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Molecular Cloning of a Gene Encoding a 45,000-Dalton Polypeptide Associated with Bile Acid 7-Dehydroxylation in *Eubacterium* sp. Strain VPI 12708

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***Eubacterium* sp. strain VPI 12708 is an intestinal anaerobic bacterium which possesses an inducible bile acid 7-dehydroxylation activity. Two cholic acid-induced polypeptides with apparent molecular weights of 27,000 and 45,000, respectively, coeluted with bile acid 7-dehydroxylation activity upon anaerobic high-performance gel filtration chromatography of crude cellular protein extracts. The 45,000-dalton polypeptide was purified to >95% homogeneity by high-performance liquid chromatography gel filtration and high-performance liquid-DEAE chromatography. The first 28 amino acid residues of the N terminus of this polypeptide were determined by gas-phase sequencing, and a corresponding mixed oligonucleotide (20-mer) was synthesized. Southern blot analysis of *Eco*RI total digests of chromosomal DNA showed a 2.6-kilobase fragment which hybridized to the ^{32}P-labeled 20-mer. This fragment was enriched for by size fractionation of an *Eco*RI total digest of genomic DNA and ligated into bacteriophage lambda gt11. Recombinant phage containing the putative gene encoding the 45,000-dalton polypeptide were detected with the ^{32}P-labeled 20-mer by plaque hybridization techniques. The insert was 2.6 kilobases in length and may contain the entire coding sequence for the 45,000-dalton polypeptide. The 2.6-kilobase insert was subcloned into pUC8 and transformed into *Escherichia coli* DH5α. However, the 45,000-dalton polypeptide was not detected in cell extracts of this organism when specific antibody was used. Preliminary nucleic acid sequence data correlated exactly with the amino acid sequence. A cholic acid-induced mRNA species of greater than 6 kilobases in size was identified by Northern (RNA) blot analysis of total RNA, suggesting that the gene coding for this polypeptide is part of a larger operon.**

Eubacterium sp. strain VPI 12708 is an intestinal anaerobic bacterium which possesses a cholic acid-inducible bile acid 7-dehydroxylation activity (32). This bacterium thus serves as a model system for studies concerning the mechanism of bile acid 7-dehydroxylation and the mechanism of bile acid-induced enzyme synthesis. Quantitatively, the most important bile acid biotransformation is the 7-dehydroxylation of cholic acid and chenodeoxycholic acid by the intestinal microflora, yielding deoxycholic acid and lithocholic acid, respectively. Deoxycholic acid and lithocholic acid make up to 20 to 25% of the total biliary bile acid pool in humans (26). These two secondary bile acids differ markedly from their 7-hydroxylated precursors in physiochemical properties as well as in physiological effects (1, 3, 9, 10, 14, 18, 20, 22).

Previous studies suggest that bile acid 7-dehydroxylation by *Eubacterium* sp. strain VPI 12708 may involve several polypeptides (21, 30), as well as several enzymatic functions (5, 13, 30, 31, 33). The induction of bile acid 7-dehydroxylation activity by sodium cholate in this bacterium coincides with the appearance of at least four new polypeptides with apparent molecular weights of 23,500, 27,000, 45,000, and 77,000 (21, 30). Immuno-inhibition studies have implicated the 27,000-dalton polypeptide in bile acid 7-dehydroxylation activity (21). Cloning of the gene which encodes the 27,000-dalton polypeptide has been recently reported (6).

In addition to the induction of bile acid 7-dehydroxylation activity, the addition of sodium cholate to the medium of cultures of *Eubacterium* sp. strain VPI 12708 results in the induction of an NADH-flavin oxidoreductase activity (13), a bile acid-Δ^6 reduction activity (30, 33), and a novel bile acid-nucleotide conjugation activity (5). The induced bile acid-Δ^6 reduction and bile acid-nucleotide conjugation (5) activities co-eluted with bile acid 7-dehydroxylation activity upon anaerobic gel filtration high-performance liquid chromatography (HPLC). The product of the bile acid-nucleotide conjugation activity has been identified as 12α-hydroxy-3-oxo-4-cholenoic acid linked at C-24 with an anhydride bond to the β phosphate (5′) of ADP-3′-phosphate (5). This bile acid adenosine nucleotide appears to be an intermediate in bile acid 7-dehydroxylation. A pathway for bile acid 7-dehydroxylation has been proposed based on the structure of the bile acid adenosine nucleotide (5) and literature precedence (19, 23, 27). In this article, we report the cloning and preliminary studies of a gene encoding a cholic acid-induced 45,000-dalton polypeptide which, with the 27,000-dalton polypeptide, coelutes with bile acid 7-dehydroxylation activity.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, plasmids, and media. *Eubacterium* sp. strain VPI 12708 was grown and bile acid 7-dehydroxylation activity was induced as described previously (32). *Escherichia coli* Y1090 (36), JM105 (35), and DH5α (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were used as host strains for bacteriophage lambda gt11, bacteriophage M13mp18, and plasmid pUC8, respectively. *E. coli* Y1090 and DH5α were grown on LB broth (15) or LB broth supplemented with ampicillin at 100 μg/ml. *E. coli* JM105 was grown on YT broth (17).

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Preparation of antibody and Western blot analysis. Antibodies specific against bile acid-induced polypeptides which coelute with bile acid 7-dehydroxylation activity were prepared as described previously (21). Enzymatically active fractions from a Spherogel-TSK 3000SW gel filtration column were used to raise rabbit antibodies. Immune serum was adsorbed with total soluble protein prepared from uninduced cultures of *Eubacterium* strain sp. VPI 12708 coupled to Sepharose 4B. Immunoglobulin specific against cholic acid-induced polypeptides was then purified from preadsorbed immune serum by protein A-Sepharose affinity chromatography (7). This immunoglobulin G preparation was used to detect the 45,000-dalton polypeptide in unfractionated and fractionated soluble protein extracts following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (30) and subsequent Western blotting (immunoblotting) (29). The secondary antibody used 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 (8).

Purification of the 45,000-dalton polypeptide. The 45,000-dalton polypeptide was purified by a previously described method (21), with modifications. Anaerobically grown cells were disrupted by two passages through a French pressure cell (16,000 lb/in^2). An $(NH_4)_2SO_4$ precipitate (35 to 55%) from soluble cellular extracts was applied to a Spherogel-TSK 3000 gel filtration column. One-milliliter fractions containing the 45,000-dalton polypeptide (fractions 19 to 23) were pooled and then applied to an Altex DEAE 545 HPLC column (15 by 0.75 cm) equilibrated in 20 mM sodium phosphate (pH 6.0). Fractions which contained the 45,000-dalton polypeptide, eluted by a gradient of 0 to 500 mM NaCl, were concentrated by ultrafiltration with a YM-10 membrane and again chromatographed on a Spherogel-TSK 3000 gel filtration column. Fractions containing the 45,000-dalton polypeptide were pooled and again chromatographed on an Altex DEAE 545 HPLC column equilibrated with 20 mM sodium phosphate (pH 5.0). Fractions which contained the 45,000-dalton polypeptide, eluted by a gradient of 0 to 500 mM NaCl, were pooled and dialyzed against four changes of HPLC-grade water containing 0.01% SDS. Approximately 50 μg of this protein 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 50 μg) was dissolved in 90 μl of 1.5% acetic acid and applied directly to a model 470A gas-phase protein sequencer.

Oligonucleotide synthesis. Oligonucleotides were synthesized with an Applied Biosystems model 380A DNA synthesizer. Oligonucleotides were deprotected by incubation at 55°C for 6 h in 1 ml of concentrated ammonia and then purified by chromatography 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), as previously described (6).

Southern blotting and plaque hybridizations. DNA restriction fragments from agarose gels were transferred to nitrocellulose as described by Southern (28). Lambda gt11 plaques were transferred to nitrocellulose filters and lysed in situ as described by Benton and Davis (2). Baked filters were prehybridized, hybridized, and washed as described by Woods (34). For phage lambda gt11 screening, filters were hybridized for 36 h with gentle shaking at 42°C. The dried filters were placed in a cassette with Kodak X-RP film and a Du Pont Cronex Lightning-Plus intensifying screen for 72 h at −70°C before the film was developed. All DNA-DNA hybridizations were performed at 42°C with 10^6 cpm of ^{32}P-labeled synthetic oligonucleotide probe per ml (10^9 cpm/μg).

Labeling of oligonucleotides and restriction fragments. Synthetic oligonucleotides were labeled with [γ-^{32}P]ATP (>3,000 Ci/mmol) with T4 polynucleotide kinase as described by Maniatis et al. (15). Gel-purified restriction fragments were nick translated with *E. coli* DNA polymerase I to a specific activity of $>10^8$ cpm/μg, using a nick translation reagent kit (Bethesda Research Laboratories). 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). 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 was digested to completion with *Eco*RI and was then size fractionated on a 1% low-melting-point agarose gel. Fragments in the desired size range were extracted by the procedure of Langridge et al. (11). The cloning of these DNA fragments into lambda gt11 arms was performed by using instructions provided by the supplier of the dephosphorylated *Eco*RI-digested lambda gt11 arms and packaging extracts (Promega Biotech, Madison, Wis.). Lambda DNA was isolated (large scale) by the method of Silhavy et al. (25). 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). *Eco*RI-digested pUC8 was dephosphorylated with calf intestinal alkaline phosphatase (15). For subcloning, *E. coli* DH5α was transformed with either pUC8- or M13mp18-derived recombinant plasmids, using a method provided by the supplier (Bethesda Research Laboratories). M13mp18-derived recombinant phage were produced with *E. coli* JM105, with *E. coli* DH5α as a transient host.

DNA sequencing. Dideoxynucleotide chain termination sequence analysis was performed by the methods of Sanger et al. (24). M13mp18 single-stranded templates were used in conjunction with chemically synthesized oligonucleotides as primers. DNA was labeled with [^{35}S]dATP (>600 Ci/mmol; New England Nuclear Corp.) during dideoxy sequencing.

Isolation of RNA from *Eubacterium* strain sp. VPI 12708 and Northern (RNA) blotting. RNA was isolated from *Eubacterium* sp. strain VPI 12708 by a method previously described for the isolation of RNA from *Clostridium pasteurianum* (12). Total RNA was isolated from exponentially growing cells which were treated with chloramphenicol and lysozyme, in the presence of 60% sucrose. RNA was stored as an ethanol precipitate at −20°C until use.

Samples of 30 μg of RNA were denatured, fractionated on 1% agarose gels containing formaldehyde, and transferred to nitrocellulose as described by Maniatis et al. (15). For 28S and 18S rRNA size markers, rat liver RNA isolated by the method of Chirgwin et al. (4) was used. Blotted RNA was hybridized to nick-translated restriction fragments or synthetic oligonucleotides (17-mers) which had been ^{32}P labeled as described above. Hybridizations with nick-translated restriction fragments as the probe were performed by using the high-stringency conditions described by Silhavy et al. (25) for Southern blot hybridizations. Hybridizations with

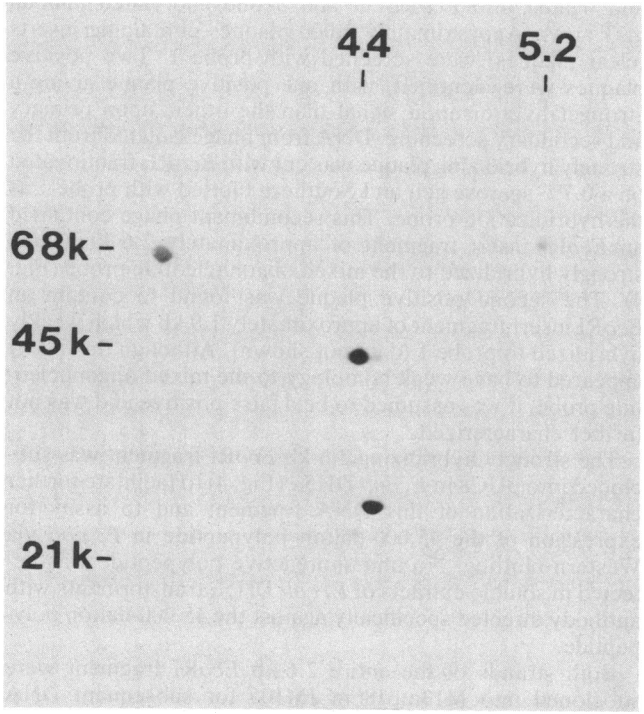

FIG. 1. Western blot of two-dimensional SDS-polyacrylamide gel of total soluble proteins from cholic acid-induced cells of *Eubacterium* strain sp. VPI 12708. The pH range of the isoelectric focusing dimension is given on the abscissa. The molecular weight range ($\times 10^3$) is given on the ordinate.

synthetic oligonucleotides as the probe were performed by the method of Woods (34), as described for Southern blot hybridizations.

RESULTS

Bile acid-induced 45,000-dalton polypeptide coelutes with bile acid 7-dehydroxylase activity. Previously described immunoprecipitation studies suggested that a cholic acid-induced 45,000-dalton polypeptide may play some role in bile acid 7-dehydroxylation (21). The appearance of a second cholic acid-induced polypeptide with apparent molecular weight of 45,000, but a slightly lower isoelectric point, has been observed with two-dimensional SDS-polyacrylamide gel electrophoresis analysis of [^{35}S]-methionine-labeled total proteins from *Eubacterium* strain sp. VPI 12708 (30). It was not clear if one or both of these polypeptides coeluted with bile acid 7-dehydroxylation activity upon HPLC gel filtration of soluble protein extracts. Western blotting of two-dimensional SDS-polyacrylamide gels of total soluble proteins from cholic acid-induced and uninduced cultures was therefore performed. Antibody directed specifically against cholic acid-induced polypeptides present in the 7-dehydroxylation-active HPLC gel filtration fractions was used to immunochemically stain these Western blots.

Western blot analysis indicated that the cholic acid-induced 27,000-dalton polypeptide, which has been previously implicated in bile acid 7-dehydroxylase catalysis (21), and one cholic acid-induced 45,000-dalton polypeptide were present in enzymatically active HPLC gel filtration fractions

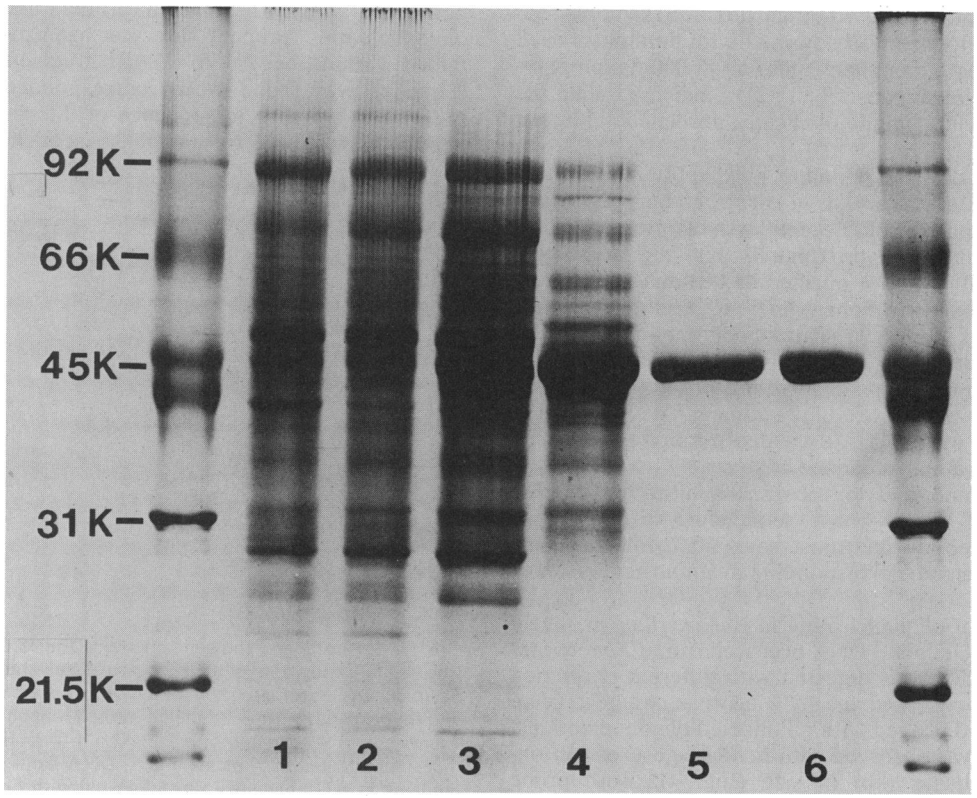

FIG. 2. Purification of the 45,000-dalton polypeptide. SDS-polyacrylamide gel is stained with Coomassie blue. Lane 1, Soluble cell extract (60 μg); lane 2, 35 to 55% ammonium sulfate precipitate (60 μg); lane 3, pooled fractions from Spherogel-TSK 3000SW gel filtration column (60 μg); lane 4, pooled fractions from Altex DEAE 545 column, pH 6.0 (30 μg); lane 5, pooled fractions from Spherogel-TSK 3000SW gel filtration column (20 μg); lane 6, pooled fractions from Altex DEAE 545 column, pH 5.0 (10 μg).

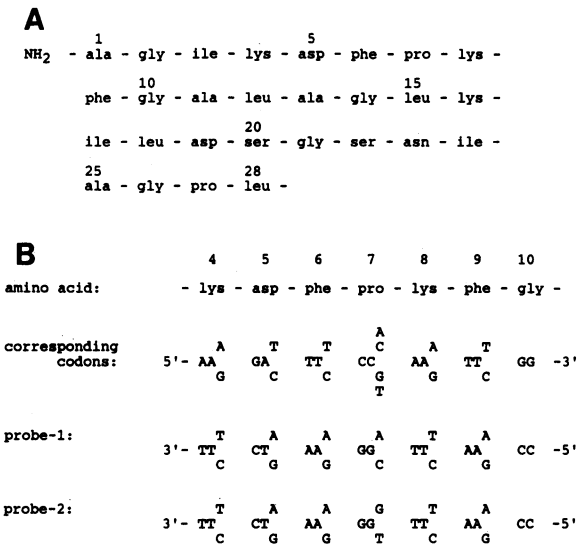

FIG. 3. N-Terminal amino acid sequence and corresponding synthetic oligonucleotide sequence for 45,000-dalton polypeptide. (A) Amino acid sequence of residues 1 through 28. (B) Synthetic oligonucleotide sequences based on amino acids 4 through 9. Each probe was 20 bases long and of 32-fold degeneracy.

(Fig. 1). Very small amounts of the cholic acid-induced 23,500- and 77,000-dalton polypeptides also appeared to be present. The immunoreactive polypeptide with an apparent molecular weight of 68,000 and a pI of <4.25 also appeared on Western blots of soluble proteins from uninduced cultures. Western blots of two-dimensional SDS-polyacrylamide gels immunochemically stained with antibody raised specifically against either the 27,000- or 45,000-dalton polypeptide, as previously described (21), indicated that the two polypeptides are immunologically unrelated (data not shown).

Purification of the 45,000-dalton polypeptide, N-terminal sequence analysis, and synthesis of corresponding oligonucleotides. Unfractionated soluble cell extracts from a 3-liter late-log-phase culture (approximately 600 mg of soluble protein) yielded 0.45 mg of purified 45,000-dalton polypeptide (Fig. 2). The second Spherogel-TSK 3000SW gel filtration step purified the 45,000-dalton polypeptide to >90% homogeneity as judged by SDS-polyacrylamide gel electrophoresis, but two minor bands were present in this preparation as judged by Coomassie blue staining. The final HPLC Altex DEAE 545 step (pH 5.0) yielded purified polypeptide suitable for N-terminal sequence analysis.

The first 28 amino acids of the 45,000-dalton polypeptide were determined by gas-phase sequencing (Fig. 3). Two 20-mer oligonucleotide mixtures, each of 32-fold degeneracy, were synthesized, corresponding to amino acid residues 4 to 10 (Fig. 3).

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 0.7% agarose, transferred to nitrocellulose, and probed with ^{32}P-labeled oligonucleotides. A discrete band was observed with *Eco*RI digests of approximately 2.6 kilobases (kb) in size (Fig. 4). Both probe mixtures yielded identical results.

Cloning of the gene encoding the 45,000-dalton polypeptide. *Eco*RI-digested DNA fragments of approximately 1.5 to 4 kb in size were extracted from a low-melting-point agarose gel and ligated into *Eco*RI-cut and dephosphorylated lambda gt11 arms. Approximately 2,000 plaques containing inserts (clear plaques) were screened with probe 1. Two positive plaques were identified, with one positive plaque giving a stronger hybridization signal than the other, upon primary and secondary screening. DNA from phage isolated from the strongly hybridizing plaque was cut with *Eco*RI, fractionated on a 0.7% agarose gel, and Southern blotted with probe 1 as the hybridization probe. This recombinant phage contained an *Eco*RI insert fragment of approximately 2.6 kb which strongly hybridized to the mixed oligonucleotide probe (Fig. 4). The second positive plaque was found to contain an *Eco*RI insert fragment of approximately 1.9 kb which weakly hybridized to probe 1 (data not shown). Although this insert appeared to have weak homology to the mixed oligonucleotide probe, it was assumed to be a false positive and was not further characterized.

The strongly hybridizing 2.6-kb *Eco*RI fragment was subcloned into pUC8 in *E. coli* DH5α (Fig. 4) to facilitate further characterization of this DNA fragment and to assay for expression of the 45,000-dalton polypeptide in *E. coli* via Western blotting. No immunoreactive polypeptide was detected in soluble extracts of *E. coli* DH5α transformants with antibody directed specifically against the 45,000-dalton polypeptide.

Both strands of the entire 2.6-kb *Eco*RI fragment were subcloned into M13mp18 in JM105 for subsequent DNA sequence analysis to verify that the correct gene had been isolated and to facilitate physical mapping of the 2.6-kb *Eco*RI fragment. *Sau*3A and *Hpa*II subfragments of the 2.6-kb *Eco*RI fragment were also subcloned into M13mp18 in JM105 for sequence analysis. DNA sequence analysis was performed with both universal primers and other synthetic oligonucleotide primers directed against sequences contained within the 2.6-kb *Eco*RI fragment. A nucleotide sequence was found which corresponded exactly to the N-terminal amino acid sequence of the 45,000-dalton polypeptide determined by gas-phase sequencing (Fig. 5). A

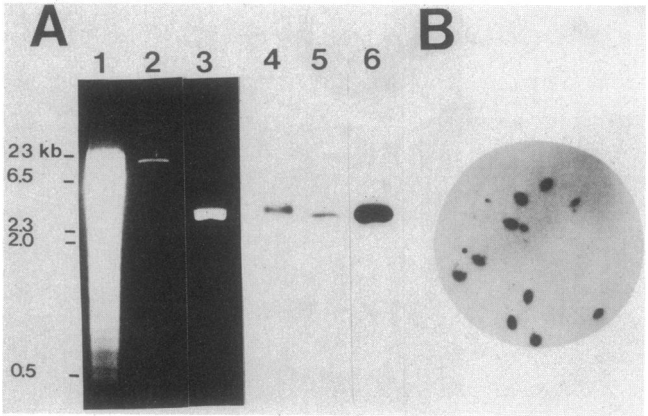

FIG. 4. Cloning of the gene encoding the 45,000-dalton polypeptide. (A) Photograph of 0.7% agarose gel stained with ethidium bromide and autoradiogram of DNA transferred to nitrocellulose and hybridized with ^{32}P-labeled mixed oligonucleotide probe 1. Lanes 1 and 4, *Eubacterium* sp. strain VPI 12708 DNA (15 μg) digested with *Eco*RI; lanes 2 and 5, DNA from positively hybridizing lambda gt11 phage (0.25 μg) digested with *Eco*RI; lanes 3 and 6, positively hybridizing insert fragment from lane 2 subcloned into pUC8 (1 μg) digested with *Eco*RI. (B) Autoradiogram of secondary screening of positively hybridizing lambda gt11 plaque containing 2.6-kb *Eco*RI insert.

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      GTAGAC      GGGGCTTACA      GACCATAATT      TTAATTTTTA
CTAAGTAGAA      TATGTGATAT      AGAAAAGGAG      ATATAAAAAC

ATG   GCT   GGA   ATA   AAA   GAT   TTT   CCA   AAA   TTC
met   ala   gly   ile   lys   asp   phe   pro   lys   phe   10
      ala   gly   ile   lys   asp   phe   pro   lys   phe

GGA   GCT   CTT   GCA   GGG   CTT   AAG   ATA   CTT   GAC
gly   ala   leu   ala   gly   leu   lys   ile   leu   asp   20
gly   ala   leu   ala   gly   leu   lys   ile   leu   asp

AGC   GGA   TCT   AAC   ATC   GCC   GGA   CCT   TTA   GGC
ser   gly   ser   asn   ile   ala   gly   pro   leu   gly   30
ser   gly   ser   asn   ile   ala   gly   pro   leu

GGA   GGC   TTC   TGG   CAG                               35
gly   gly   phe   trp   gln
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FIG. 5. Nucleotide sequence which corresponds to the N-terminal sequence of the 45,000-dalton polypeptide and the upstream flanking sequence. Both strands were sequenced. Only the noncoding strand is shown. The nucleotide residues overlined are those present in a subfamily of the probe mixture 1 degenerate mix. The lower amino acid sequence corresponds to those from the N-terminal amino acid sequence data.

methionine codon was found directly adjacent to the alanine codon which corresponds to the N-terminal amino acid of the 45,000-dalton polypeptide. DNA sequence analysis and restriction mapping techniques (15) were used to determine the orientation and location of the gene which encodes the 45,000-dalton polypeptide on the cloned 2.6-kb *Eco*RI fragment (Fig. 6). This cloned fragment may contain the coding sequence for the entire 45,000-dalton polypeptide.

Identification of the *Eubacterium* mRNA species which encodes the 45,000-dalton polypeptide. The mRNA species which encodes the 45,000-dalton polypeptide was identified by Northern blot analysis of total RNA from *Eubacterium* sp. strain VPI 12708, using two unrelated DNA probes which contained sequences within the coding region for the 45,000-dalton polypeptide (Fig. 7). Both probes hybridized to a cholic acid-induced mRNA species which appeared to be larger than 6 kb in size. There was detectable hybridization in the region of the 23S and 16S rRNAs. However, this was apparent only in RNA prepared from cultures grown in the presence of sodium cholate (Fig. 7B and C, lanes I).

DISCUSSION

Two lines of evidence indicate that we indeed cloned the structural gene which codes for the cholic acid-induced polypeptide with apparent molecular weight of 45,000. First, the N-terminal amino acid sequence (first 28 residues) determined by gas-phase sequencing corresponds exactly to an amino acid sequence derived from the DNA sequence of the cloned DNA fragment (Fig. 5). The isolated protein has an NH_2-terminal alanine, and the deduced protein sequence

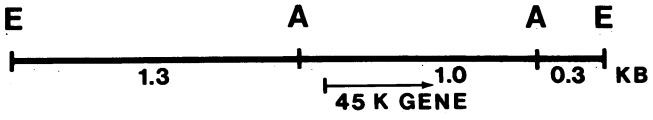

FIG. 6. Partial restriction map of strongly hybridizing, cloned 2.6-kb *Eco*RI fragment. The arrow indicates the location and orientation of the gene encoding the 45,000-dalton polypeptide. Abbreviations: A, *Acc*I; E, *Eco*RI. The *Acc*I site upstream of the gene encoding the 45,000-dalton polypeptide corresponds to the first six bases shown in Fig. 5.

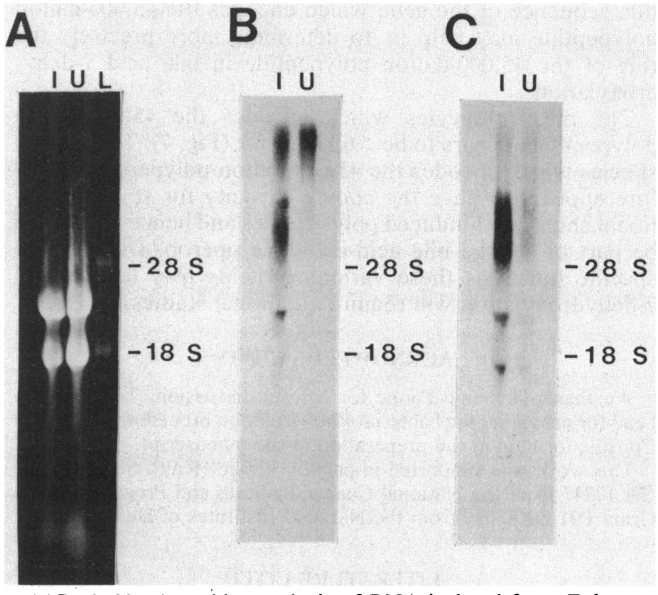

FIG. 7. Northern blot analysis of RNA isolated from *Eubacterium* sp. strain VPI 12708. Abbreviations: I, total RNA isolated from a log-phase cholic acid-induced culture (30 μg); U, total RNA isolated from a log-phase uninduced culture (30 μg); L, rat liver RNA (5 μg). Eucaryotic 28S and 18S rRNA bands are indicated. (A) Formaldehyde–1% agarose gel stained with ethidium bromide. (B) Autoradiogram of RNA transferred to nitrocellulose and hybridized with ^{32}P-labeled, nick-translated, 1.0-kb *Acc*I fragment. (C) Autoradiogram of RNA transferred to nitrocellulose and hybridized with ^{32}P-labeled synthetic oligonucleotide (17-mer), the sequence of which is found on the antisense strand within the 0.3-kb *Acc*I-*Eco*RI fragment, starting 144 bases from the *Eco*RI site. The probe sequence is 5′-CCAAGGCCTGTGATATG-3′.

predicts an alanine following the initiator methionine. Aside from this, the amino acid sequence agrees completely with the known N-terminal sequence of the protein. Second, we were able to identify a cholic acid-induced mRNA species from *Eubacterium* sp. strain VPI 12708 which hybridizes to the cloned gene (Fig. 7). It is not clear why we were not able to detect expression of the cloned gene in *E. coli*. To answer this question will require additional studies.

Several lines of indirect evidence implicate a role for the 45,000-dalton polypeptide in bile acid 7-dehydroxylation. Of the cholic acid-induced polypeptides we have identified by two-dimensional SDS-polyacrylamide gel electrophoresis analysis of total proteins (30), only the 27,000-dalton polypeptide and one 45,000-dalton polypeptide appear to coelute with bile acid 7-dehydroxylation activity (Fig. 1). Antibody specific for the 27,000-dalton polypeptide has been shown to inhibit bile acid 7-dehydroxylation activity in crude protein extracts (21). Antibody specific for the 45,000-dalton polypeptide had no detectable effect on bile acid 7-dehydroxylation activity. However, antibody specific for the 27,000-dalton polypeptide immunoprecipitated both that polypeptide and smaller amounts of the 45,000-dalton polypeptide from crude protein extracts (21). Finally, we have identified a cholic acid-induced bile acid nucleotide conjugation activity which coelutes with bile acid 7-dehydroxylation activity. The structure of the bile acid-nucleotide conjugate strongly suggests a new mechanism for bile acid 7-dehydroxylation (5). This proposed multiple-step mechanism implies the involvement of several polypeptides in bile acid 7-dehydroxylation. Determination of the complete nucleo-

tide sequence of the gene which encodes the 45,000-dalton polypeptide may help us to determine more precisely the role of the 45,000-dalton polypeptide in bile acid 7-dehydroxylation.

The mRNA species which encodes the 45,000-dalton polypeptide appears to be >6 kb in size (Fig. 7). The mRNA species which encodes the 45,000-dalton polypeptide therefore appears to have the coding capacity for several additional cholic acid-induced polypeptides and hence appears to be part of a large bile acid-inducible operon. Defining the specific functions these various proteins play in bile acid 7-dehydroxylation will require additional studies.

ACKNOWLEDGMENTS

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