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7α-HYDROXYSTEROID DEHYDROGENASE FROM *ESCHERICHIA COLI* B: PRELIMINARY STUDIES

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SUMMARY

7α-Hydroxysteroid dehydrogenase was examined in crude preparations of one strain of *Escherichia coli* B which was devoid of both 3α- and 12α-dehydrogenase activities as shown by substrate specificity studies, complete oxidation studies and examination of oxidation products by thin-layer chromatography. Extraction of enzyme from non-induced cells by sonication was shown to be more effective than grinding with alumina or silica. Sonication released approximately 90 units of 7α-hydroxysteroid dehydrogenase per g dry weight of bacteria or 300 units/10^{10} cells. Measurement of activity as a function of pH revealed a broad, flat optimal activity range between 9.0 and 10.0 for oxidation of cholate and a similar optimal range for glycocholate. Michaelis constants for trihydroxybile acids and conjugates were in the range of 0.8–1.1 mM, whereas dihydroxybile acids and conjugates gave a range of values one order of magnitude lower. Polyacrylamide disc gel electrophoreses indicate only one major active band of identical mobility for all 7α-hydroxy substrates studied, suggesting the presence of a single enzymatic entity for the oxidation of both conjugated and free 7α-hydroxy substrates. No oxidation of purified 3α,7β-dihydroxycholanic acid was observed.

INTRODUCTION

Since the purification of 3α- and 3β-hydroxysteroid dehydrogenase from induced strains of *Pseudomonas testosteroni*[1–3], these enzymes have been well studied and characterized[3–5], and now are commercially available in partially purified form. 3α-Hydroxysteroid dehydrogenase has been exploited by investigators for the quantitative measurement of bile acids[6] and other steroids[7] spectrophotometrically and later applied to bile acid measurement in other biological fluids using a fluorometric assay[8–10]. Subsequently these systems have been employed in combination with thin-layer chromatography[11–13] to individually measure bile acids from naturally occurring mixtures.

It would appear advantageous to use the NAD^+ dependent 7α-hydroxysteroid

dehydrogenase activity, demonstrated in intestinal anaerobes and in enterobacteria[14], to quantitatively measure the 7α-OH containing bile acids in biological fluids. This enzyme, in combination with 3α-hydroxysteroid dehydrogenase could be employed to measure the individual components in naturally occurring mixtures of glycine conjugates of 3α,7α-dihydroxy- and 3α,12α-dihydroxycholanic acid, thus far inseparable by thin-layer chromatography. Similarly this method will quantify the individual components of mixtures of corresponding taurine conjugates. In general 7α-dehydrogenase has only been sparsely documented and has not yet been exploited for analytical purposes. This communication describes the presence of 7α-hydroxysteroid dehydrogenase in one strain of *E. coli* and some of its kinetic properties.

MATERIALS

Todd–Hewitt broth, subsequently referred to as "broth", was obtained from Bioquest Co. Ltd. Todd–Hewitt agar for pouring plates was prepared by adding 1.5% Bacto agar to the broth medium before autoclaving. Phenazine methosulphate, nitro blue tetrazolium, NAD^+, Coomassie brilliant blue and barbituric acid (veronal) were obtained from Sigma Chemical Co. Hydrochloric and phosphoric acids, mono- and dibasic sodium phosphates, sucrose and acrylamide were purchased from Baker Co.; glycine, NaOH and EDTA, from Fisher Chemicals; and mono-, di- and trihydroxybile acids were obtained from Calbiochemicals.

METHODS

Growth and harvest of E. coli B

E. coli B was obtained from the American Type Culture Collection. This strain was grown on brain–heart infusion agar (Difco) from which subcultures were made to broth for respective experiments. Small volume starter cultures (30 ml) were prepared by emulsifying some of the growth from the infusion plate in broth and allowing growth to occur for 2–2.5 h at 37 °C. 25 ml of this culture were then added to a 500-ml volume of broth at 37 °C (5% inoculum) and shaken at 150 rev./min in a New Brunswick Metabolyte water bath. Growth curves were constructed both on the basis of the absorbance of the culture at 660 nm (Coleman Model B spectrophotometer) and the number of viable *E. coli* cells per ml of culture medium determined by serial dilution and plating. The bacteria, unless otherwise designated, were harvested at $t = 4.5$ h, centrifuged at 6000 × *g* for 20 min at 4 °C in an International B 20 centrifuge, gently resuspended in 20 ml of 0.1 M sodium phosphate buffer, pH 7.0, and recentrifuged as before. The final sediment was resuspended in 15 ml of 0.1 M sodium phosphate buffer, pH 7.0.

Extraction of 7α-hydroxysteroid dehydrogenase from E. coli

The 15 ml volume of washed cells described above was sonicated with a Fisher ultrasonic probe at 100 W for a period of 4 min and then centrifuged at 10 000 × *g* for 10 min to sediment the cell debris. The supernatant fluid was collected for enzyme studies.

Alternatively, the bacteria obtained by centrifuging a 500-ml culture were ground with a mortar and pestle using silica or coarse alumina as an abrasive[15]. The

cell paste was suspended in 10 ml of 0.1 M sodium phosphate buffer and centrifuged 10 000 × *g*. The supernatant fluid was decanted. All crude preparations, unless otherwise designated, were prepared and maintained at 4 °C.

Assay for 7α-hydroxysteroid dehydrogenase activity

The formation of NADH was followed at 22 °C at 340 nm using a Beckman spectrophotometer with a 10-inch Beckman recorder. Each assay cuvette contained, unless otherwise indicated, $1.7 \cdot 10^{-3}$ M NAD^+, 0.17 M glycine–NaOH buffer, pH 9.5, $1.0 \cdot 10^{-3}$ M bile acid and 100 μl of crude *E. coli* supernatant fluid in a final volume of 3.0 ml. One unit of enzyme is defined as the amount of enzyme required to yield 1 μmole of NADH per min under the above described conditions with cholic acid as substrate. An estimated extinction coefficient of $6.2 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ was used for NADH[16].

Assay for protein concentration

The concentration of protein in crude supernatants was determined by the procedure of Lowry *et al.*[17] with crystalline bovine serum albumin (Sigma) as standard.

Dry weight of bacterial cells

500-ml volumes of *E. coli* cultures were harvested as above, washed with water, and the sediment was collected on a Millipore membrane filter (0.45 μm porosity) and dried at 100 °C to constant weight.

Polyacrylamide disc gel electrophoresis studies

Disc gel electrophoresis with 5% acrylamide gels was performed by the procedure similar to that outlined by Davis[18] and by Gabriel[19]. Gel buffers were identical to those designated by Gabriel as System II. However, Solution 13 was adjusted (4 g barbital and 0.44 g Tris per liter, pH 7.0). The running pH of the stacking gel was 7.0 and the running pH of the separating gel was 8.0. Electrophoresis required approximately 90 min for completion.

Staining of the gels

After electrophoresis, the gels were removed from their tubes and stained either for proteins or for dehydrogenase activity with a selected substrate.

The staining procedure for proteins was identical to that described by Chrambach[20]. Gels were incubated 30 min in 12.5% trichloroacetic acid and 1 h in a freshly prepared solution of Coomassie brilliant blue made by dilution of stock 0.1% Coomassie brilliant blue into 12.5% trichloroacetic acid (1:20, v/v).

The staining procedure for 7α-hydroxysteroid dehydrogenase activity was designated on the basis of that of Gabriel[22]. Gels were incubated in the dark for 15 min with a reaction mixture consisting of 0.1 mg/ml phenazine methosulphate, 0.4 mg/ml nitro blue tetrazolium, $1.7 \cdot 10^{-3}$ M NAD^+ and 0.17 M glycine–NaOH, pH 9.5, and $1.0 \cdot 10^{-3}$ M bile acid of desired substitution*. The total reaction mixture volume was 4.0 ml. Gels treated with Coomassie brilliant blue were destained with

* Solutions of phenazine methosulphate and nitro blue tetrazolium were freshly made and added to the reaction mixture after all the other reagents.

10% trichloroacetic acid[20] and activity-stained gels were destained with 7% acetic acid[21].

Complete oxidation studies

Both di- and trihydroxysubstrates at $6 \cdot 10^{-5}$ M were allowed to undergo oxidation in the presence of 0.16 units of crude 7α-hydroxysteroid dehydrogenase till no further absorbance at 340 nm was evident (approx. 10 min). The total change in absorbance, (corrected for the after-subtraction of the blank run in parallel) directly quantified the total amount of bile acid in the cuvette.

Thin-layer chromatography of oxidation products

Oxidation of 10^{-3} M chenodeoxycholic acid (3α,7α-dihydroxycholanic acid) and ursodeoxycholic acid (3α,7β-dihydroxycholanic acid) was performed with (a) 7α-hydroxysteroid dehydrogenase and (b) 7α- and 3α-hydroxysteroid dehydrogenase together. A cuvette containing 10^{-3} M bile acid, $3.4 \cdot 10^{-3}$ M NAD^+ and 0.16 units of 7α-hydroxysteroid dehydrogenase or 0.16 units of both 7α- and 3α-hydroxysteroid dehydrogenase (total volume 3 ml) was incubated overnight at room temperature. The reaction mixture was then acidified with 1.0 ml of 12.5% trichloroacetic acid and the reaction products were extracted with 0.5 ml chloroform–methanol (2:1, v/v) and spotted onto thin-layer plates (20 cm × 20 cm).

The extracts (lower phase), as well as substrates, were chromatographed on thin-layer silica plates with Solvent S11 as described by Eneroth[13] (trimethylpentane–ethyl acetate–acetic acid, 10:10:2.0, v/v/v). Plates were dried and sprayed with concentrated H_2SO_4 and charred at 240 °C for 2 h.

RESULTS

Growth of bacteria and extraction of the enzyme

In contrast to the 3α- and 3β-hydroxysteroid dehydrogenase activities from *P. testosteroni*, 7α-dehydrogenase activities appear to be constitutive or non-inducible, as the inclusion of 0.01% cholic acid in the growth medium did not alter the obtainable enzyme levels.

In a separate experiment, no difference in 7α-dehydrogenase activities could be observed when a 1.5-h culture was split into two subcultures, 0.01% cholic acid added to one, and both cultures were harvested as previously described 3 h later. Cholic acid, therefore, does not appear to affect the rate of growth or yield of bacteria (Fig. 1). Supporting these observations, the actual time of harvest during the stationary growth phase of *E. coli* did not appear to be of primary importance to the yield of enzyme activities.

The efficiency and reproducibility of enzyme extraction by sonication greatly exceeded that obtained by grinding with either alumina or silica*. 90 units/g dry weight of bacteria or 300 units/10^{10} *E. coli* cells can be extracted with 4 min of sonication (Table I). Very few intact bacteria were visible under the microscope at this time. No activity was found in spent culture media.

* Our results are similar to those of Marcus and Talalay[2] who were using both methods for extraction of 3α-hydroxysteroid dehydrogenase from *P. testosteroni*.

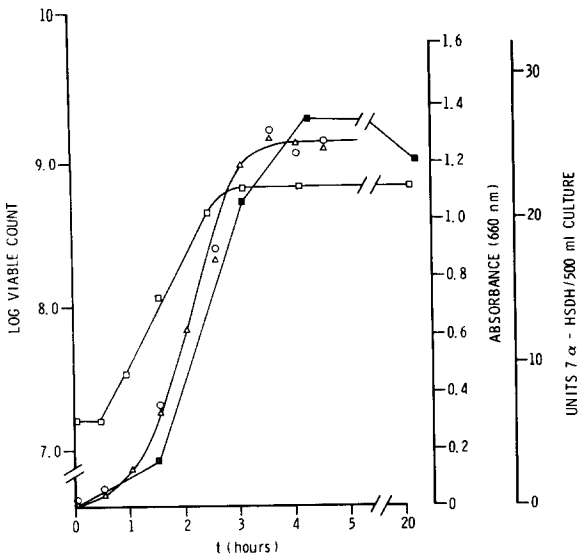

Fig. 1. Growth curves for 500 ml shaking cultures of *E. coli* B: log viable cell counts (□); absorbance at 660 nm for the culture with 0.01% cholic acid (△); and for a control culture (○); total units 7α-hydroxysteroid dehydrogenase obtained by sonication of harvested bacteria with respect to time (■) (see Methods for further details). HSDH, hydroxysteroid dehydrogenase.

TABLE I

RELATIVE YIELDS OF *E. coli* 7α-HYDROXYSTEROID DEHYDROGENASE OBTAINED BY DIFFERENT EXTRACTION PROCEDURES

Procedure Sonication (min)	*Units/10^{10} cells*	*Units/g dry cells*	*Units/mg protein*
0.5	150	43	0.12
1.0	204	59	0.12
1.5	240	68	0.12
2.0	288	83	0.15
3.0	300	86	0.16
4.0	318	90	0.16
8.0	310	88	0.12
Grinding with alumina	30	8.0	0.36
Grinding with silica	31	8.3	0.38

Effect of pH on activity

7α-Hydroxysteroid dehydrogenase activity toward cholic acid was linear with protein concentration in the cuvette over a considerable range as determined at either pH 9.3 and 10 (Fig. 2). Variation of pH value confirmed a broad activity curve, optimal between pH 9.0 and 10.0, for oxidation of cholic acid and a similar, somewhat sharper pH optimal for oxidation of glycocholic acid (Fig. 3).

Stability

Table III shows the stability of 7α-hydroxysteroid dehydrogenase under different storage conditions with time. Inclusion of EDTA, and to some extent GSH, stabilized the enzyme. Freezing abolished 7α-dehydrogenase activity.

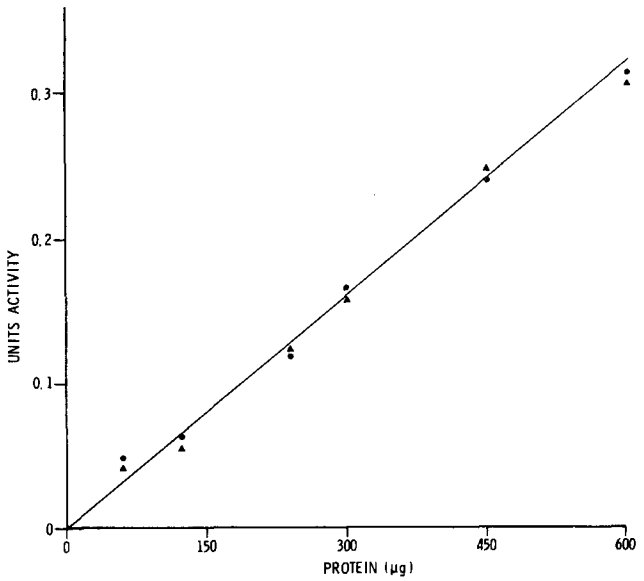

Fig. 2. Units of 7α-hydroxysteroid dehydrogenase activity *versus* µg protein in the assay cuvette at pH 9.3 (▲); and at pH 10 (●).

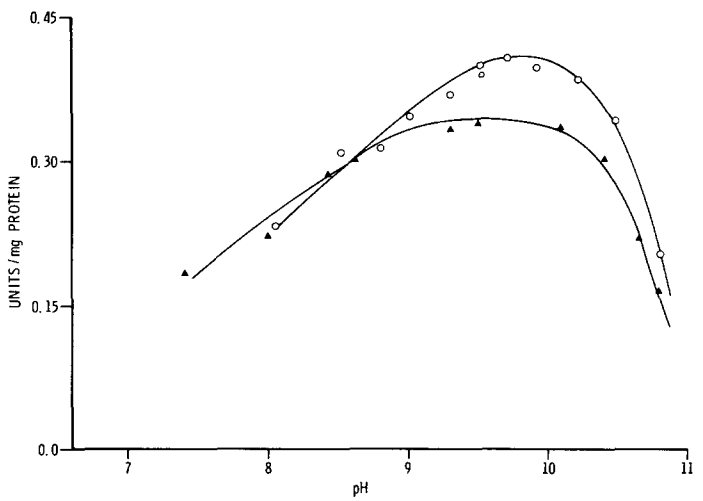

Fig. 3. Units of 7α-hydroxysteroid dehydrogenase activity per mg protein *versus* pH value for 1 mM cholate (▲) and glycocholate (○).

Substrate specificity and impurity detection

A substrate specificity study (Table II) revealed the oxidation of the 7α-hydroxyl group of free bile acids and their glycine and taurine conjugates by the cell-free extract. No trace of 3α- or 12α-hydroxysteroid dehydrogenase activity was detected. Group specificity of the 7α-dehydrogenase was confirmed by the complete oxidation of a $6.0 \cdot 10^{-5}$ M chenodeoxycholate with crude supernatant fluid and sequential oxidation with 3α-dehydrogenase (Worthington). A stoichiometric yield

TABLE II

RELATIVE 7α-HYDROXYSTEROID DEHYDROGENASE ACTIVITIES AND MICHAELIS CONSTANTS FOR BILE ACIDS

Substrate	*Relative activities**		*Km* × 10^{-3} *M*	*Inhibition by [substrate] > 10^{-3} M*
	10^{-3} *M substrate*	3 × 10^{-3} *M substrate*		
Cholate	1.0	1.6	0.80	—
Glycocholate	1.27	1.9	1.0	—
Taurocholate	1.24	2.2	1.0	—
Chenodeoxycholate	0.64	0.55	0.060	+
Glycochenodeoxycholate	0.98	0.78	0.085	+
Taurochenodeoxycholate	0.85	0.70	0.24	+
Deoxycholate	0	0		
Glycodeoxycholate	0	0		
Taurodeoxycholate	0	0		
Lithocholate	0	0		
Glycolithocholate	0	0		
Taurolithocholate	0	0		

* All activities are relative to the rate of oxidation of cholate at 10^{-3} M, designated as unity.

TABLE III

STABILITY STUDIES FOR 7α-HYDROXYSTEROID DEHYDROGENASE

Storage conditions	*Temp. (°C)*	*7α-Hydroxysteroid dehydrogenase activity (units/ml)*		
		Day 1	*Day 9*	*Day 23*
Enzyme, alone	4	2.0	1.6	0.52
Enzyme + 10^{-3} M GSH	4	2.0	1.7	0.84
Enzyme + 10^{-3} M EDTA	4	2.0	1.8	1.3
Enzyme + 10^{-3} M EDTA, 10^{-3} M GSH	4	2.0	1.8	1.4
Enzyme, alone	— 20	2.0	0	0

of NADH was obtained in each oxidation step by absorbance measurements at 340 nm.

Unpurified ursodeoxycholic acid showed considerable activity at 10^{-3} M with 7α-dehydrogenase. However, when subjected to complete oxidation studies either at $6 \cdot 10^{-5}$ M or 10^{-3} M, only 14–16% of the compound underwent oxidation at the 7α-position compared to the 100% oxidation observed with chenodeoxycholate (Fig. 5). The percentage completion was determined by the ratio of the observed to theoretical absorbances of NADH. After chromatographing crude unoxidized ursodeoxycholate, a major component of an R_F value slightly below that of chenodeoxycholate as well as an unresolved minor component of R_F value corresponding to chenodeoxycholate were demonstrated (Fig. 4A and 4B) and after chromatographing the 7α-dehydrogenase assay mixture extract, the R_F value for the main component of crude ursodeoxycholate did not change (Fig. 4D). The R_F of the minor component corresponded to the R_F of oxidized chenodeoxycholate (at the 7α-position) (Fig. 4C).

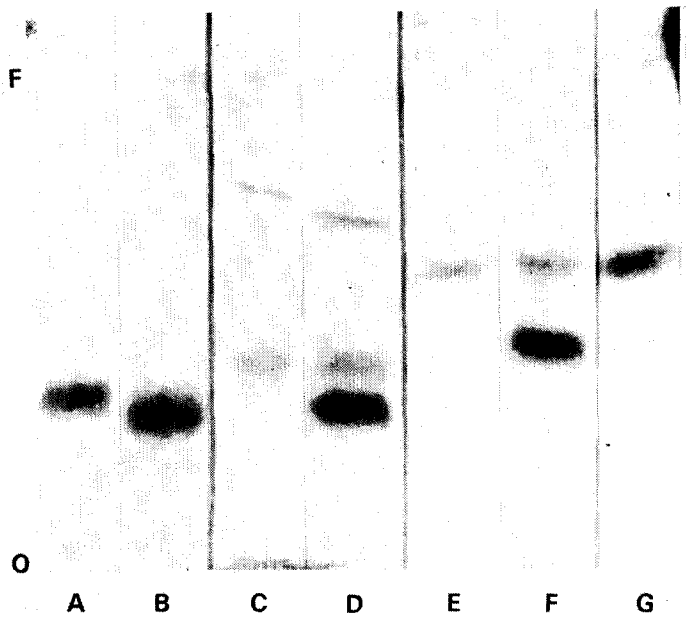

Fig. 4. Thin-layer chromatography of chenodeoxycholic acid, crude ursodeoxycholic acid, and products of oxidation by 7α-hydroxysteroid dehydrogenase and by both 7α- and 3α-hydroxysteroid dehydrogenase (Worthington) and commercial 3,12-diketocholanic acid. Hydroxy- and ketocholanic acid derivatives enumerated in order of origin, O: A, 3α-, 7α-; B, 3α-, 7β-; 3α-, 7α-(contaminant); C, 3α-, 7-keto-; D, 3α-, 7β-; 3α-, 7-keto- (oxidized contaminant); E, 3, 7-diketo-; F, 3keto-, 7β-; 3,7-diketo- (twice oxidized contaminant) and G, 3,12-diketo- (Applied Science Laboratories Inc.). Minor spots are due to impurities in starting materials.

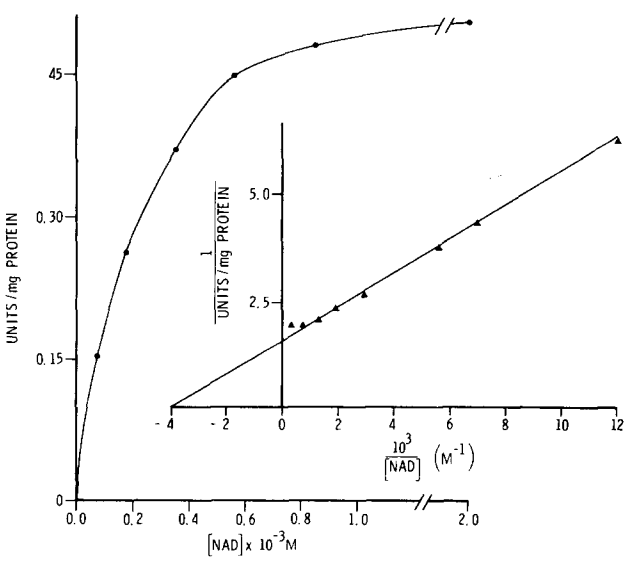

Fig. 5. Michaelis and Lineweaver–Burk plots for NAD^+ as cofactor for 7α-hydroxysteroid dehydrogenase, substrate cholic acid.

Oxidation of ursodeoxycholate at both the 3α- and 7α-positions, again yielded a minor component of R_F value corresponding to that of twice oxidized chenodeoxycholate as well as the major oxidation product (Fig. 4E and 4F). Ursodeoxycholate, purified by treatment with 7α-dehydrogenase, scraped from thin-layer plates and eluted from silica with methanol was not a substrate for 7α-dehydrogenase.

Polyacrylamide disc gel electrophoresis

Polyacrylamide disc gel electrophoresis studies with crude enzyme supernatant indicate only one active band for conjugated and unconjugated 7α-hydroxybile acids, (Fig. 6). Predictably, no active band was observed for deoxycholic acid (3α,12α-dihydroxycholanic acid) or for lithocholic acid (3α-monohydroxycholanic acid). No difference could be observed in the mobilities of the active bands for the entire range of substrates studied.

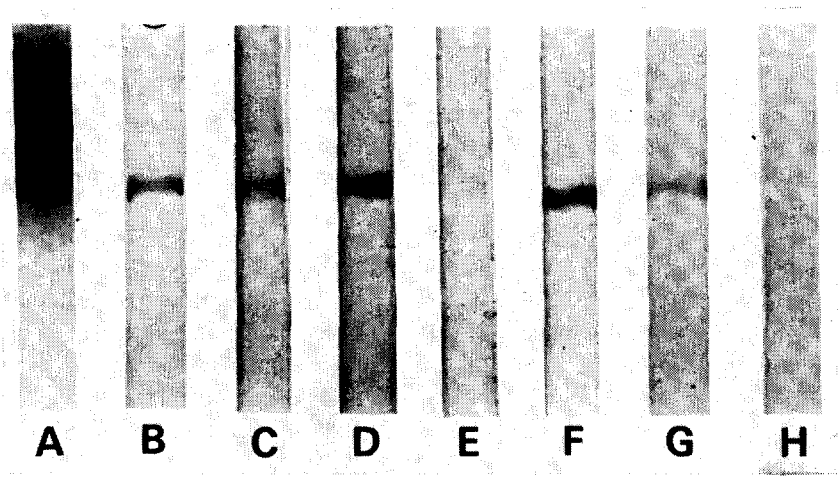

Fig. 6. Polyacrylamide disc gel electrophoresis of 7α-hydroxysteroid dehydrogenase, 500 μg protein each, stained for total proteins (A); and activity with cholate (B); GC (C); GCDC (D); GDC (E); TCDC (F); TC (G); and TLC (H).

DISCUSSION

Although 7α-hydroxysteroid dehydrogenase activity has previously been demonstrated in *E. coli* and in anaerobes by Aries and Hill[14], several differences are evident in our experimental approach and results. These investigators grew enterobacteria anaerobically in the presence of 0.01% cholic acid and showed the presence of a 7α-hydroxysteroid dehydrogenase with very little activity for conjugated substrates. We have shown that in one strain, there is considerable activity against conjugated substrates and a preference for oxidation of dihydroxy substrates over trihydroxy substrates. The extent of hydroxylation of the substrates has a greater effect on the kinetic behaviour of the enzyme than the state of conjugation or the nature of the conjugate (Table II).

On preparation of the enzyme, it became immediately obvious that freezing of

7α-hydroxysteroid dehydrogenase must be avoided. Although we have found moderate success in protecting this enzyme from heavy metals and oxidation by the inclusion of EDTA and GSH, other reagents are also being examined.

The optimal pH 9.0–10 for cholic acid is somewhat higher than that obtained by Aries and Hill[14] (pH 9.0–9.5). The 7α-dehydrogenase from this particular strain of *E. coli* B was shown to be completely devoid of contaminating 3α- and 12α-dehydrogenase. This was demonstrated by substrate specificity studies (Table II) and verified by the stoichiometric production of NAD^+ on sequential tandem oxidation of chenodeoxycholate with *E. coli* supernatant fluid and with commercial 3α-dehydrogenase. Thin-layer chromatography of substrates and reaction products further supports this observation.

In agreement with Aries and Hill[14,23], 7α-dehydroxylase activity does not appear to be present in significant quantity, as a stoichiometric amount of NADH was obtained on complete oxidation studies and no lithocholic acid was observed on incubating chenodeoxycholic acid with crude enzyme and chromatographing the extract on thin layer plates.

It is apparent that in commercial ursodeoxycholate there is approximately 15% impurity oxidizable by *E. coli* 7α-dehydrogenase and that purified ursodeoxycholate is not oxidized by 7α-dehydrogenase. Therefore, unlike the 3α- and 3β-dehydrogenase from *P. testosteroni*[1–5], there does not appear to be two enzymes of different stereospecificities present for 7-hydroxysteroid oxidation in this strain of *E. coli*.

The impurity in commercial ursodeoxycholate appears to be chenodeoxycholate. This is not surprising since the chemical synthesis of ursodeoxycholate commonly used is reduction of 3α-hydroxy-7-ketocholanic acid with Na in propanol to selectively, but not specifically, yield 3α,7β-dihydroxycholanic acid[24]. Clearly then, 7α-dehydrogenase, even in its crude form is useful for preliminary characterization of bile acids of doubtful or unknown identity and indicating the presence of 7α-dehydrogenase oxidizable impurities in 7α-dehydrogenase-non-oxidizable bile acids, not easily separable chromatographically. Moreover, we have demonstrated that the enzyme, in combination with chromatography will purify such 7α-dehydrogenase-resistant substrates by selectively oxidizing such impurities.

7α-Hydroxysteroid dehydrogenase in *E. coli* appears to be a non-inducible intracellular enzyme of broad substrate specificity. Purification of this enzyme and further substrate specificity studies are presently being pursued in our laboratories. A method using this enzyme for analyzing mixtures of conjugates of dihydroxybile acids has been submitted for publication.

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REFERENCES

1 Talalay, P. and Dobson, M. M. (1953) *J. Biol. Chem.* 205, 823–837
2 Marcus, P. I. and Talalay, P. (1956) *J. Biol. Chem.* 218, 661–674
3 Delin, S., Squire, P. G. and Porath, J. (1964) *Biochim. Biophys. Acta* 89, 398–408
4 Talalay, P. and Marcus, P. I. (1956) *J. Biol. Chem.* 218, 398–408
5 Squire, P. G., Delin, S. and Porath, J. (1964) *Biochim. Biophys. Acta* 89, 409–421
6 Iwata, T. and Yamasaki, K. (1964) *J. Biochem.* 56, 424–431
7 Hurlock, B. and Talalay, P. (1957) *J. Biol. Chem.* 227, 37–52
8 Palmer, R. H. (1969) *Methods in Enzymology* (Clayton, R. B., ed.), 15, pp. 280–288, Academic Press, London and New York
9 Turnberg, L. A. and Anthony-Mote, A. (1969) *Clin. Chim. Acta* 24, 253–259
10 Murphy, E. M., Billing, B. H. and Baron, D. N. (1970) *J. Clin. Pathol.* 23, 594–598
11 Gregg, J. A. (1966) *J. Lipid Res.* 7, 579–581
12 Hofmann, A. E. (1962) *J. Lipid Res.* 3, 127–128
13 Eneroth, P. (1963) *J. Lipid Res.* 4, 11–16
14 Aries, V. and Hill, M. J. (1970) *Biochim. Biophys. Acta* 202, 535–543
15 McIlwain, H. (1948) *J. Gen. Microbiol.* 2, 288–291
16 Horecker, B. L. and Kornberg, A. (1948) *J. Biol. Chem.* 175, 385–390
17 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
18 Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* 121, 404–427
19 Gabriel, O. (1971) in *Methods of Enzymology*, (Jakoby, W. B. ed) Vol. 22, pp. 565–578
20 Chrambach, A., Reisfeld, R. A., Wyckoff, M. W. and Zaccari, J. (1967) *Anal. Biochem.* 20, 150–154
21 Gabriel, O. (1971) in *Methods of Enzymology*, (Jacoby, W. B. ed.), Vol. 22, pp. 578–604
22 Lineweaver, H. and Burk, D. (1934) *J. Am. Chem. Soc.* 56, 658–666
23 Aries, V. and Hill, M. J. (1970) *Biochim. Biophys. Acta* 202, 526–534
24 Kritchevsky, D. and Nair, P. P. (1971) in *The Bile Acids* (Nair, P. P. and Kritchevsky, D., eds), 1–10 Plenum Press, New York, London