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Molecular Cloning of Bile Acid 7-Dehydroxylase from *Eubacterium* sp. Strain VPI 12708

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***Eubacterium* sp. strain VPI 12708 is a human intestinal bacterium which contains an inducible bile acid 7-dehydroxylase. Two-dimensional polyacrylamide gel electrophoresis showed that at least four new polypeptides were synthesized after exposure of growing cells to sodium cholate. One of these, of molecular weight 27,000 (PP-27), was implicated in 7-dehydroxylase catalysis. PP-27 was purified to >95% homogeneity by DEAE-cellulose chromatography, high-pressure liquid chromatographic gel filtration, high-pressure liquid chromatography-DEAE chromatography, and preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The first 33 amino acid residues of the N terminus of PP-27 were determined with a gas-phase sequencer, and a corresponding mixed oligonucleotide (17-mer) was synthesized. Southern blot analysis of *Eco*RI total digests of chromosomal DNA showed a 2.2-kilobase fragment which hybridized to the ^{32}P-labeled 17-mer. This fragment was enriched for by size fractionation of an *Eco*RI total digest of genomic DNA, ligated into the bacterial plasmid pUC8, and used to transform *Escherichia coli* HB101. Transformants containing the putative 7-dehydroxylase gene were detected with the ^{32}P-labeled 17-mer by colony hybridization techniques. The insert was 2.2 kilobases in length and contained the first 290 bases of the PP-27 gene. Preliminary nucleic acid sequence data correlate with the amino acid sequence. The entire gene was cloned on a 1,150-base-pair *Taq*I fragment. Western blot analysis of *E. coli* strains containing these plasmids indicated that PP-27 is expressed in *E. coli* but is not regulated by bile acids under the conditions used.**

Eubacterium sp. strain VPI 12708 is an intestinal anaerobic bacterium that possesses bile acid 7-dehydroxylase activity which is induced by culturing in the presence of C_{24} bile acids containing a 7α-hydroxy group. The presence of this activity in the intestinal microflora results in the 7α-dehydroxylation of cholic acid and chenodeoxycholic acid, yielding deoxycholic acid and lithocholic acid, respectively. These two secondary bile acids differ markedly from their 7α-hydroxylated precursors in physiochemical properties as well as physiological effects (1, 4, 8, 9, 14, 17, 18, 20), and they make up approximately 20 to 25% of the total biliary bile acid pool in humans (22). Because the 7α-dehydroxylation reaction is an important physiological reaction in the intestinal ecosystem, the properties of this enzyme and the manner of regulation of its synthesis are of considerable interest.

The induction of 7α-dehydroxylase activity in *Eubacterium* sp. strain VPI 12708 coincides with the appearance of at least four new polypeptides with molecular weights of 23,500, 27,000, 45,000, and 77,000 (19, 29). Immunoprecipitation and immunoinhibition experiments have implicated the 27,000-dalton polypeptide as the major catalytic component of bile acid 7α-dehydroxylase (19). As an initial step to studies of the regulation of bile acid 7-dehydroxylase expression and activity, this report describes the cloning of the gene encoding the 27,000-dalton polypeptide. This represents the first report of the cloning of a gene involved in bile acid metabolism as well as the first cloned gene from a *Eubacterium* species.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and media. *Eubacterium* sp. strain VPI 12708 was grown as described previously (24). *Escherichia coli* HB101 (3), JM105 (32), and DH5α (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were used as host strains. *E. coli* strains were grown on LB broth (15) or LB broth supplemented with ampicillin at 100 μg/ml for selecting transformants. The plasmid used for cloning was pUC8 (26).

Purification of 27,000-dalton polypeptide from *Eubacterium* sp. strain VPI 12708. The purification of the 27,000-dalton polypeptide was a modification of a previously described method for production of the polypeptide for antibody generation (19). The presence of the polypeptide at all stages of the purification was monitored by indirect enzyme-linked immunosorbent assays (27). Alternatively, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (29) followed by Western blotting (25) was used to detect the 27,000-dalton polypeptide. Anti-27,000-dalton polypeptide immunoglobulin G was prepared as described by Paone and Hylemon (19). The second antibody used in both techniques was goat anti-rabbit antibody conjugated to horseradish peroxidase (Bio-Rad Laboratories, Richmond, Calif.). Cell extract protein concentrations were determined by the method of Kalb and Bernlohr (7).

Eubacterium sp. strain VPI 12708 (20 g [wet weight]) grown anaerobically in medium containing 0.1 mM sodium cholate was suspended in 60 ml of 50 mM sodium phosphate (pH 6.8) containing 0.5 mM phenylmethylsulfonyl fluoride and 0.1 mg each of DNase and RNase A. The cells in suspension were disrupted by two passages through a French pressure cell (16,000 lb/in^2). Unbroken cells and

debris were removed by centrifugation at 27,000 × *g* for 30 min at 4°C. The supernatant fluid was removed and adjusted to 35% saturation with solid ammonium sulfate. After equilibration for 1 h at 4°C, the precipitate was removed by centrifugation at 12,000 × *g* for 10 min. The supernatant fluid was adjusted to 55% saturation with ammonium sulfate. After equilibration and centrifugation as above, the pellet was suspended in 25 ml of 50 mM sodium phosphate (pH 6.0) (buffer A) containing 0.5 mM phenylmethylsulfonyl fluoride and dialyzed overnight at 4°C against 4 liters of this buffer. The dialyzed extract was added to 10 g (dry weight) of DE-52 cellulose equilibrated with buffer A. After equilibration for 1 h at 25°C, the DEAE-cellulose was pelleted by centrifugation at 3,000 × *g* for 5 min. The DEAE-cellulose was then treated consecutively with 50-ml aliquots of buffer A containing increasing amounts of NaCl (0.1, 0.15, 0.2, 0.3, and 0.5 M). Each buffer was equilibrated with the ion exchanger for 1 h at 25°C before centrifugation. The 0.3 M NaCl wash was brought to 75% saturation with solid ammonium sulfate. After equilibration as before, the precipitate was removed by centrifugation (12,000 × *g*, 10 min) and dissolved in a small volume of 50 mM sodium phosphate–100 mM sodium chloride (buffer B). This material was injected over a Spherogel-TSK 3000 SW gel filtration column (60 by 0.75 cm) fitted with a precolumn (10 by 0.75 cm) which had been equilibrated with buffer B at a flow rate of 0.85 ml/min. Fractions (0.85 ml) containing the 27,000-dalton polypeptide (fractions 23 and 24) were combined and dialyzed against 1 liter of 50 mM sodium phosphate (pH 6.0) before being applied to an Altex DEAE 545 high-pressure liquid chromatography column (15 by 0.75 cm) equilibrated with this same buffer at a flow rate of 0.85 ml/min. Protein was eluted with a linear salt gradient of 0 to 0.5 M NaCl over 100 min. Fractions (0.85 ml) were collected, and those containing 27,000-dalton polypeptide (28 to 43) were combined, concentrated by ultrafiltration with an Amicon stirred cell with a YM-10 membrane, and dialyzed overnight against 2 liters of 10 mM sodium phosphate (pH 6.8). To the dialyzed material were added SDS and 2-mercaptoethanol to a final concentration of 1% each. After heating at 100°C for 5 min, the solution was loaded onto a 1.5-mm-thick SDS-polyacrylamide gel (7 to 20% polyacrylamide gradient). Electrophoresis was complete when the tracking dye (bromphenol blue) had migrated to the bottom of the gel, using the Laemmli buffer system (10). The gel was removed, and a small strip was stained with Coomassie blue to determine the position of the 27,000-dalton polypeptide. The region of the gel containing the polypeptide was excised, and electroelution was performed as described by Hashizume et al. (6). The eluted protein was dialyzed against several changes of high-pressure liquid chromatography grade water containing 0.01% SDS. The material was then lyophilized before N-terminal sequence analysis.

Polypeptide sequencing. The N-terminal sequence analysis was performed by Applied Biosystems (Foster City, Calif.).The protein (approximately 40 μg) was dissolved in 90 μl of 1.5% acetic acid and applied directly to a model 470 A gas-phase protein sequencer.

Oligonucleotide synthesis. Oligonucleotides were synthesized with an Applied Biosystems model 380A DNA synthesizer. The oligonucleotides were deprotected by incubation at 55°C for 6 h in 1 ml of concentrated ammonia. After lyophilization to dryness the material was dissolved in 1 ml of sterile water, and 250 μl (approximately 200 μg) was streaked and chromatographed on a silica gel plate (250-μm-thick layer with fluorescent indicator; J. T. Baker Diagnostics, Bethlehem, Pa.) in a solvent system consisting of *n*-propanol–concentrated ammonia–water (55:35:10, vol/vol/vol). The oligonucleotide bands were visualized under shortwave UV light, and the band closest to the origin was scraped off and extracted twice with 1 ml of sterile water. The nucleotide concentration was calculated from the A_{260}.

Southern blotting and colony hybridizations. DNA restriction fragments from agarose gels were transferred to nitrocellulose as described by Southern (23). Transformed colonies on agar plates were transferred to nitrocellulose filters as described by Maniatis et al. (15), and the colonies were lysed in situ as described by Berent et al. (2). Baked filters were washed, prehybridized, and hybridized as described by Woods (31). Hybridizations were done at 37°C with 10^6 cpm of ^{32}P-labeled probe per ml (>10^9 cpm/μg).

Labeling of oligonucleotides. Oligonucleotides were labeled with [γ-^{32}P]ATP (>3,000 Ci/mmol) with T4 polynucleotide kinase as described by Maniatas et al. (15). Unincorporated label was removed with Nensorb 20 cartridges (New England Nuclear Corp., Boston, Mass.) according to the instructions of the manufacturer.

Recombinant DNA methods. *Eubacterium* sp. strain VPI 12708 DNA was purified by the method of Marmur (16). Plasmid DNA was isolated (mini- and large scale) by the Ish-Horowitz modification of the method of Birnboim and Doly (15). Highly purified plasmids were obtained by CsCl gradient centrifugation (15). Restriction digests, ligations, and electrophoresis of DNA were carried out according to the suggestions of the enzyme suppliers and of Maniatis et al. (15). *Eubacterium* sp. strain VPI 12708 genomic DNA digested with *Eco*RI was fractionated by electrophoresis on

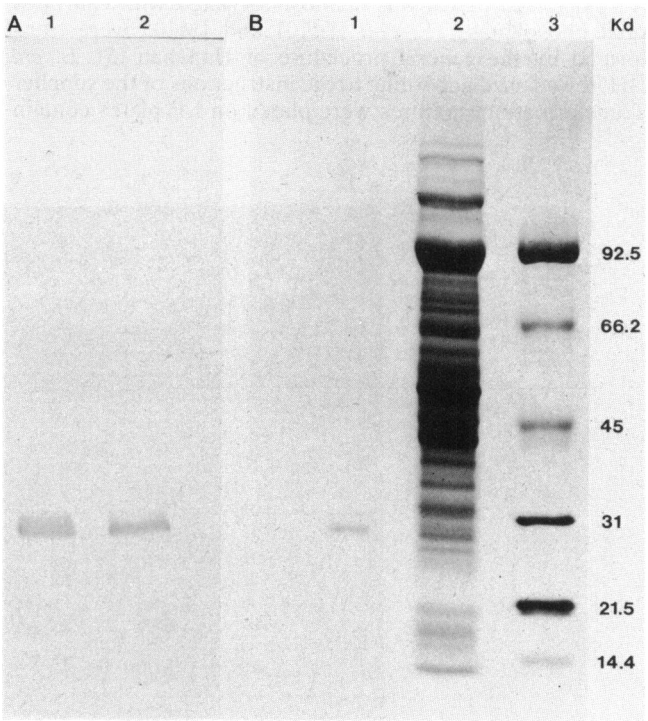

FIG. 1. Purification of the 27,000-dalton polypeptide. (A) Western blot of SDS-PAGE-separated protein with anti-27,000-dalton polypeptide immunoglobulin G. Lanes: 1, purified polypeptide, 0.5 μg; 2, cholic acid-induced *Eubacterium* sp. strain VPI 12708 extract, 50 μg. (B) Coomassie blue stain of SDS-PAGE-separated protein. Lanes: 1, purified polypeptide, 2 μg; 2, sodium cholate-induced *Eubacterium* sp. strain VPI 12708 extract, 50 μg; 3, molecular size markers. Molecular sizes are indicated in kilodaltons (Kd).

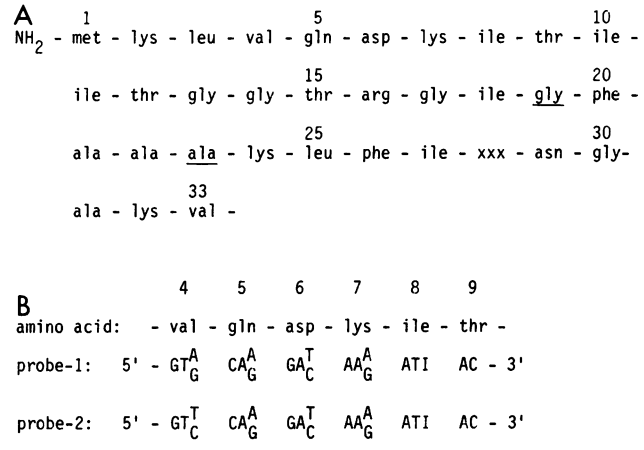

FIG. 2. N-terminal amino acid sequence and corresponding synthetic oligonucleotide sequence for 27,000-dalton polypeptide. (A) Amino acid sequence of residues 1 to 33. Underlined residues or xxx indicate residues not accurately determined by gas-phase sequencing owing to high background. (B) Synthetic oligonucleotide sequences based on amino acids 4 to 9. Each probe was 17 bases long and of 16-fold degeneracy with one inosine residue.

1% low-melting-point agarose, and fragments in the desired size range were extracted by the procedure of Langridge et al. (11). *Taq*I-digested genomic DNA was fractionated on 1.5% agarose, and fragments in the desired size range were isolated by electroelution onto DEAE membranes (NA-45; Schleicher & Schuell, Inc., Keene, N.H.) (13). *Eco*RI- and *Acc*I-digested pUC8 was dephosphorylated with calf intestinal phosphatase. *E. coli* HB101 and JM105 were transformed by the general procedure of Hanahan (5). *E. coli* DH5α was used according to the instructions of the supplier. Transformation mixtures were plated on LB plates containing ampicillin (100 μg/ml) and, for strains JM105 and DH5α, isopropyl-β-D-thiogalactopyranoside and 5-bromo-4-chloro-3-indoyl-β-galactoside at final concentrations of 50 mM and 0.005% (wt/vol), respectively.

DNA sequencing. DNA sequences were obtained by the dideoxy sequencing method (21) with linearized plasmid as described by Wallace et al. (28). DNA was labeled with ^{35}S-dATP (>600 Ci/mmol; New England Nuclear Corp.) during dideoxy sequencing.

Expression of *Eubacterium* genes in *E. coli*. Recombinant *E. coli* strains were grown in anaerobic brain heart infusion medium with and without sodium cholate (0.1 mM) to the late log phase. The cells were harvested by centrifugation and washed once with phosphate-buffered saline. The cells were suspended in 1 volume of 100 mM sodium phosphate (pH 7.0) containing 1% (wt/vol) SDS and 1% (vol/vol) 2-mercaptoethanol and lysed by incubation in a boiling water bath for 5 min. Cell debris was removed by centrifugation, and the supernatant was analyzed by electrophoresis on 5 to 20% polyacrylamide gradient gels. The presence of cross-reacting material was determined by Western blotting with the specific antibody (19).

RESULTS

Purification of 27,000-dalton polypeptide. The purification procedure through the high-pressure liquid chromatography-DEAE step resulted in a preparation of 27,000-dalton polypeptide which contained several minor contaminating proteins, as judged by SDS-PAGE. These were effectively removed by preparative SDS-PAGE and electroelution. SDS-PAGE of this material followed by Coomassie blue staining showed a single protein band which comigrated with the 27,000-dalton polypeptide present in crude extracts as judged by immunoblotting of the SDS-PAGE-separated material (Fig. 1).

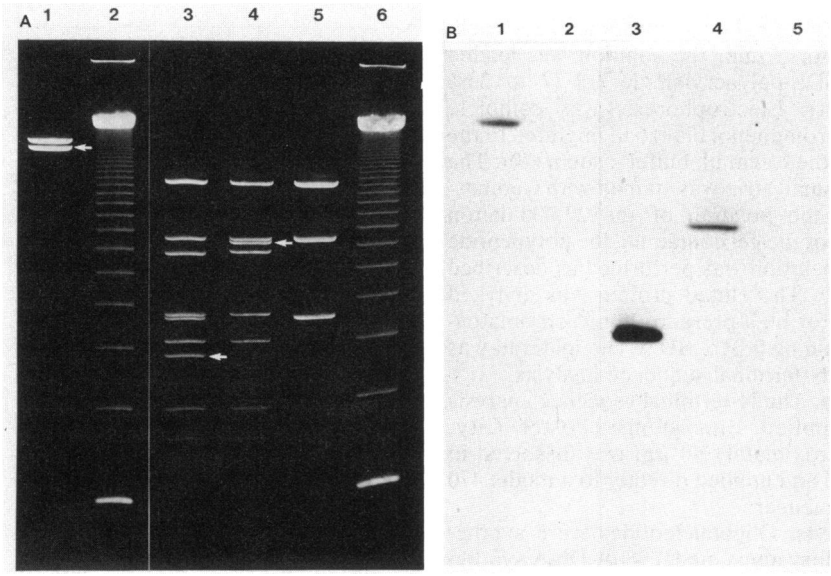

FIG. 3. Restriction fragments of pUC8-22. Restriction enzyme-digested plasmids were run on 5% polyacrylamide slabs (1.5 by 100 mm). After staining with ethidium bromide the gels were electroblotted onto nylon membranes (Gene Screen; New England Nuclear Corp.) according to the instructions of the manufacturer. The membranes were then hybridized with oligonucleotide probe mixture 1. (A) Ethidium bromide-stained gel. Lanes: 1, pUC8-22, *Eco*RI digested; 2, 123-bp ladder; 3, pUC8-22, *Eco*RI-*Taq*I digested; 4, pUC8-22, *Taq*I digested; 5, pUC8, *Taq*I digested; 6, 123-bp ladder. The arrows indicate the bands which hybridize to probe mixture 1. (B) Autoradiogram of probe-hybridized membrane. Lanes: 1, pUC8-22, *Eco*RI digested; 2, 123-bp ladder; 3, pUC8-22, *Eco*RI-*Taq*I digested; 4, pUC8-22, *Taq*I digested; 5, pUC8, *Eco*RI digested.

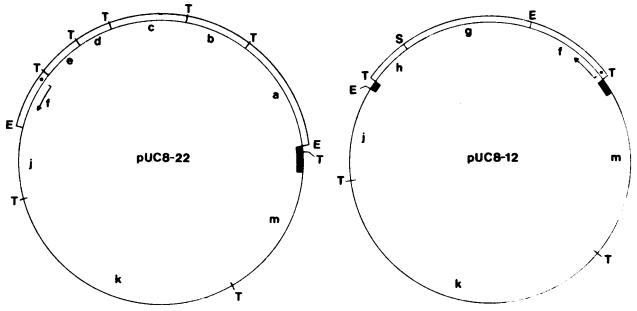

FIG. 4. Partial mapping of plasmids pUC8-22 and pUC8-12. The solid line indicates the vector (pUC8) DNA. The open regions indicate the 2,200-bp insert (pUC8-22) or the 1,150-bp insert (pUC8-12). The dark shaded region indicates the position of the multiple cloning site. The solid dot and arrow refer to the position of the initiation codon and direction of transcription, respectively, for the 27,000-dalton polypeptide reading area. Sizes of fragments are as follows: a, 715 bp; b, 380 bp; c, 465 bp; d, 185 bp; e, 235 bp; f, 345 bp; g, 565 bp; h, 245 bp; j, 455 bp; k, 1,440 bp; m, 807 bp. Restriction enzyme sites are indicated as follows: E, *Eco*RI; T, *Taq*I; S, *Sma*I.

N-terminal sequence analysis and synthesis of corresponding oligonucleotides. The first 33 amino acids of the 27,000-dalton polypeptide were determined by gas-phase sequencing (Fig. 2). Two 17-mer oligonucleotide mixtures, each of 16-fold degeneracy, were synthesized, corresponding to amino acid residues 4 through 9 (Fig. 2). Inosine was incorporated into each at the third position of the isoleucine codon.

Hybridization of oligonucleotide to *Eubacterium* sp. strain VPI 12708 DNA. Genomic DNA prepared from *Eubacterium* sp. strain VPI 12708 was digested with different restriction enzymes, electrophoresed on 0.8% agarose, transferred to nitrocellulose, and probed with ^{32}P-labeled oligonucleotides. Discrete bands were observed with *Eco*RI digests (~2.2 kilobases [kb]) and with *Mbo*I digests (~0.5 kb) with probe mixture 1. No hybridization was observed with probe mixture 2.

Cloning of gene for 27,000-dalton polypeptide. Initial cloning experiments were attempted with *Eco*RI total digests. Digested DNA was first size fractionated (see Materials and Methods) and ligated to *Eco*RI-digested and phosphatase-treated pUC8. After transformation into *E. coli* HB101, colonies were screened by in situ lysis on nitrocellulose filters and hybridization with ^{32}P-labeled oligonucleotide probe 1. Four colonies that hybridized strongly were isolated. Recombinant plasmids were isolated from each and digested with *Eco*RI or *Taq*I. Agarose gel electrophoresis indicated that all four recombinant plasmids contained a 2.2-kb insert and that all were in the same orientation. One was selected for further analysis and is designated pUC8-22. Double digests of the plasmid with *Eco*RI and *Taq*I followed by agarose gel electrophoresis and Southern blotting indicated that the hybridization site (and thus the N-terminal region of the polypeptide) was on a 345-base-pair (bp) *Taq*I-*Eco*RI fragment adjoining the vector (Fig. 3). Since the synthetic oligonucleotide probe hybridized to a terminal *Taq*I fragment of the *Eco*RI insert, Southern blotting of *Taq*I partial digests of the 2.2-kb *Eco*RI insert should yield a series of fragments which hybridize to the probe and correspond to the order of the *Taq*I fragments on the 2.2-kb insert. The resulting map is shown in Fig. 4. Because approximately 675 bp are required for coding for a 27,000-dalton polypeptide, the 345-bp *Taq*I-*Eco*RI fragment was subcloned into *Acc*I-*Eco*RI-digested pUC8 and sequenced to determine the reading direction and to confirm the amino acid sequence data. If the gene is read from the 345-bp fragment toward the remainder of the insert, then sufficient coding potential is present. However, if reading is in the opposite direction,

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 TC  GAT  ACG  ATA  CTT  TGG  CAG  ATA  TGA  TAA

GCC  AAA  GGA  AAA  GAA  AGG  AAG  GAA  AAG  TTC

                ---  ---  ---  ---  ---  ---
ATG  AAA  CTT  GTA  CAG  GAC  AAA  ATT  ACA  ATT
met  lys  leu  val  gln  asp  lys  ile  thr  ile      10
met  lys  leu  val  gln  asp  lys  ile  thr  ile

ATC  ACA  GGC  GGA  ACC  CGT  GGA  ATC  GGA  TTC
ile  thr  gly  gly  thr  arg  gly  ile  gly  phe      20
ile  thr  gly  gly  thr  arg  gly  ile  gly  phe

GCA  GCA  GCA  AAA  CTC  TTT  ATT  GAG  AAT  GGA
ala  ala  ala  lys  leu  phe  ile  glu  asn  gly      30
ala  ala  ala  lys  leu  phe  ile  xxx  asn  gly

GCA  AAA  GTC  TCC  ATA  TTT  GGC  GAG  ACC  CAG
ala  lys  val  ser  ile  phe  gly  glu  thr  gln      40
ala  lys  val

GAA  GAG  GTA  GAC  ACA  GCG  CTG  GCT  CAG  TTA
glu  glu  val  asp  thr  ala  leu  ala  gln  leu      50


AAG  GAA  CTC  TAT  CCG
lys  glu  leu  tyr  pro
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FIG. 5. Partial nucleotide sequence of 345-bp *Taq*I-*Eco*RI fragment. The first 233 nucleotides of the *Taq*I-*Eco*RI fragment are indicated as well as the corresponding amino acid sequence. The lower amino acid sequence corresponds to those from the N-terminal sequence analysis data. The nucleotide residues overlined are those present in a subfamily of the probe mixture 1 degenerate mix.

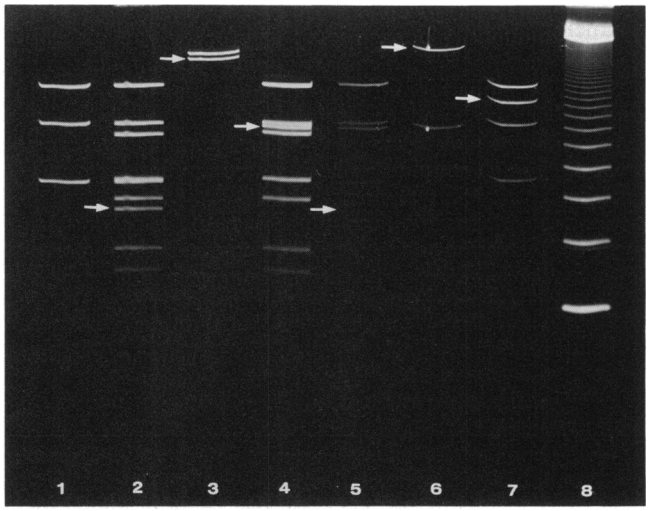

FIG. 6. *Eco*RI-*Taq*I digests of pUC8-22 and pUC8-12. Plasmid (1 μg) was cut with 5 units of enzyme for 1 h before running on 5% polyacrylamide gels (1.5 mm by 10 cm). The arrows indicate the bands which hybridized to the synthetic oligonucleotide probe. Lanes: 1, pUC8 digested with *Eco*RI and *Taq*I; 2, pUC8-22 digested with *Eco*RI and *Taq*I; 3, pUC8-22 digested with *Eco*RI; 4, pUC8-22 digested with *Taq*I; 5, pUC8-12 digested with *Eco*RI and *Taq*I; 6, pUC8-12 digested with *Eco*RI; 7, pUC8-12 digested with *Taq*I; 8, 123-bp ladder.

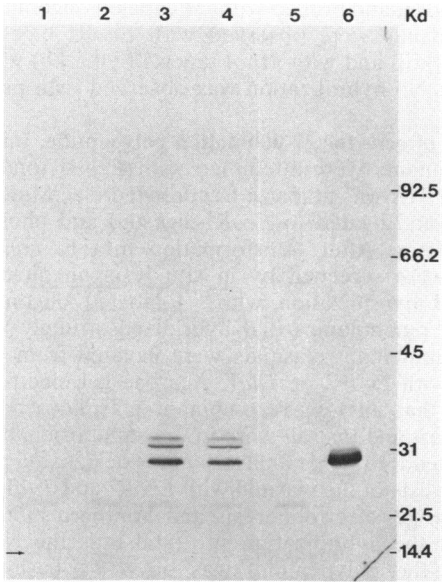

FIG. 7. Expression of 27,000-dalton polypeptide by *E. coli*. Western blots of SDS-PAGE-separated lysates of *E. coli* HB101 containing plasmids were run with anti-27,000-dalton polypeptide immunoglobulin G. Cells were grown anaerobically in brain heart infusion medium with or without sodium cholate (0.1 mM). All lanes contain 50 μg of cell extract protein. Lanes: 1, HB101(pUC8-22), without cholate; 2, HB101(pUC8-22), with cholate; 3, HB101(pUC8-12), without cholate; 4, HB101(pUC8-12), with cholate; 5, HB101(pUC8), with cholate; 6, *Eubacterium* sp. strain VPI 12708, with cholate. Kd, Kilodaltons.

then the gene most likely has an internal *Eco*RI site and therefore only part of the gene was closed. DNA sequence data (Fig. 5) show that the gene had been truncated, with approximately 350 bp missing. Because the 345-bp fragment is bordered on one end by a *Taq*I site, a *Taq*I digest of genomic DNA was Southern blotted to determine the size of fragment hybridizing to the synthetic oligonucleotide probe. The probe hybridized to a DNA fragment corresponding to a molecular size of approximately 1.2 kb. This fragment, which contained sufficient coding potential for a 27,000-dalton polypeptide, was cloned into pUC8 (*Acc*I and phosphatase treated). A single colony was isolated that hybridized to the synthetic oligonucleotide probe. The recombinant plasmid (pUC8-12) had a 1,150-bp insert and yielded a 345-bp fragment when cut with *Eco*RI and *Taq*I (Fig. 6).

Expression of cloned 27,000-dalton polypeptide gene. *E. coli* containing plasmid pUC8-22 produced low levels of a protein with a molecular weight of ~9,000 which was recognized by anti-27,000-dalton polypeptide immunoglobulin G (Fig. 7). The synthesis was independent of the presence of bile acid in the growth medium. *E. coli* containing plasmid pUC8-12 synthesized a protein of the same molecular weight as that from *Eubacterium* and which was also recognized by the specific antibody. As with the truncated gene, synthesis was independent of the presence of bile acid in the growth medium. The presence of two minor bands at higher molecular weights is interesting; however, the nature of these secondary bands is unknown at this time.

DISCUSSION

We have previously shown that cholic acid, but not deoxycholic acid, rapidly induces bile acid 7α-dehydroxylase (30) and NADH:flavin oxidoreductase (12) activities in *Eubacterium* sp. strain VPI 12708. Rabbit antibodies prepared against high-pressure liquid chromatography gel filtration fractions containing 7α-dehydroxylase activity reacted strongly with two bile acid-inducible polypeptides of 27,000 and 45,000 daltons. Specific antibodies were prepared against the 27,000- and 45,000-dalton polypeptides (19). Of these, only the antibodies prepared against the 27,000-dalton polypeptide inhibited enzyme activity in a concentration-dependent manner. These data strongly implicate the involvement of the 27,000-dalton polypeptide in the catalytic function of bile acid 7α-dehydroxylase. To gain further insight into the role of this polypeptide in bile acid 7α-dehydroxylation, we attempted to clone the gene coding for this protein into *E. coli*.

Several lines of evidence show that we indeed cloned the structural gene which codes for the 27,000-dalton polypeptide. First, the N-terminal amino acid sequence (first 33 residues) determined by gas-phase sequencing corresponds exactly to the amino acid sequence derived from the DNA sequence of the cloned DNA fragment. Second, *E. coli* strains harboring the recombinant plasmid pUC8-12 synthesized a 27,000-dalton polypeptide that was recognized by antibodies specific for the *Eubacterium* sp. strain VPI 12708 27,000-dalton protein. Finally, we have recently shown that cholic acid induces, in *Eubacterium* sp. strain VPI 12708, mRNA specific for the 27,000-dalton polypeptide (unpublished data). However, cholic acid does not appear to regulate significantly the amount of the 27,000-dalton polypeptide in *E. coli* (Fig. 7). Whether this is due to differences in RNA polymerase specificities or other factors will require additional study.

Future investigations of the regulation of bile acid 7α-dehydroxylase in *Eubacterium* sp. strain VPI 12708 and other intestinal bacteria are now possible. Moreover, the DNA sequence of the 1,150-bp *Taq*I fragment will yield the complete amino acid sequence of the 27,000-dalton polypeptide and may help define the role of this polypeptide in bile acid 7α-dehydroxylation.

ACKNOWLEDGMENTS

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