

Biotransformation of Bile Acids by *Bacteroides* sp. Strain T-40 Isolated from Human Microflora

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The effects of *Bacteroides* sp. strain T-40 isolated from human feces on the biotransformation of bile acids were examined in an anaerobic culture system. *Bacteroides* sp. T-40 oxidized cholic acid (CA) and chenodeoxycholic acid (CDCA) to 3 α ,12 α -dihydroxy-7-oxo-5 β -cholanoic acid and 3 α -hydroxy-7-oxo-5 β -cholanoic acid, and reduced these oxo-bile acids to CA and CDCA, respectively. However, the reduction activities were lower than the oxidation activities. Hyocholic acid was dehydrogenated, but to a lesser extent than CA or CDCA. On the other hand, α -muricholic acid, which has a hydroxyl group at the position of 7 α , was not dehydrogenated. Glycocholic acid was converted to free 3 α ,12 α -dihydroxy-7-oxo-5 β -cholanoic acid but any glycine conjugated 7-oxo product was not detected. These data indicate that *Bacteroides* sp. T-40 possesses bile acid hydrolase and 7 α -hydroxysteroid dehydrogenase, by which conjugated bile acids are initially deconjugated, and then undergo oxidization of the 7 α -hydroxy group.

Key words: *Bacteroides*; bile acid; biotransformation; dehydrogenase; hydrolase

Ursodeoxycholic acid (UDCA), which is present in small amounts in human bile and intestine, is a useful therapeutic agent for dissolution of cholesterol gallstones (Sugata and Shimizu, 1974) and for treatment and prevention of hepatic diseases (Angulo et al., 1999). It is speculated that UDCA is generated from chenodeoxycholic acid (CDCA) through two-step reaction processes via 3 α -hydroxy-7-oxo-5 β -cholanoic acid (7=O-LCA) (by microbial biotransformation reactions) in hu-

man intestine as shown in Fig. 1. The first step, oxidation of the 7 α -hydroxy group of CDCA, is carried out by 7 α -hydroxysteroid dehydrogenase (7 α -HSDH), and the second step, reduction of the 7-oxo group of 7=O-LCA, by 7 β -hydroxysteroid dehydrogenase (7 β -HSDH). In a previous paper, we reported the oxidation/reduction characteristics of 7 α -hydroxyl bile acids by *Escherichia coli*, a facultative anaerobe, under aerobic and anaerobic conditions (Ogura et al., 2005). Meanwhile,

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GLC, gas-liquid chromatography; G- β α , glycine-conjugated 7 β ,12 α -dihydroxy-5 β -cholanoic acid; HCA, hyocholic acid; HSDH, hydroxysteroid dehydrogenase; LCA, lithocholic acid; α -MCA, α -muricholic acid; β -MCA, β -muricholic acid; Me-DMES, methyl ester dimethylethylsilyl ether; MPYG, modified peptone yeast extract glucose; 7=O-DCA, 3 α ,12 α -dihydroxy-7-oxo-5 β -cholanoic acid (7-oxodeoxycholic acid); 7=O-LCA, 3 α -hydroxy-7-oxo-5 β -cholanoic acid (7-oxolithocholic acid); PHP GEL, piperidinohydroxypropyl dextran gel; T- α β , taurine-conjugated 7 α ,12 β -dihydroxy-5 β -cholanoic acid; UDCA, ursodeoxycholic acid; β β , 7 β ,12 β -dihydroxy-5 β -cholanoic acid

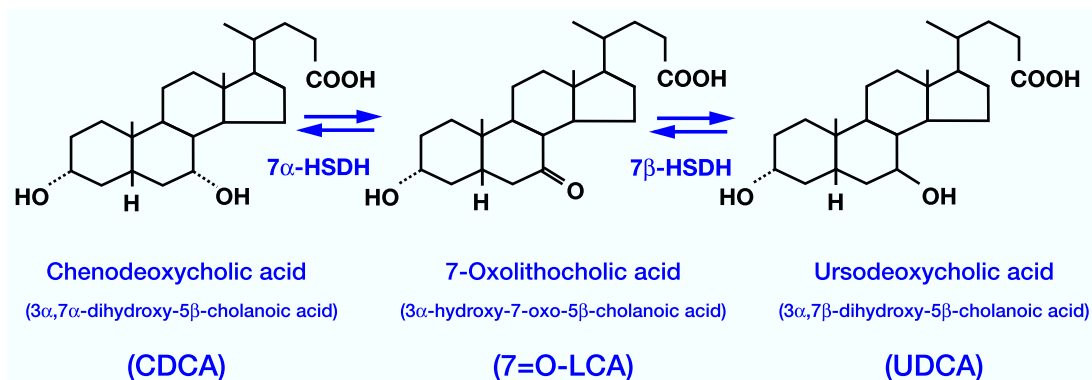


Fig. 1. Reaction sequences of chenodeoxycholic acid (CDCA), 3α-hydroxy-7-oxo-5β-cholanoic acid (7=O-LCA) and ursodeoxycholic acid (UDCA). 7α-HSDH, 7α-hydroxysteroid dehydrogenase; 7β-HSDH, 7β-hydroxysteroid dehydrogenase.

one strain of *Bacteroides* (*Bacteroides* sp. strain T-40) was isolated from healthy human feces, in which a relatively high amount of UDCA was detected, and this bacterium was found to have the highest 7α-HSDH activity among bacteria isolated from the feces. In the present experiments, we examined the effect of *Bacteroides* sp. T-40, an obligate anaerobe, on bile acid biotransformation (the first step for production of UDCA) in an anaerobic culture system.

Materials and Methods

Chemicals

CA, CDCA, hyocholic acid (HCA), and glycocholic acid (GCA) were purchased from Sigma Chemical, St. Louis, MO. α-Muricholic acid (α-MCA) and β-muricholic acid (β-MCA) were synthesized according to the methods reported by Iida et al. (1989). 3α,12α-Dihydroxy-7-oxo-5β-cholanoic acid (7=O-DCA) and 7=O-LCA were prepared by the oxidation of CA and CDCA with *N*-bromosuccinimide (Fieser and Rajagopalan, 1949), respectively. 7β,12β-Dihydroxy-5β-cholanoic acid (ββ), glycine-conjugated 7β,12α-dihydroxy-5β-cholanoic acid (G-βα), and taurine-conjugated 7α,12β-dihydroxy-5β-cholanoic acid (T-αβ) were synthesized as described previously

(Arimoto et al., 1982; Yamaga et al., 1987), and used as internal standards for analysis of bile acids by capillary gas-liquid chromatography (GLC). These bile acids were estimated to be more than 96% pure so far examined by capillary GLC. Dimethylethylimidazole was purchased from Tokyo Kasei Kogyo, Tokyo, Japan. Piperidinohydroxypropyl dextran gel (PHP GEL) was purchased from Shimadzu, Kyoto, Japan. The other reagents and solvents of analytical grade were obtained from Wako Pure Chemical Industries, Osaka, Japan. If not otherwise stated, the solvents were distilled once before use.

Bacteriological procedures

Eighty-four strains (*E. coli*: 4, *Enterococcus*: 12, Bacteroidaceae 38, *Bifidobacterium*: 8, *Eubacterium*: 5, *Clostridium*: 1 and unidentified anaerobes: 6) were isolated from a human fecal sample using 3 non-selective and 7 selective agar plates (Narushima et al., 2006) and characterized on bases of colony form, Gram stain, morphology, growth in air and spore formation (Mitsuoka et al., 1965). Each strain was cultured anaerobically for 3 days in a modified peptone yeast extract glucose (MPYG) medium (Hirano et al., 1981; Takahashi and Morotomi, 1994) containing CA and the bile acids transformed from CA were examined by thin-layer chromatography (Van den

Ende et al., 1982) and GLC (Ogura et al., 2003). The colony (*Bacteroides* sp. T-40) which showed the highest production of 7=O-DCA was selected for the present experiment. Strain T-40 was finally identified by approximately 1,500 bp of the 16S rDNA gene according to methods published previously (Miyamoto and Itoh, 2000). Although this sequence suggested strong similarity for the species in *Bacteroides* cluster, the sequence homology between strain T-40 and type strains in the *Bacteroides* cluster was less than 95%. *E. coli* K-12 which possesses 7 α -HSDH (Ogura et al., 2003) was obtained from the American Type Culture Collection, Manassas, VA. Both bacteria, the strain T-40 and *E. coli* K-12, were precultured anaerobically in MPYG medium (Hirano et al., 1981; Takahashi and Morotomi, 1994) at 37°C for 1 day by the gas-pack method (BBL Gas Pak Anaerobic System; Becton Dickinson, Sparks, MD). Aliquots of the growth of the strain T-40 ($8 \times 10^7/10 \mu\text{L}$) and K-12 ($3 \times 10^7/10 \mu\text{L}$) were added to 10 mL of MPYG medium containing 0.5 mM bile acid and cultured anaerobically at 37°C for 4 days.

Analytical methods

Bile acids were extracted from the medium as previously described (Ogura et al., 2003). A portion of the cultured medium, 100 μL , was treated with 8 volumes of ethanol by warming for 10 min and then filtered. The filtrate was evaporated to dryness under a stream of nitrogen and the residue was hydrolyzed in alkaline solution. After hydrolysis, the reaction mixture was acidified with diluted hydrochloric acid and bile acids were extracted with diethylether. In the PHP GEL analysis, prior to the analysis of bile acids, $\beta\beta$, G- $\beta\alpha$ and T- $\alpha\beta$ as internal standards were added to 100 μL of the culture medium. The analytical sample with the three internal standards was treated with 8 volumes of ethanol by warming for 10 min and then filtered. The filtrate was evaporated to dryness under a stream of nitrogen and the residue was subjected to PHP GEL column

chromatography to separate bile acids into free, glycine-conjugate and taurine-conjugate fractions (Yamaga et al., 1987). Glycine-conjugate fraction was hydrolyzed in alkaline solution (Yamaga et al., 1997). The hydrolysate and free fraction were acidified with diluted hydrochloric acid, and the bile acids were extracted with diethylether. The extracted bile acids were converted into methyl ester dimethylethylsilyl ether (Me-DMES) derivatives as described previously (Yamaga et al., 1987, 1996). The methyl ester of 7=O-LCA was converted to its methoxime derivative as described by Horning et al. (1968), to separately determine this compound and CDCA by GLC.

Capillary GLC

An aliquot of the bile acid derivatives dissolved in n-hexane was injected into a gas chromatograph (Model G14A; Shimadzu) equipped with a flame ionization detector, a solventless injector, and a computerized data system (Model C-R4A; Shimadzu). A Hicap CBP-1 capillary column (25 m \times 0.25 mm I.D.; Shimadzu) was used. The column temperature was maintained at 285°C and helium was used as the carrier gas.

Experiments with cell-free enzyme preparation

Crude 7 α -HSDH was prepared from *Bacteroides* sp. T-40 according to the method of Macdonald et al. (1973). The enzyme activity was assayed for bile acid transformation by a slight modification of the method reported by Macdonald et al. (1974, 1975). The reaction was performed at 37°C for 10 min with 7 mM NAD⁺, 0.2 M glycine-NaOH buffer (pH 9.5), 0–4 mM bile acid and 45 μL of the crude enzyme preparation in final volume of 1 mL. One unit of enzyme activity of 7 α -dehydrogenation was defined as the amount of enzyme required to yield 1 nmole of NADH per min under the conditions described above.