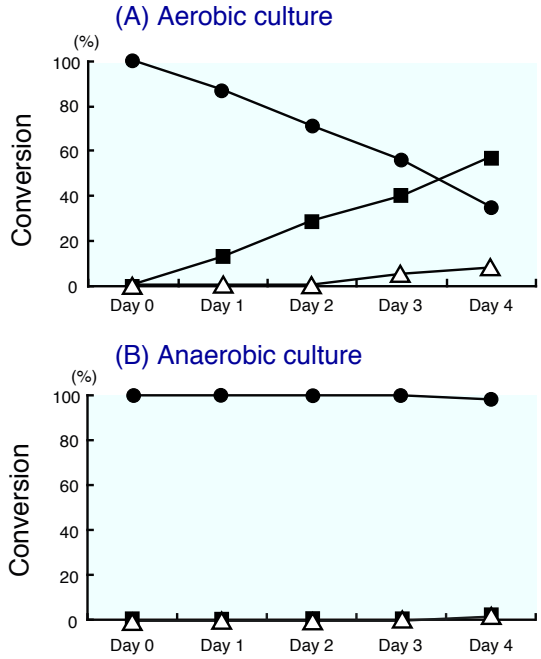

Fig. 2. Conversion of taurocholic acid (TCA) to taurine-conjugated 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid (T-3α12α7=O) by *Escherichia coli*. Changes in percentage composition of individual bile acids were followed during the course of (**A**) aerobic and (**B**) anaerobic incubation for 4 days. ●, TCA; ■, T-3α12α7=O; △, Unknown.

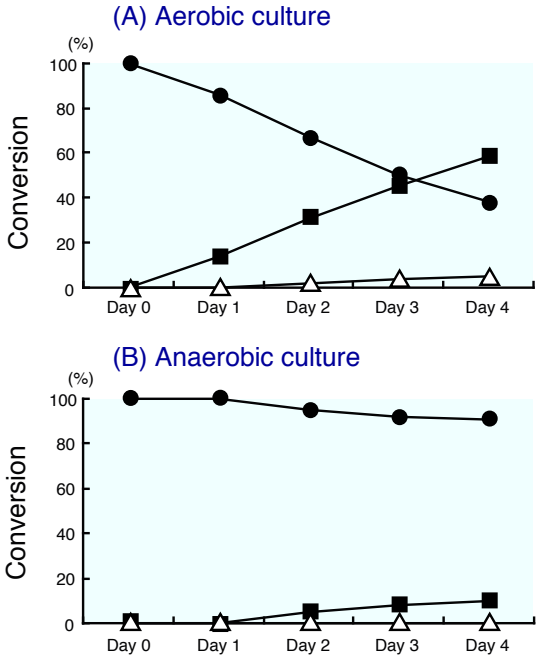

Fig. 3. Conversion of glycocholic acid (GCA) to glycine-conjugated 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid (G-3α12α7=O) by *Escherichia coli*. Changes in percentage composition of individual bile acids were followed during the course of (**A**) aerobic and (**B**) anaerobic incubation for 4 days. ●, GCA; ■, G-3α12α7=O; △, Unknown.

to ββ was detected only in the nonamidate fraction, and a peak corresponding to βα only in the glycine-conjugate fraction. No more than two internal standard peaks were detected in each fraction. These gas chromatograms demonstrate that the bile acids in the analytical samples were separated completely by PHP GEL column chromatography. This was also confirmed in the experiments with GCA and free CA.

Figure 2 shows the time course of conversion of TCA by *E. coli*. In aerobic culture, TCA was dehydrogenated to T-3α12α7=O, and the conversion proceeded almost linearly at a rate of about 15% a day (or 0.15 μmol/day under our experimental conditions). Small amounts of unidentified compounds were formed. However, there was little dehydrogenation of TCA in anaerobic culture.

Figure 3 shows the conversion of GCA by *E. coli*. GCA was dehydrogenated to the corresponding glycine-conjugated 7-oxo-bile acid. The conversion rate was almost the same as that of TCA in aerobic culture, but GCA was slightly more susceptible to the reaction than TCA, especially in anaerobic culture.

Figure 4 shows the conversion of free CA by *E. coli*. In this case, the reaction occurred much more quickly than with TCA or GCA, with 80% conversion after 1 day in aerobic culture and about 50% conversion after 2 days in anaerobic culture.

Medium cultured for 4 days without CA was filtered through a Millipore filter (MILLEX-GV0.22) and the filtrate was incubated aerobically for 1 to 4 days with 1 mM GCA without addition of cofactors. No oxidation was detected at any time of incubation (data not shown), suggesting that no enzyme reaction took place in the medium.

These results suggest that conjugated bile acids are taken up by *E. coli* as conjugate forms, dehydroge-

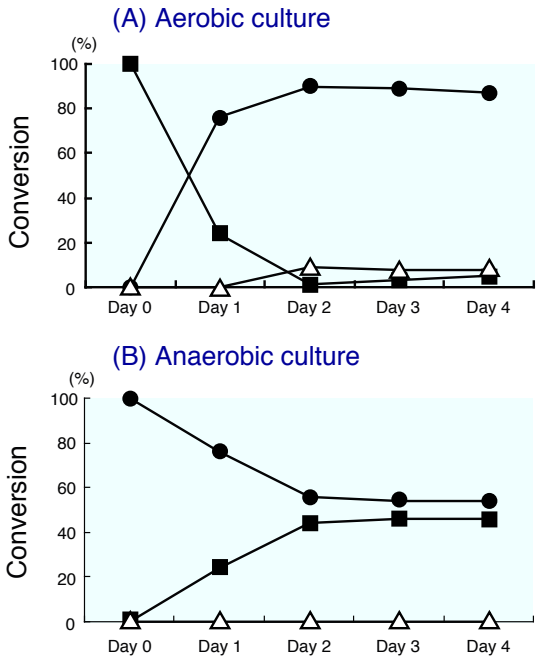

Fig. 4. Conversion of cholic acid (CA) to 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid (3α12α7=O) by *Escherichia coli.* Changes in percentage composition of individual bile acids were followed during the course of (**A**) aerobic and (**B**) anaerobic incubation for 4 days. ●, CA; ■, 3α12α7=O; △, Unknown.

nated as conjugate forms and excreted from the cell as conjugate forms.

Discussion

The present experiments demonstrated that *E. coli* dehydrogenates taurine- or glycine-conjugated CA to the corresponding 3α12α7=O without deconjugation. These reactions occurred only under aerobic conditions, and the conversion rates were lower than for free CA. TCA and GCA were about 60% dehydrogenated after 4 days (Fig. 1), whereas free CA was about 80% dehydrogenated after 1 day and almost completely dehydrogenate after 4 days (Ogura et al., 2003).

Haslewood and Haslewood (1976) showed that 7α-hydroxysteroid dehydrogenase prepared from *E. coli* oxidizes both conjugated and free 7α-hydroxyl bile acids at the same rate in an in vitro system. The difference in rates between the present report and the previous report presumably represents differences in substrate penetration into living cells.

It is well known that *E. coli* possesses no hydrolase activity that will hydrolyze conjugated bile acids (Drasar and Hill, 1966; Midtvedt and Norman, 1967; Imamura et al., 1979; Chikai et al., 1987; Kayahara et al., 1994; Uchida et al., 1999). Accordingly, in the present experiments, no deconjugation of TCA and GCA was detected. *E. coli* possesses 7α-hydroxysteroid dehydrogenase (7α-HSDH) (Aries et al., 1969; Macdonald et al., 1973; Prabha et al., 1989) as a non-inducible intracellular enzyme that requires NAD^+ as a cofactor (Macdonald et al., 1973; Prabha et al., 1989). Disruption of *E. coli* during culture might release 7 α-HSDH into the medium, but the present experiments ruled out the possibility that released enzyme activity catalyzes the conversion, since the filtrate prepared from medium cultured for 4 days was completely ineffective in 7α-dehydrogenation of GCA.

If conjugated bile acids are taken up by *E. coli*, the corresponding conjugated 7-oxo-bile acids are formed and excreted into the culture medium. However, it remains unclear how conjugated bile acids are take up by *E. coli.* Bile acids may cross the cell membrane by ionic or nonionic diffusion, as demonstrated with intestinal bile acid absorption (Dietschy, 1968). Nonionized bile acids may penetrate the membrane easily, but conjugated bile acids are unlikely to pass through lipid bilayers because their pKa values are low (taurine-conjugated, < 1.5; glycine-conjugated, 3.5–5.2) and they are ionized at the pH of the present culture medium. The pH in our experiments increased to about 9 in aerobic cultures, whereas it decreased to around 6.5 in anaerobic cultures (Ogura et al., 2003). However, the rate of dehydrogenation of conjugated bile acids, presumably reflecting the rate of influx of substrate, was greatest in aerobic culture and least in anaerobic culture.

These observations suggest that *E. coli* possesses an influx mechanism for conjugated bile acids. Mallonee and Hylemon (1996) reported that a bile acid transporter in *Eubacterium* sp. strain

VPI 12708 showed a much higher activity for free bile acids than for glycocholic acid or 7-oxocholic acid. Conversely, Thanassi et al. (1997) demonstrated that *E. coli* possesses a mechanism to actively pump out TCA. T-3α12α7=O was not examined in that report, but it is presumed that this oxo bile acid is excreted by the same mechanism. It is not yet known whether this efflux mechanism is also responsible for influx of bile acids, but if this efflux mechanism is a rate-limiting step, influx of conjugated bile acids will be restricted under anaerobic conditions.

As shown in the present paper, conjugated bile acids are dehydrogenated by *E. coli* without deconjugation. On the other hand, 7α-dehydroxylation of bile acids by intestinal bacteria is considered to take place after deconjugation (Aries and Hill, 1970), and 7α-dehydroxylating bacteria show much higher activities when cultured with bacteria possessing deconjugation activity than when cultured alone (Hirano and Masuda, 1982; Narushima et al., 2002); nevertheless, this dehydroxylation reaction has also been suggested to occur without deconjugation in vivo (Hepner et al., 1972).

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