Mechanism of intestinal 7α-dehydroxylation of cholic acid: evidence that allo-deoxycholic acid is an inducible side-product

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Abstract We previously reported that the 7α-dehydroxylation of cholic acid appears to be carried out by a multi-step pathway in intestinal anaerobic bacteria both in vitro and in vivo. The pathway is hypothesized to involve an initial oxidation of the 3α-hydroxy group and the introduction of a double bond at C4–C5 generating a 3-oxo-4-cholenoic bile acid intermediate. The loss of water generates a 3-oxo-4,6-choldienoic bile acid which is reduced (three steps) yielding deoxycholic acid. We synthesized, in radiolabel, the following putative bile acid intermediates of this pathway 7α,12α-dihydroxy-3-oxo-4-cholenoic acid, 7α,12α-dihydroxy-3-oxo-5β-cholanoic acid, 12α-dihydroxy-3-oxo-4,6-choldienoic acid, and 12α-hydroxy-3-oxo-4-cholenoic acid and showed that they could be converted to 3α,12α-dihydroxy-5β-cholanoic acid (deoxycholic acid) by whole cells or cell extracts of *Eubacterium* sp. VPI 12708. During studies of this pathway, we discovered the accumulation of two unidentified bile acid intermediates formed from cholic acid. These bile acids were purified by thin-layer chromatography and identified by gas-liquid chromatography–mass spectrometry as 12α-hydroxy-3-oxo-5α-cholanoic acid and 3α,12α-dihydroxy-5α-cholanoic (allo-deoxycholic acid). Allo-deoxycholic acid was formed only in cell extracts prepared from bacteria induced by cholic acid, suggesting that their formation may be a branch of the cholic acid 7α-dehydroxylation pathway in this bacterium. —**Hylemon, P. B., P. D. Melone, C. V. Franklund, E. Lund, and I. Björkhem.** Mechanism of intestinal 7α-dehydroxylation of cholic acid: evidence that allo-deoxycholic acid is an inducible side-product. *J. Lipid Res.* 1991. **32:** 89–96.

Supplementary key words *Eubacterium* • secondary bile acids • bile acid 7α-dehydroxylation pathway

Cholic acid and chenodeoxycholic acid are primary bile acids synthesized from cholesterol in the human liver. However, during their enterohepatic circulation, these primary bile acids are 7α-dehydroxylated yielding deoxycholic and lithocholic acid. Deoxycholic acid constitutes 20–25% of the total biliary bile acid pool in humans (1). 7-Dehydroxylated bile acids are much more hydrophobic than their corresponding primary bile acids (2) and have been shown to be more powerful regulators of hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase activities in rats (3).

The mechanism of bile acid 7α-dehydroxylation was initially reported to involve a diaxial *trans* elimination of water (7α-hydroxy, 6β-hydrogen) yielding a Δ^6-bile acid intermediate which is then reduced (4) to the secondary bile acid. However, recent studies in our laboratories strongly suggest that the 7α-dehydroxylation of primary bile acids is a multi-step pathway in the human intestinal *Eubacterium* VPI 12708. We have demonstrated that deoxycholic acid is converted into 12α-hydroxy-3-oxo-4-cholenoic acid while linked to an adenosine nucleotide (5). We proposed that this compound may be an intermediate in bile acid 7α-dehydroxylation. In addition, we showed that [3β-^{3}H]- and [5β-^{3}H]-labeled cholic acid lose the tritium during the conversion into deoxycholic acid both in vitro and in vivo studies in humans (6). Based on this information, we proposed that the mechanism of bile acid 7α-dehydroxylation involves an initial two-step oxidation of the primary bile acid generating a 3-oxo-Δ^4-intermediate. The 7α-hydroxy group would be labilized and the loss of water generating a 3-oxo-4,6-choldienoic

Abbreviations: TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; CA, cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; DCA, deoxycholic acid 3α,12α-dihydroxy-5β-cholanoic acid; 7-oxo-CA, 7-oxo-cholic acid, 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid; 3-oxo-DCA, 3-oxo-deoxycholic acid, 7α-hydroxy-3-oxo-5β-cholanoic acid; 3-oxo-CA, 3-oxo-cholic acid, 7α,12α-dihydroxy-3-oxo-5β-cholanoic acid; 3-oxo-Δ^4CA, 3-oxo-Δ^4-cholic acid, 7α,12α-dihydroxy-3-oxo-4-cholenoic acid; 3-oxo-Δ^4DCA, 3-oxo-Δ^4-deoxycholic acid, 12α-hydroxy-3-oxo-4-cholenoic acid; 3-oxo-$\Delta^{4,6}$DCA, 3-oxo-$\Delta^{4,6}$-deoxycholic acid, 12α-hydroxy-3-oxo-4,6-choldienoic acid; allo-DCA, allo-deoxycholic acid, 3α,12α-dihydroxy-5α-cholanoic acid; allo-3-oxo-DCA, allo-3-oxo-deoxycholic acid, 12α-hydroxy-3-oxo-5α-cholanoic acid.

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bile acid intermediate which is reduced (three steps) would yield deoxycholic acid (6).

In the current communication, we report additional evidence for this pathway and the discovery that allo-deoxycholic acid is formed during the bile acid 7α-dehydroxylation of cholic acid in *Eubacterium* sp. VPI 12708. Allo-bile acids are 5α-cholanoic acids in which the hydrogen at C5 is α-oriented rather than β-oriented. Allo-bile acids are widely distributed in nature and are the predominant bile acids in lower animals and some fishes (7). Small amounts of allo-bile acids have been detected in certain mammals including humans (8, 9).

MATERIALS AND METHODS

Materials

[24-^{14}C]cholic acid (50 mCi/mmol), [24-^{14}C]deoxycholic acid (50 mCi/mmol), and [24-^{14}C]lithocholic acid (50 mCi/mmol) were obtained from Amersham Corp. [24-^{14}C]12α-Hydroxy-3-oxo-5β-cholanoic acid and [24-^{14}C]-7α,12α-dihydroxy-3-oxo-5β-cholanoic acid were enzymatically synthesized from [^{14}C]deoxycholic acid and [^{14}C]cholic acid, respectively, by treatment with 3α-hydroxysteroid dehydrogenase (Sigma Chemical Co., St. Louis, MO). [24-^{14}C]3α,12α-Dihydroxy-7-oxo-5β-cholanoic acid was synthesized from [^{14}C]cholic acid using purified 7α-hydroxysteroid dehydrogenase isolated from *Eubacterium* sp. VPI 12708 (10). [24-^{14}C]12α-Hydroxy-3-oxo-4-cholenoic acid (0.4 mCi/mmol) was synthesized according to Kallner (11) from the above [24-^{14}C]deoxycholic acid. This method involves Oppenhauer oxidation to yield the corresponding 3-oxo-bile acid followed by dehydrogenation with selenium dioxide. The produce obtained had the expected UV-absorbance at 244 nm and a mass spectrum (as methyl ester-trimethylsilyl derivative) identical to that of authentic 12α-hydroxy-3-oxo-4-cholenoic acid (a generous gift from Dr. Anders Kallner). [24-^{14}C]7α,12α-Dihydroxy-3-oxo-4-cholenoic acid (2 mCi/mmol) was synthesized from the above [24-^{14}C]cholic acid according to the same method as for [24-^{14}C]12α-hydroxy-3-oxo-4-cholenoic acid. The final product had the expected UV-absorbance and a mass spectrum (as methyl ester-trimethylsilyl derivative) identical to that of the authentic compound (a generous gift from Dr. Anders Kallner). [24-^{14}C]7α,12α-Dihydroxy-3-oxo-4-cholenoic acid was converted into [24-^{14}C]12α-hydroxy-3-oxo-4,6-choldienoic acid by dehydration with 2 M KOH in methanol (24 h at room temperature). The product obtained had the expected UV-absorbance at 285 nm (corresponding to a 3-oxo-$\Delta^{4,6}$ structure) and a mass spectrum as expected for a C_{24}-bile acid containing one hydroxy group and two double bonds. All the above bile acid intermediates were purified by preparative thin-layer chromatography (in some cases also by HPLC) prior to use and had a purity higher than 98% as judged by gas–liquid chromatography and thin-layer chromatography. All unlabeled bile acid intermediates were synthesized according to the same methods as for the corresponding labeled compounds. Allo-deoxycholate was synthesized according to Kallner (11) and had the expected mass spectrum and chromatographic properties. 12α-hydroxy-3-oxo-5α-cholanoic acid was formed from allo-deoxycholate by treatment with 3α-hydroxysteroid dehydrogenase. Sodium cholate was purchased from Calbiochem. Silica gel 1B thin-layer chromatography plates, *p*-dioxane, and benzene were obtained from J. T. Baker.

Growth of bacteria and preparation of cell extracts

Eubacterium sp. VPI 12708 was cultured under anaerobic conditions as previously described (12), except the growth medium was modified by replacing Brain Heart Infusion media with Tryptic Soy Broth (30 g/l). One liter of culture medium was inoculated with 3 ml of a −70°C, 33% (v/v) glycerol stock grown to late exponential phase (16 h), and diluted in 3 liters of fresh media. Bacterial growth was monitored using a Klett-Summerson colorimeter. Cultures were induced by the addition of 0.1 mM sodium cholate at 60, 90, and 120 Klett units. Cells were harvested at 150 Klett Units (6000 *g*, 4°C, 40 min) and suspended in 4 ml anaerobic phosphate buffer [20 mM sodium phosphate (pH 6.8), 0.5 M NaCl, 5% glycerol (v/v), 10 mM 2-mercaptoethanol]. Cell extracts were prepared by passing the suspension of cells twice through a pre-cooled French Pressure Cell (14,000 psi) and centrifugation at 100,000 *g*, 4°C, 2 h. Protein concentrations of extracts were determined by the method of Bradford (13). Aliquots of extracts were then stored at −70°C until used.

Thin-layer chromatography of bile acid intermediates

Bile acid metabolites were twice extracted from reaction mixtures using two volumes of ethyl acetate, dried under a stream of nitrogen gas, and suspended in 100 μl ethyl acetate. Under our extraction conditions, there was no detectable degradation of bile acid intermediates as determined by TLC and autoradiography. Extracted [24-^{14}C]-labeled bile acids were separated by TLC using silica gel 1 B plates developed in solvent system (S-1) described by Eneroth (14). Bile acids were detected by autoradiography using Kodak XRP film with 24- and 72-h exposures. This film was then scanned using a Shimadzu laser densitometer. Under these conditions, the density of each spot is proportional to the radioactivity of each bile acid metabolite.

High performance liquid chromatography of bile acid intermediates

Radiolabeled bile acids or synthesized intermediates were separated by a C-18 reverse phase HPLC using a

Whatman Partisil 10 ODS-3 column (4.6 mm × 25 cm) and a solvent system of 2-propanol–10 mM potassium phosphate (pH 7.0) 8:17 (v/v). Fractions (0.5 ml) were collected at a flow rate of 1.0 ml/min and radioactivity was determined by liquid scintillation spectrometry.

Gas–liquid chromatography–mass spectrometry

The steroids were methylated with diazomethane in diethyl ether–methanol (9:1 v/v) and trimethylsilylated with pyridine–hexamethylsilazane–trimethylchlorosilane 3:2:1 (v/v) (15). Gas–liquid chromatography–mass spectrometry was performed using an LKB 2091 GLC–MS operating in the electron impact mode at 70 eV. The gas chromatograph was fitted with a 30 m (0.25 mm i.d., 0.25 μM phase) DB-1301 column. The oven temperature was 180°C for 2 min, then programmed at 8°C/min to 290°C.

RESULTS

Accumulation of bile acid intermediates in cell extracts

We previously proposed a multistep pathway for bile acid 7α-dehydroxylation in the intestinal *Eubacterium* sp. VPI 12708 (5, 6). In the present study, we attempted to detect, characterize, and identify the various bile acid intermediates of this putative pathway. The data in **Fig. 1** show the relative migration rates of various bile acid intermediates formed from [4-^{14}C]cholic acid in cell extracts prepared from induced bacterial cultures or uninduced controls. We have previously shown that the 7α-dehydroxylation of primary bile acids is induced in this bacterium when grown in the presence of cholic acid (12). At least five new radiolabeled bile acid intermediates are resolved by TLC and detected under these assay conditions as compared to cell extracts prepared from uninduced cultures (Fig. 1). Essentially the same pattern of bile acid metabolites was observed when broken cells or crude membranes were used as the source of enzymes (data not shown).

Each metabolite was scraped from the TLC plate, extracted with ethyl acetate, and characterized with regard to the following: *1*) TLC and HPLC migration rates compared to known standards (**Table 1**); *2*) individual treatment with stereospecific 3α-, 3β-, 7α-, and 12α-hydroxysteroid oxidoreductase in both the oxidative and reductive direction followed by TLC; and *3*) gas–liquid chromatography–mass spectrometry (GLC–MS) using procedures described in Materials and Methods. The following metabolites were rigorously identified using the above methods: 12α-hydroxy-3-oxo-4-cholenoic acid, 12α-hydroxy-3-oxo-5β-cholanoic acid, 3α,12α-dihydroxy-5β-cholanoic acid, and 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid. However, 7α,12α-dihydroxy-3-oxo-4-cholenoic acid and 12α-hydroxy-3-oxo-4,6-choldienoic acid did not accumulate to significant amounts to allow for detection. In addition, two unknown metabolites were discovered.

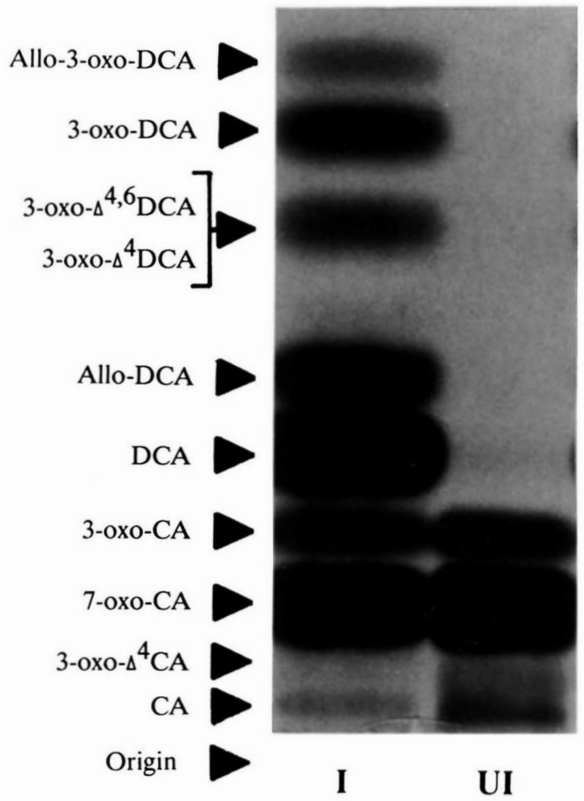

Fig. 1. Thin-layer chromatogram of [^{14}C]cholic acid metabolites formed by cell extracts prepared from induced (I) and uninduced (UI) cultures of *Eubacterium* sp. VPI 12708. Radiolabeled cholic acid (5 × 10^4 dpm, 0.5 nmol) was added to cell extracts, incubated for 15 min, and extracted with ethyl acetate. Radiolabeled metabolites of cholic acid were separated by TLC and detected by autoradiography as described in Materials and Methods. Abbreviations: CA, cholic acid; 3-oxo-Δ^4-7α,12α, 3-oxo-Δ^4-cholic acid; 7-oxo-CA, 7-oxo-cholic acid; 3-oxo-CA, 3-oxo-cholic acid; DCA, deoxycholic acid; allo-DCA, allo-deoxycholic acid; 3-oxo-Δ^{4}DCA, 3-oxo-Δ^4-deoxycholic acid; 3-oxo-DCA, 3-oxo-deoxycholic acid; and allo-3-oxo-DCA, allo-3-oxo-deoxycholic acid. Arrows indicate where various authentic bile acid intermediates migrated in this solvent system.

Identification of unknown metabolites as allo-bile acids

The two unknown bile acid metabolites had TLC and HPLC migration rates similar, but not identical, to deoxycholic acid and 3-oxo-deoxycholic acid (Fig. 1), respectively. Treatment of each metabolite with a panel of hydroxysteroid oxidoreductases also showed that they had the same oxo/hydroxy group profile as deoxycholic acid and 3-oxo-deoxycholic acid (data not shown). Conclusive identification of these bile acid metabolites required GLC–MS. The unknown bile acid metabolite migrating slightly faster than deoxycholic acid on TLC (Fig. 1) had a GLC retention time identical to that of allo-deoxycholic

TABLE 1. TLC and HPLC characteristics of various bile acids and intermediates in 7α-dehydroxylation of cholic acid

Bile Acid Intermediate	TLC[a]	HPLC[b] Elution Volume
	R_f	*ml*
3α,7α,12α-Trihydroxy-5β-cholanoic acid	0.13	6.0
7α,12α-Dihydroxy-3-oxo-4-cholenoic acid	0.34	6.0
3α,12α-Dihydroxy-7-oxo-5β-cholanoic acid	0.47	4.5
7α,12α-Dihydroxy-3-oxo-5β-cholanoic acid	0.61	5.5
3α,12α-Dihydroxy-5β-cholanoic acid	1.0	12.0
12α-Hydroxy-3-oxo-4-cholenoic acid	1.47	5.5
12α-Hydroxy-3-oxo-4,6-choldienoic acid	1.47	5.5
12α-Hydroxy-3-oxo-5β-cholanoic acid	1.74	9.5
3α-Hydroxy-5β-cholanoic acid	2.06	21.0
12α-Hydroxy-3-oxo-5α-cholanoic acid	1.9	7.0
3α,12α-Dihydroxy-5α-cholanoic acid	1.15	11.0

[a]Solvent system: benzene–dioxane–acetic acid 75:20:2 (v/v/v).
[b]Solvent system: 2-propanol–10 mM potassium phosphate (pH 7.0) 160:340 (v/v); flow rate 1 ml/min.

acid (as methyl ester trimethylsilyl derivative). The mass spectrum of the compound was identical to that of the derivative of authentic allo-deoxycholic acid (**Fig. 2**). It should be pointed out that the mass spectrum of methyl allo-deoxycholate (as trimethylsilyl ether) is clearly different from that of the corresponding derivative of deoxycholate with much more prominent ions at *m/z* 345, *m/z* 535 (M–15), *m/z* 460 (M–90), and *m/z* 429 (M–90–31). In contrast, the ion at *m/z* 370 (M–2 × 90) is more intense in the mass spectrum of the derivative of deoxycholate (16).

The bile acid metabolite migrating slightly faster on TLC (Fig. 1) than 3-oxo-deoxycholic acid had a GLC retention time identical to that of allo-3-oxo-deoxycholic acid (as methyl ester trimethylsilyl derivative). Furthermore, the mass spectrum of the compound was identical to that of the derivative of allo-3-oxo-deoxycholic acid (**Fig. 3**). It may be pointed out that the mass spectrum of the isomeric 3-oxo-deoxycholic acid is very similar to that of allo-3-oxo-deoxycholic acid. However, the two compounds separated both on TLC (Fig. 1) and on gas–liquid chromatography.

Metabolism of radiolabeled bile acid intermediates

Various radiolabeled bile acids or synthesized bile acid intermediates (see Materials and Methods) were individually added to cell extracts, incubated for 15 min, extracted, separated by TLC, and quantitated by laser densitometry. The data in **Table 2** show the relative

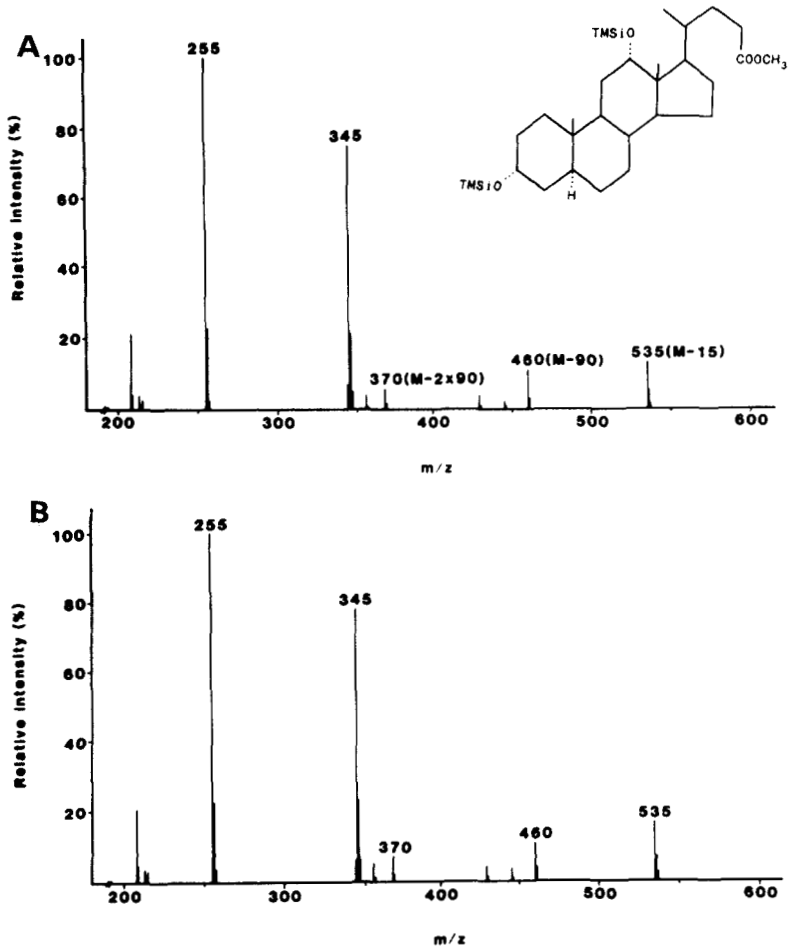

Fig. 2. Mass spectrum of allo-deoxycholic acid standard (A) and unknown bile acid metabolite (B). Cell extracts of *Eubacterium* sp. VPI 12708 were incubated with [^{14}C]cholic acid (5 × 10^4 dpm, plus 50 μM unlabeled) for 15 min; bile acid metabolites were extracted with ethyl acetate and separated by TLC as described in Materials and Methods. Bile acid metabolites were then extracted from TLC plates and subjected to gas–liquid chromatography–mass spectrometry as described in Materials and Methods.

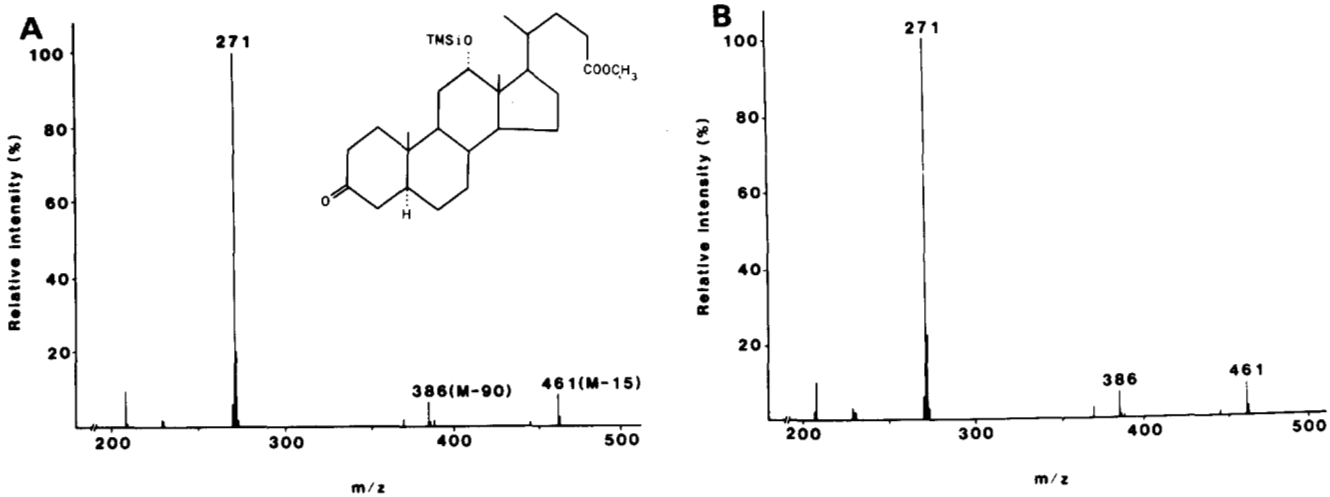

Fig. 3. Mass spectrum of allo-3-oxo-deoxycholic acid standard (A) and unknown bile acid metabolite (B). This metabolite was formed from cholic acid in cell extracts of *Eubacterium* sp. VPI 12708, extracted, and separated by TLC as described in Fig. 2. Gas–liquid chromatography–mass spectrometry was carried out as described in Materials and Methods.

amounts of each produce formed (vertical column) from the bile acid metabolite added (horizontal column). Each metabolite added was capable of being converted to deoxycholic acid by cell extracts prepared from induced cultures. Although only a small amount of 12α-dihydroxy-3-oxo-4,6-choldienoic acid and 12α-hydroxy-3-oxo-4-cholenoic acid was converted to deoxycholic acid in cell extracts, both were quantitatively reduced to deoxycholic acid or allo-deoxycholic acid by whole cells (data not shown). There was no detectable deoxycholic acid formed when these same bile acid intermediates were added to extracts prepared from control cultures. In addition, no deoxycholic acid or allo-deoxycholic acid was formed when these intermediates were added to cell extracts prepared from cultures grown in the presence of deoxycholic acid (data not shown). Therefore, we believe that the formation of allo-deoxycholic acid is induced by cholic acid and is a branch of the cholic acid 7α-dehydroxylation pathway in this bacterium (**Fig. 4**). Moreover these data are all consistent with the pathway proposed in Fig. 4, in that, when each proposed intermediate was synthesized and added to cell extracts or whole cell, it could be converted ultimately to deoxycholic acid.

DISCUSSION

Early studies on the mechanism of 7α-dehydroxylation of cholic acid suggested that the reaction proceeded via a diaxial *trans*-elimination of water (6βH, 7αOH) yielding a Δ^6-steroid intermediate followed by reduction via a transhydrogenation (4). Indeed, studies by White et al. (17) supported this mechanism by showing the reduction of chemically synthesized 3α,12α-dihydroxy-5β-6-cholenoic acid by cell extracts of *Eubacterium* sp. VPI 12708. Surprisingly, the 7α-dehydroxylation of cholic acid and the reduction of 3α,12α-dihydroxy-5β-6-cholenoic acid by cell extracts of this bacterium were highly stimulated by

TABLE 2. Relative amounts of bile acid intermediates formed during 7α-dehydroxylation of cholic acid

Bile Acid Intermediate Formed	Bile Acid or Intermediate Added							
	CA	3-Oxo-CA	3-Oxo-Δ^4CA	7-Oxo-CA	DCA	3-Oxo-$\Delta^{4,6}$DCA	3-Oxo-Δ^4DCA	3-Oxo-DCA
7-Oxo-CA	1	4	<1	1	<1	<1	<1	<1
DCA	54	35	19	60	63	2	7	50
Allo-DCA	4	<1	1	4	12	<1	2	2
3-Oxo-Δ^4DCA[a] 3-Oxo-$\Delta^{4,6}$DCA[a]	9	32	57	8	5	90	74	12
3-Oxo-DCA	24	26	20	20	13	2	10	35
Allo-3-oxo-DCA	6	2	3	4	5	4	2	2
Other (unknown)	2	<1	<1	3	2	<1	5	<1

Approximately 20,000 cpm of each bile acid or intermediate was added to cell extracts, incubated for 15 min, extracted, separated by TLC, exposed to X-ray film, and quantitated by laser densitometry as described in Materials and Methods.

[a]These bile acid intermediates were not resolved by TLC with the solvent system used.

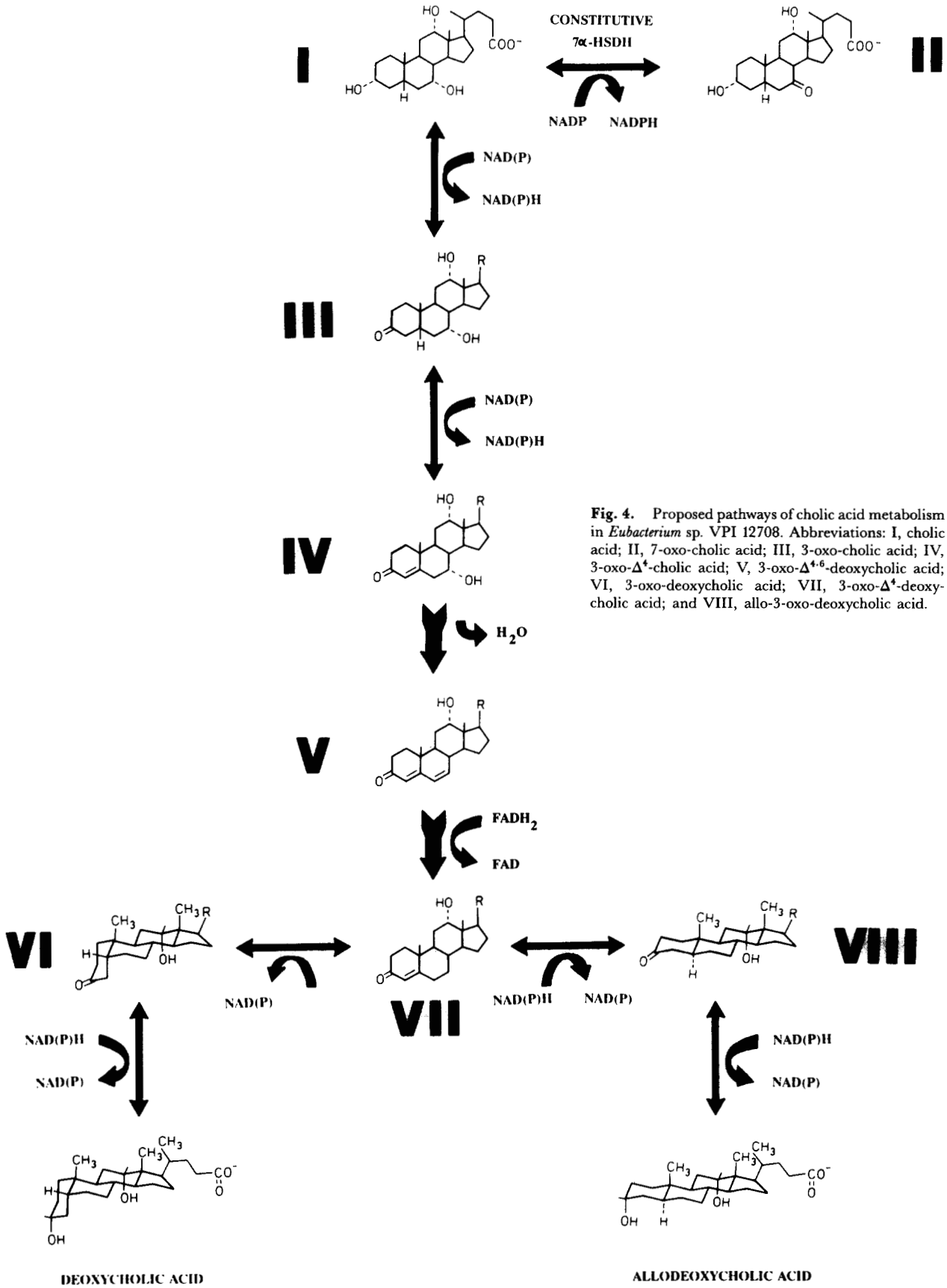

Fig. 4. Proposed pathways of cholic acid metabolism in *Eubacterium* sp. VPI 12708. Abbreviations: I, cholic acid; II, 7-oxo-cholic acid; III, 3-oxo-cholic acid; IV, 3-oxo-Δ^4-cholic acid; V, 3-oxo-$\Delta^{4,6}$-deoxycholic acid; VI, 3-oxo-deoxycholic acid; VII, 3-oxo-Δ^4-deoxycholic acid; and VIII, allo-3-oxo-deoxycholic acid.

the addition of NAD^+. These same biotransformations were inhibited by excess NADH (17). These results were difficult to explain until it was discovered that bile acid modifications were being carried out while the bile acid was linked to an adenosine nucleotide (5). The major bile acid intermediate linked to the adenosine nucleotide was 12α-hydroxy-3-oxo-4-cholenoic acid. These data, in conjunction with studies that show a 7α-hydroxy-3-oxo-4-steroid easily loses the 7α-hydroxy group, allowed us to propose that 7α-dehydroxylation of primary bile acids occurs after two sequential oxidations at the 3α-hydroxy group, as water, then yields a 12α-hydroxy-3-oxo-4,6-choldienoic acid, which is subsequently reduced in three steps (5). Additional evidence for this pathway in *Eubac*-steps (5). Additional evidence for this pathway in *Eubacterium* sp. VPI 12708 and in humans was gained by use of specifically labeled [5β-^{3}H]cholic acid. It was demonstrated that the 5β-^{3}H is differentially lost during the formation of deoxycholic acid, indicating the formation of a 3-oxo-4-steroid intermediate during 7α-dehydroxylation. In the current study, we isolated and rigorously identified several of the putative bile acid intermediates of this pathway. Moreover, we synthesized each proposed intermediate in this pathway and showed that it could be metabolized to deoxycholic acid by either cell extracts or whole cells (Table 2).

The most surprising aspect of the current study was the discovery that bile acid 7α-dehydroxylation can result in the formation of allo-deoxycholic acid. Allo-deoxycholic acid was first discovered in normal rabbits by Kishi (18) in 1936. This bile acid was isolated from rabbit bile by Danielsson, Kallner, and Sjövall (19) in 1963; its structure was confirmed by gas–liquid chromatographic and mass spectrometric data and by partial chemical synthesis. Hofmann and Mosbach (20) reported that allo-deoxycholic acid is a major component of gallstones in rabbits induced by feeding cholestanol. Finally, Kallner (21) showed that allo-deoxycholic acid could be formed from deoxycholic acid after intracecal administration to rats. He proposed that allo-deoxycholic acid was formed by the intestinal microflora. The most unexpected finding in the present study is that the formation of allo-deoxycholic acid appears to be under inductive control by cholic acid in the human intestinal *Eubacterium* sp. VPI 12708. However, it should be emphasized that this may not necessarily apply to all 7α-dehydroxylating intestinal bacteria. We propose that allo-deoxycholic and allo-3-oxo-deoxycholic acid are formed from 12α-hydroxy-3-oxo-4-cholenoic acid, which is an intermediate in the 7α-dehydroxylation pathway by a cholic acid-inducible 12α-hydroxy-3-oxo-5α-hydroxysteroid oxidoreductuase (Fig. 4). An analogous conversion of a 7α-hydroxylated 3-oxo-Δ^4-steroid into a corresponding dehydroxylated 5α-saturated steroid has been reported in connection with formation of cholestanol from an accumulated bile acid intermediate in the rare inborn disease cerebrotendinous xanthomatosis (22, 23). In this conversion the accumulated C_{27}-steroid 7α-hydroxy-4-cholesten-3-one is dehydroxylated in the liver by a microsomal enzyme and the product, cholesta-4,6-dien-3-one, is reduced by microsomal enzymes to give the 5α-saturated end product, cholestanol. In contrast to the analogous intestinal conversion, no 5β-saturated product seems to be formed from the 3-oxo-$\Delta^{4,6}$ steroid in the liver. The present results further suggest that allo-deoxycholic acid is formed in the large bowel of humans during the formation of secondary bile acids. However, the physiological importance of allo-bile acid formation in humans is currently unknown, although these bile acids have very different physicochemical properties than 5β-cholanoic acids (7).

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