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Dehydrogenation of Conjugated Cholic Acid by *Escherichia coli*

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7α-Dehydrogenation of taurocholic acid and glycocholic acid by *Escherichia coli (E. coli)* was examined in aerobic and anaerobic culture conditions. Bile acids in the culture medium of *E. coli* were extracted, separated into free, glycine-conjugate and taurine-conjugate fractions by piperidinohydroxypropyl dextran gel column chromatography, hydrolyzed in alkali and analyzed by gas-liquid chromatography. Both conjugated cholic acids were dehydrogenated to the corresponding 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid without deconjugation and no deconjugation of both conjugated cholic acid was detected in aerobic cultures. But there was little transformation in anaerobic cultures. These data suggest that conjugated cholic acids are taken up by *E. coli* in an aerobic culture as conjugate forms, dehydrogenated without deconjugation and excreted from the cell as conjugate forms.

Key words: dehydrogenation; *Escherichia coli*; glycocholic acid; taurocholic acid

Primary bile acids synthesized from cholesterol in the liver are conjugated mainly with taurine or glycine, and excreted with bile into the duodenum. Intestinal microorganisms are well known to transform sterols and bile acids in human and animal gut (Macdonald et al., 1983; Groh et al., 1993; Hylemon and Harder, 1999). In the gut, these primary conjugated bile acids undergo various transformations by intestinal microorganisms. The major reactions are deconjugation, oxidation of hydroxyl groups (dehydrogenation), reduction of oxo groups, and dehydroxylation.

Escherichia coli (*E. coli*) has no deconjugation activity (Drasar and Hill, 1966; Midtvedt and Norman, 1967; Imamura et al., 1979; Chikai et al., 1987; Kayahara et al., 1994; Uchida et al., 1999), but has dehydrogenation activity that forms 7-oxo-bile acids (Macdonald et al., 1973; Heslewood and Haslewood, 1976; Imamura et al., 1979). In a previous paper, we reported that *E. coli* dehydrogenates the 7α-hydroxyl group of both unconjugated cholic acid and taurocholic acid (TCA) (Ogura et al., 2003). In addition, we found that TCA is dehydrogenated by *E. coli* to taurine-conjugated

Abbreviations: CA, cholic acid; GCA, glycocholic acid; GLC, capillary gas-liquid chromatography; G-βα, glycine-conjugated 7β,12α-dihydroxy-5β-cholanoic acid; 7α-HSDH, 7α-hydroxysteroid dehydrogenase; Me-DMES, methyl ester dimethylethylsilyl ether; PHP GEL, piperidinohydroxypropyl dextran gel; TCA, taurocholic acid; T-αβ, taurine-conjugated 7α,12β-dihydroxy-5β-cholanoic acid; T-3α12α7=O, taurine-conjugated 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid; 3α12α7=O, 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid; ββ, 7β,12β-dihydroxy-5β-cholanoic acid

Table 1. Relative retention time of bile acid derivatives

	Derivative	Retention time (min)	Relative retention time		
Internal standard	7β,12β-Dihydroxy-5β-cholanoic acid Me-DMES	12.61	1.08	**1.00**	1.28
	7β,12α-Dihydroxy-5β-cholanoic acid Me-DMES	11.62	**1.00**	0.92	1.18
	7α,12β-Dihydroxy-5β-cholanoic acid Me-DMES	9.82	0.85	0.78	**1.00**
Substrate and product	Cholic acid Me-DMES		1.59	1.46	1.88
	3α,12α-Dihydroxy-7-oxo-5β-cholanoic acid Me-DMES		1.72	1.58	2.03
	Unknown		1.90	1.76	2.25

Me-DMES, methyl ester dimethylethylsilyl ether.

3α,12α-dihydroxy-7-oxo-5β-cholanoic acid (T-3α 12α7=O) without deconjugation.

In the present experiments, we examined in detail the dehydrogenation of glycine- and taurine-conjugated cholic acids by *E. coli*.

Material and Methods

Chemicals

Glycocholic acid (GCA), TCA and cholic acid (CA) were purchased from Sigma Chemicals Co., St. Louis, MO. 3α,12α-Dihydroxy-7-oxo-5β-cholanoic acid (3α12α7=O) was prepared by the oxidation of CA with *N*-bromosuccinimide (Fieser and Rajagopalan, 1949). 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). Piperidino hydroxypropyl dextran gel (PHP GEL) was purchased from Shimadzu, Kyoto, Japan and dimethylethylsilylimidazole from Tokyo Kasei Kogyo Co. Ltd., Tokyo, 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

E. coli K-12 was obtained from the American Type Culture Collection (Manassas, VA). Brain-heart infusion (BHI; Becton Dickinson, Sparks, MD) was used for all cultures. *E. coli* was precultured aerobically in BHI medium at 37˚C for 1 day. Aliquots (3×10^7/10 μL) of *E. coli* were added to 2 mL of BHI medium containing 1 mM bile acids, and the bacteria were cultured at 37˚C for 4 days. Aerobic culture was performed by incubation in a thermostat, and anaerobic culture was performed by the gas-pack method (BBL GasPak Pouch System; Becton Dickinson).

Analytical methods

Prior to analysis of bile acids, ββ, G-βα and T-αβ 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 at 80˚C for 10 min, and 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 nonamidate (free), glycine-conjugate and taurine-conjugate fractions (Yamaga et al., 1987). Glycine- and taurine-conjugate fractions were hydrolyzed individually in alkaline solution (Yamaga et al., 1997). The hydrolysates and nonamidate fraction were acidified with dilute hydrochloric acid, and the bile acids were extracted with ethyl ether. The extracted bile acids were converted into

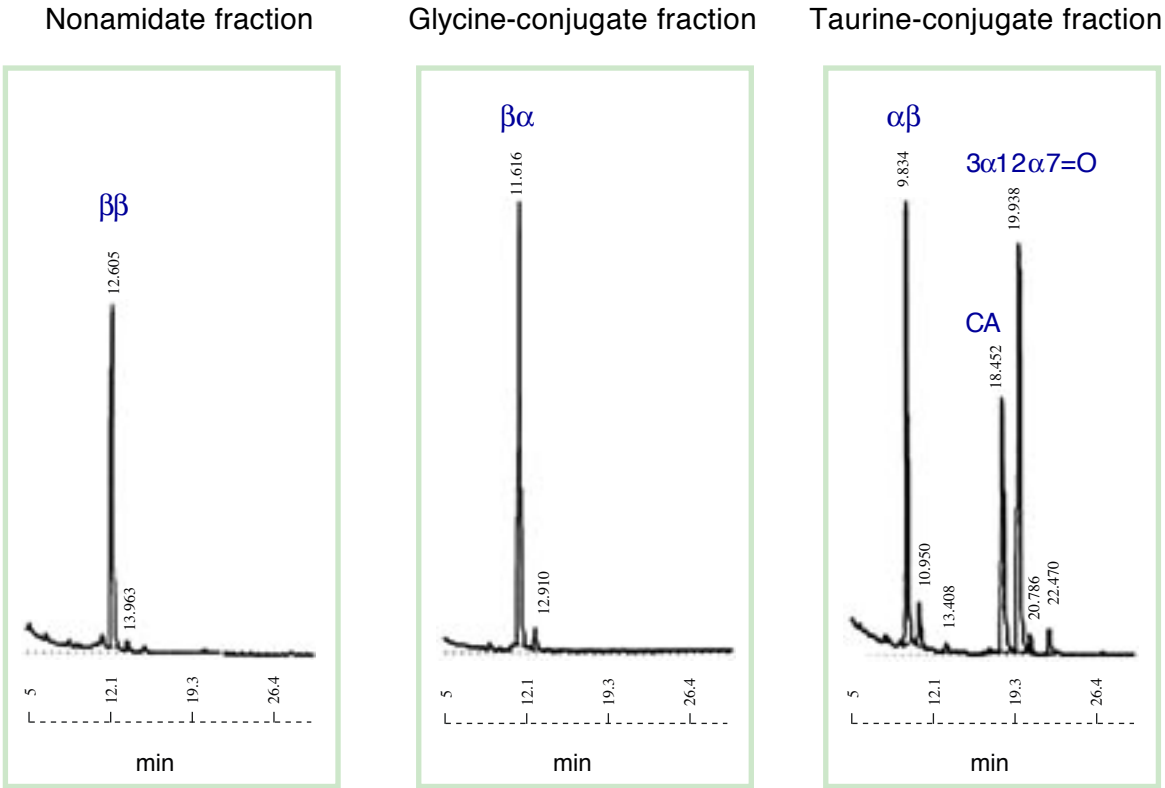

Fig. 1. Gas chromatograms of bile acids in nonamidate, glycine- and taurine-conjugate fractions after a piperidinohydroxypropyl dextran gel (PHP GEL) column of bile acids. The medium cultured with taurocholic acid (TCA) for 4 days added three internal standard compounds, 7β,12β-dihydroxy-5β-cholanoic acid (ββ), glycine-conjugated 7β,12α-dihydroxy-5β-cholanoic acid (G-βα) and taurine-conjugated 7α,12β-dihydroxy-5β-cholanoic acid (T-αβ). The sample was fractionated by PHP GEL column. Each fraction was hydrolyzed, and the bile acid extracts were converted into Me-DMES derivatives. See Table 1 for gas chromatographic condition. CA, cholic acid; 3α12α7=O, 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid.

methyl ester dimethylethylsilyl ether (Me-DMES) derivatives as described previously (Yamaga et al., 1987; Yamaga et al., 1996). Briefly, bile acids were methylated with diazomethane, and then left in 70 μL of dimethylethylsilylimidazole in an air-tight vessel at room temperature overnight.

Capillary gas liquid chromatography

An aliquot of the bile acid derivatives dissolved in *n*-hexane was injected into a gas-liquid chromatograph (model GC-14A; 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 × 0.25 mm i.d.; Shimadzu) was used. The column temperature was maintained at 285°C and helium was used as the carrier gas.

Relative retention times of bile acid derivatives in the present GLC analysis are given in Table 1.

Results

Figure 1 shows gas chromatograms of the bile acids in the nonamidate, glycine- and taurine-conjugate fractions obtained by PHP GEL column chromatography of the medium after culture with TCA for 4 days. Peaks corresponding to CA, 3α12α7=O and αβ (internal standard) were detected in the taurine-conjugate fraction. A peak corresponding