S. Smith. 1978. Properties of the thioesterase component obtained by limited trypsinization of the fatty acid synthetase multienzyme complex. *J. Biol. Chem.* **253:** 1954–1962.
24. Dunaway-Marino, D., and P. C. Rabbitt. 1994. On the origins and functions of the enzymes of the 4-chlorobenzoate to 4-hydroxybenzoate converting pathway. *Biodegradation.* **5:** 259–276.
25. Ferri, S. R., and E. A. Meighen. 1991. A lux-specific myristoyl transferase in luminescent bacteria related to eukaryotic serine esterase. *J. Biol. Chem.* **266:** 12852–12857.
26. Donadio, S., M. J. Staver, J. B. McAlpine, S. J. Swanson, and L. Katz. 1991. Modular organization of genes required for complex polyketide biosynthesis. *Science.* **252:** 675–679.
27. Camp, L. A., and S. L. Hofmann. 1993. Purification and properties of a palmitoyl-protein thioesterase that cleaves palmitate from H-ras. *J. Biol. Chem.* **268:** 22566–22574.
28. Cho, H., and J. E. Cronan. 1993. *Escherichia coli* thioesterase I, molecular cloning and sequencing of the structural gene and identification as a periplasmic enzyme. *J. Biol. Chem.* **268:** 9238–9245.
29. Reichel, C. R., R. Brugger, H. Bang, G. Geisslinger, and K. Brune. 1997. Molecular cloning and expression of a 2-arylpropionyl-coenzyme A epimerase: a key enzyme in the inversion metabolism of ibuprofen. *Mol. Pharmacol.* **51:** 576–582.