Formation of cholic acid from 3α,7α,12α-trihydroxy-5β-cholestanoic acid by rat liver peroxisomes

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Abstract In a previous study, it was shown that the peroxisomal fraction of rat liver, isolated by Percoll gradient centrifugation of a light mitochondrial fraction, was able to catalyze conversion of 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA) into cholic acid (Pedersen, J. I., and J. Gustafsson, 1980. *FEBS Lett.* **121:** 345–348). In the present work, this peroxisomal THCA-oxidizing system has been studied in more detail. The peroxisomes were prepared by sucrose gradient centrifugation. By use of different marker enzymes, it was confirmed that the major part of the activity in the light mitochondrial fraction was located in the peroxisomes. The reaction was absolutely dependent on the presence of Mg^{2+}, CoA, ATP, and NAD^+ in the reaction medium. In addition to cholic acid, small amounts of 3α,7α,12α,24-tetrahydroxy-5β-cholestanoic acid were detected as product. Provided the peroxisomes were preincubated with ATP and CoA, the reaction was linear with time up to 75 min. It was linear with peroxisomal protein and the pH optimum was 8. The reaction was stimulated by FAD (ca. 50%), by cytsolic protein (about twofold), by microsomal protein (about twofold), bovine serum albumin (about sevenfold), and by KCN (75% at 1 mM). In the absence of bovine serum albumin in the medium the K'_m for the overall reaction was 1.4×10^{-6} M and the maximum rate was 4.3 nmol × mg^{-1} × hr^{-1}. In the presence of bovine serum albumin, the K'_m increased to 6.3×10^{-6} M and the maximum rate to about 32 nmol × mg^{-1} × hr^{-1}. It is concluded that rat liver peroxisomes contain enzymes able to catalyze the cleavage of the side chain of THCA. The enzymes involved may be similar to but not necessarily identical with those responsible for β-oxidation of fatty acids. The findings strongly suggest that peroxisomes are important in the normal formation of bile acids.—**Kase, F., I. Björkhem, and J. I. Pedersen.** Formation of cholic acid from 3α,7α,12α-trihydroxy-5β-cholestanoic acid by rat liver peroxisomes. *J. Lipid Res.* 1983. **24:** 1560–1567.

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The oxidative cleavage of the side chain of 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA) is considered to be the last step in the biosynthesis of cholic acid (1, 2). 3α,7α,12α,24-Tetrahydroxy-5β-cholestanoic acid (24-OH-THCA) has been proposed as an intermediate in the reaction (3–5). Introduction of the 24-hydroxyl group into THCA has been demonstrated both with the mitochondrial (5, 6) and the microsomal (5) fraction of rat liver in combination with the 100,000 *g* supernatant fluid. The 24-hydroxylation reaction catalyzed by the latter system has been shown to be due to a combined action of a desaturase and a hydratase (5).

The final cleavage of the side chain of 24-OH-THCA may be catalyzed by the mitochondrial fraction (4, 6, 8), the soluble fraction (4, 6), or by a combination of the microsomal fraction and the 100,000 *g* supernatant (8).

Recently we found that a peroxisomal-enriched fraction was the most active subcellular fraction of rat liver in catalyzing the overall conversion of THCA to cholic acid (9). The reaction was dependent on the presence of ATP, CoA, NAD^+, and Mg^{2+} in the incubation medium (9), suggesting a certain similarity to the fatty acid oxidizing system present in the peroxisomal fraction of rat (10, 11) and human (12) liver.

In the present work we have further characterized the THCA-oxidizing system in rat liver peroxisomes. Our findings may explain the variable results previously published in this field. Furthermore, the results suggest that peroxisomes are important in the biosynthesis of the primary bile acids.

EXPERIMENTAL PROCEDURES

Materials

3α,7α,12α-Trihydroxy-5β-[7β-^{3}H]cholestanoic acid (0.2 mCi/μmol) as well as the unlabeled compound were prepared as described (8). The ^{3}H-labeled compound was purified immediately before use by high pressure liquid

Abbreviations: GLC–MS, gas–liquid chromatography–mass spectrometry; HPLC, high pressure liquid chromatography; EDTA, ethylenediaminetetraacetic acid; Hepes, N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid; THCA, 3α,7α,12α-trihydroxy-5β-cholestanoic acid; 24-OH-THCA, 3α,7α,12α,24-tetrahydroxy-5β-cholestanoic acid.

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chromatography (HPLC) using acetate buffer in methanol as eluting solvent as described below. With phosphate buffer in methanol as eluent (see below), it was found to consist of about 28% of the 25*R* and about 72% of the 25*S* isomer. The two C-25 stereoisomers were not separated since both forms are converted equally well into cholic acid by rat liver subcellular fractions (8). This was confirmed also with the present peroxisomal fractions.

^{14}C-Labeled cholic acid was from The Radiochemical Centre, Amersham, England. NAD^+, ATP, and CoA were from Sigma Chemical Co., St. Louis, MO. Dextran T 40 was from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Maxidens was from Nyegaard & Co. A/S, Oslo, Norway. All solvents were analytical or HPLC grade. Other chemicals were standard commercial high purity materials.

Preparation of liver subcellular fractions

Male Wistar rats weighing 200–250 g were used. They were given a commercial pellet diet. The liver was finely minced and homogenized by one stroke in a Potter Elvehjem homogenizer. From the 10% liver homogenate in 0.25 M sucrose, 1 mM EDTA (pH 6.5 with Trizma Base), a postnuclear supernatant was prepared by centrifugation at 2200 rpm (600 g_{av}), for 10 min in the HB 4 rotor in a Sorvall RC2-B centrifuge. The pellet was rehomogenized and the suspension was recentrifuged. The combined superanatants were centrifuged at 6500 rpm (4900 g_{av}) for 10 min in the same rotor. The 4900 g_{av} supernatant was centrifuged at 16,200 rpm (24,200 g_{av}) for 10 min in the SS 34 rotor. The resulting pellet (light mitochondrial fraction) was washed once and resuspended in the homogenization medium. The 24,200 g_{av} supernatant was centrifuged at 29,000 rpm (75,000 g_{av}) for 1 hr in the 30 rotor of a Beckman ultracentrifuge. The resulting microsomal pellet was resuspended in 0.25 M sucrose, 15 mM Hepes, pH 7.4. The supernatant was used as cytosolic fraction.

Preparation of peroxisomes

Peroxisomal-enriched fractions were separated on a linear sucrose gradient (24–48% (w/w)) with Dextran T 40 (2–3.8% (w/w)) (13) in a vertical rotor (Beckman VTi 50). The light mitochondrial fraction was layered on the top of the gradient and centrifuged at a time integral of $rpm^2\ dt = 3.0 \times 10^{10}\ s^{-1}$ at 20,000 rpm (35,000 g_{av}) in a Sorvall OTD 50B centrifuge (14). Fractions of 3 ml were collected in a Beckman universal fraction recovery system. The tubes were punctured in the bottom and Maxidens was used as displacement fluid.

The fractions with the highest activities of the peroxisomal marker enzymes were pooled (no. 13–15, see Fig. 2) and diluted 1:3 with 0.25 mM sucrose and 15 mM Hepes, pH 7.4. The peroxisomes were subsequently sedimented at 75,000 g_{av} for 1 hr in the 30 rotor of a Beckman ultracentrifuge. The resulting pellet was resuspended in a minimal volume of the dilution medium to a concentration of 10–20 mg of protein/ml.

The following marker enzymes were used: cytochrome *c* oxidase (15), monoamine oxidase (16), rotenone-insensitive NADPH-cytochrome *c* reductase (17), urate oxidase (18), catalase (19), and β-N-acetyl-D-glucosaminidase (20). Protein was determined by the method of Lowry et al. (21).

Incubation, extraction, and chromatographic procedures

All incubations were performed in duplicate. Under standard conditions the incubation mixture contained the following in 1.5 ml of 0.1 M Tris-HCl buffer, pH 8.0: 7.5 mM ATP, 2.6 mM CoA, 10 mM $MgCl_2$, 0.5 mg of peroxisomal protein, and 8.7 μM THCA with 50,000–100,000 cpm of labeled THCA in 5 μl of ethanol. Alterations are given in the figure legends. After 15 min preincubation at 37°C, the reaction was started by the addition of 2 mM NAD^+ and the incubation was continued for 60 min. The reaction was terminated by the addition of 30 μl of 6 N KOH. After 30 min of hydrolysis at 50°C, the mixture was acidified by HCl and extracted twice with 9 ml of ethyl acetate. The ethyl acetate was washed twice with water, evaporated under N_2, and the residue was redissolved in 100 μl of methanol. Two to ten percent of the radioactivity was lost in the water phase. Omission of the hydrolysis step resulted in the loss of an appreciable proportion of the radioactivity.

Aliquots of the extracts were injected into a Spectra-Physics HPLC instrument fitted with a Zorbax ODS column (5.0 × 250 mm) and eluted with 18.5–20% 10 mM acetate buffer (pH 4.37) in methanol (1 ml/min) and 1-ml fractions were collected. In some experiments 24% 25 mM H_3PO_4/KH_2PO_4, pH 3.4, in methanol was used as eluent. This system resolved the 25*R* and the 25*S* form of THCA.

Counting solution (Packard Insta-Gel II, 6 ml) was added and the fractions were counted in a Packard Tri-Carb liquid scintillation spectrometer. Conversion was calculated from the percentage distribution of radioactivity. To locate the cholic acid formed, ^{14}C-labeled cholic acid was used as a reference. Recovery of radioactivity from the HPLC column was essentially complete.

Combined gas–liquid chromatography–mass spectrometry

Aliquots of the extracts were converted into the methyl ester-trimethylsilyl ether derivatives (22) and analyzed by combined GLC–MS using a 1.5% SE column at 280°C and the LKB 9000 instrument (LKB Instruments Inc., Stockholm, Sweden) equipped with a multiple ion detec-

tor. For identification of the derivative of cholic acid, the multiple ion detector was focused on the ions at m/e 368 (M − 3 × 90), m/e 253, m/e 623 (M − 15), and 638 (M). For identification of the derivative of 24-OH-THCA, the instrument was focused on the ions at m/e 253, m/e 498 (M − 3 × 90), and m/e 588 (M − 2 × 90).

RESULTS

After incubation of a peroxisomal-enriched fraction with THCA in the presence of ATP, CoA, NAD^+, and Mg^{2+}, two products were obtained as shown by HPLC of the incubation extract (**Fig. 1**). The elution volume of the major product (peak I in Fig. 1) was identical to that of cholic acid. The identity of this product as cholic acid was confirmed by analysis of the trimethylsilyl ether derivative of the methyl ester by the selected ion monitoring technique. In similarity with the corresponding derivative of authentic cholic acid, the material contained ions at m/e 253, m/e 368 (M − 3 × 90), m/e 623 (M − 15), and m/e 638 (M). The peak obtained in all the above tracings had a retention time identical with that of the derivative of authentic cholic acid. In order to identify the material in the smaller peak II (Fig. 1), the extract was chromatographed on a Zorbax-ODS column with a solvent consisting of 24% 25 mM potassium phosphate, pH 3.4, in methanol. In this system most of the activity of peak II eluted in one fraction shortly after cholic acid. After derivatization as above, the major part of this material was shown to be identical with the derivative of authentic 24-OH-THCA by use of the ions at m/e 253, m/e 498 (M − 3 × 90), and m/e 588 (M − 2 × 90). The smaller peak in the HPLC-chromatogram corresponding to 24-OH-THCA was consistently seen in all incubations. It increased with time up to 45–60 min, when it leveled off.

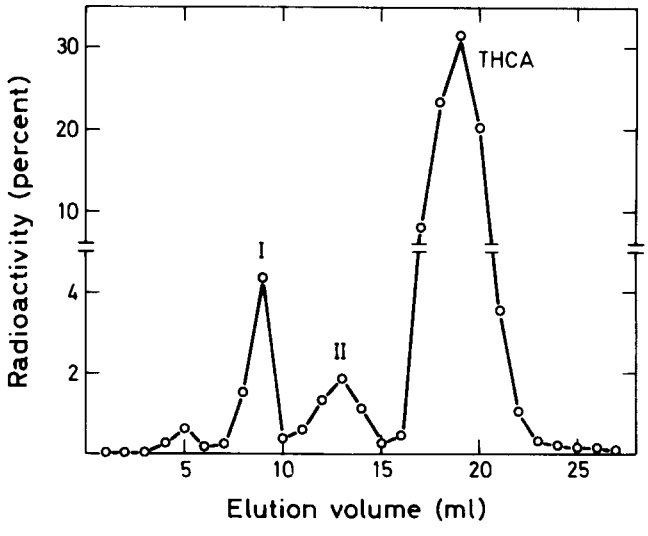

Fig. 1. Reversed phase HPLC of ethyl acetate extract of incubation with THCA and rat liver peroxisomes. The incubation conditions, extraction, and chromatographic procedures were as described in Experimental Procedures. The product peak I corresponds to cholic acid, peak II contains 24-OH-THCA.

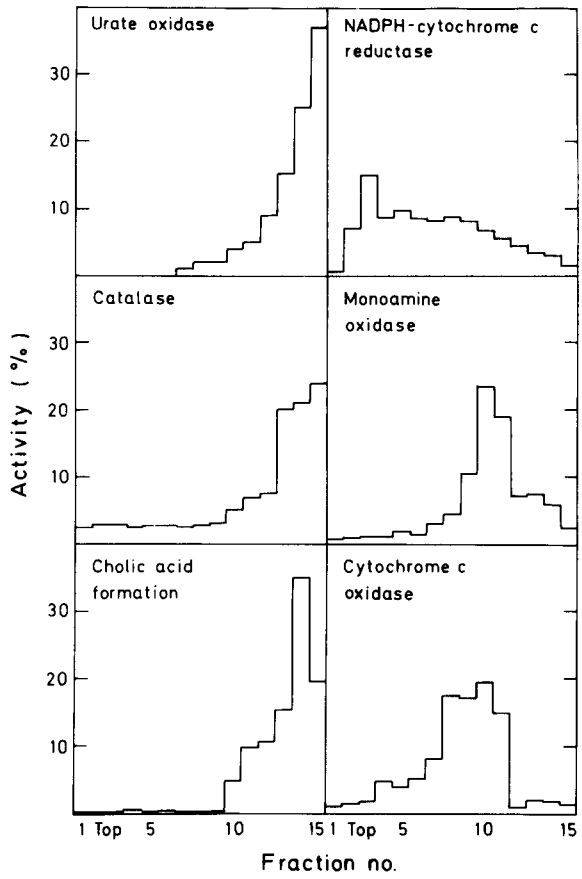

Fig. 2. Profiles of enzyme activity obtained after sucrose gradient centrifugation. A light mitochondrial fraction from rat liver was layered on top of a linear sucrose gradient (24–48% w/w) and centrifuged in a vertical rotor as described in Experimental Procedures. The gradient was fractionated into 3-ml fractions. Enzyme activities are expressed as percentage of the amount in the whole gradient.

Localization of the THCA-oxidizing system to the peroxisomes was evident from the profile of activity obtained after the sucrose gradient centrifugation of the light mitochondrial fraction. The capacity for cholic acid formation in the different fractions closely followed the enzyme markers for peroxisomes, urate oxidase, and catalase (**Fig. 2**).

The rate of cholic acid formation was linear with time up to 75 min provided that the peroxisomes and the substrate were preincubated with CoA, ATP, and Mg^{2+} for 15 min prior to the addition of NAD^+ (**Fig. 3A**). Without this preincubation a lag phase of 10–15 min duration was observed before the reaction entered a linear phase. This lag phase probably corresponds to the time

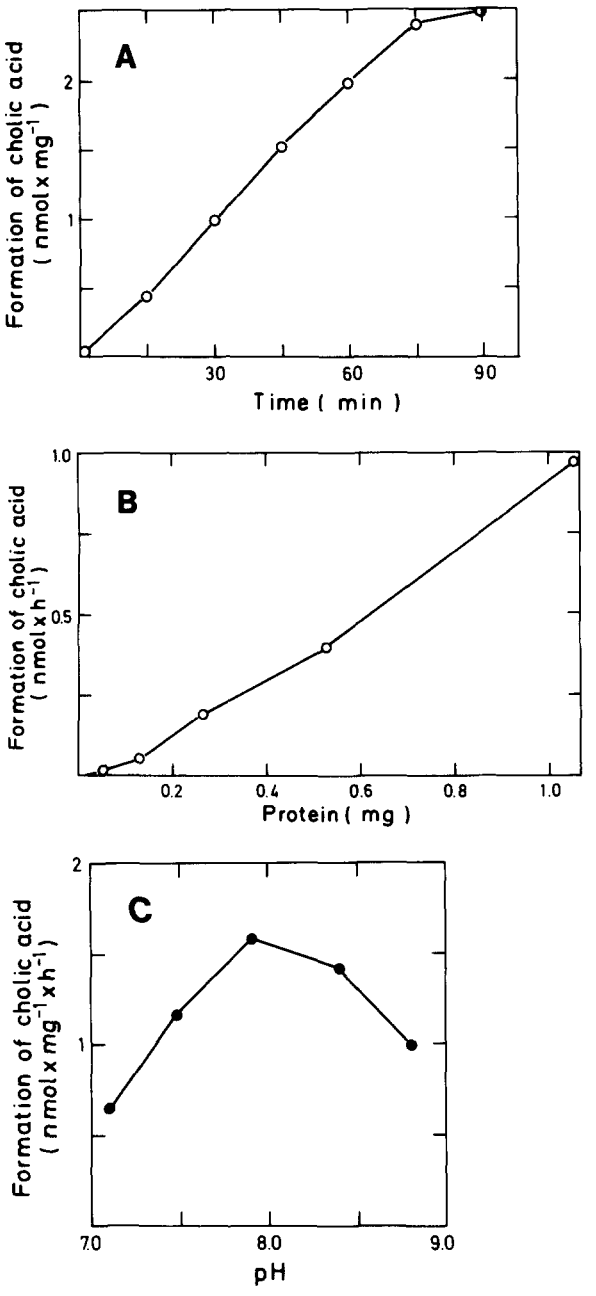

Fig. 3. Effect of time (A), protein (B), and pH (C) on cholic acid formation from THCA by rat liver peroxisomes. Standard incubation conditions were used with the following alterations: CoA, 0.9 mM; NAD^+, 1 mM; ATP, 2.5 mM; volume (in B), 0.75 ml; and pH (in A and B), 8.5. Otherwise the conditions were varied as shown in the figures.

required for formation of sufficient amounts of THCA-CoA to saturate the oxidase. The formation of cholic acid was linear with the amount of peroxisomal protein (Fig. 3B), and the optimum pH was about 8 (Fig. 3C).

Cholic acid formation from THCA by the peroxisomal protein was dependent on the presence of Mg^{2+} (**Fig. 4A**), as well as of ATP (Fig. 4B). In the latter case, 5 mM was required to saturate the enzyme system. Likewise the reaction was stimulated by CoA up to 2.5×10^{-3} M (Fig. 4C), after which there was a slight inhibition. The effect of NAD^+ is shown in Fig. 4D. The K_m for NAD^+ was found to be 1.7×10^{-4} M. $NADP^+$ could not replace NAD^+. The reaction was slightly (approx. 50%) stimulated by FAD (Fig. 4E), and FMN did not have any effect. The presence of bovine serum albumin (**Fig. 5**) or cytosolic protein (**Fig. 6**) in the incubation medium greatly stimulated the reaction. The stimulatory effect of bovine serum albumin approached a saturation level at about 7 mg/ml (Fig. 5), while that of cytosolic protein was reached at 0.07 mg/ml. Higher concentrations of cytosolic protein inhibited the reaction (Fig. 6). Microsomal protein also stimulated the reaction (**Table 1**). Potassium cyanide, 1 mM, stimulated the reaction about 75%. Carnitine, 0.5 mM, had no effect on the rate of the reaction. Triton X-100 stimulated the cholic acid formation about 40% at a concentration of 0.01% (v/v), with no further stimulation at higher concentrations. The peroxisomal β-oxidation of fatty acids has been reported to be stimulated tenfold by Triton X-100 (11).

The effect of substrate concentration on the rate of cholic acid formation was highly dependent on serum albumin (or cytosolic protein) in the incubation medium. The substrate saturation curve in the absence of serum albumin is shown in **Fig. 7A.** A saturation level was reached at about 8×10^{-6} M THCA. From the reciprocal plotting of the data (Fig. 7A, inset) an apparent K'_m of 1.4×10^{-6} M was obtained. The maximum rate was 4.3 nmol $\times$ mg^{-1} $\times$ hr^{-1}. The maximum rate varied between 3 and 8 nmol $\times$ mg^{-1} $\times$ hr^{-1} when several peroxisomal preparations were tested under similar conditions.

When bovine serum albumin was present in the incubation medium, the maximum rate at substrate saturation (Fig. 7B) was sevenfold higher than that obtained in the absence of added protein. Also the K'_m was increased to 6.3×10^{-6} M.

DISCUSSION

In a previous study we showed that the peroxisomal fraction apparently had the highest capacity of all the subcellular fractions of rat liver to catalyze the conversion of THCA to cholic acid (9). The present study has confirmed the presence of a peroxisomal THCA-oxidizing system and improved conditions for the overall reaction have been determined.

The in vitro requirements established in the present work indicate that the conversion of THCA to cholic acid encompasses several steps, in agreement with current concepts of cholic acid formation (1, 2, 4–8).

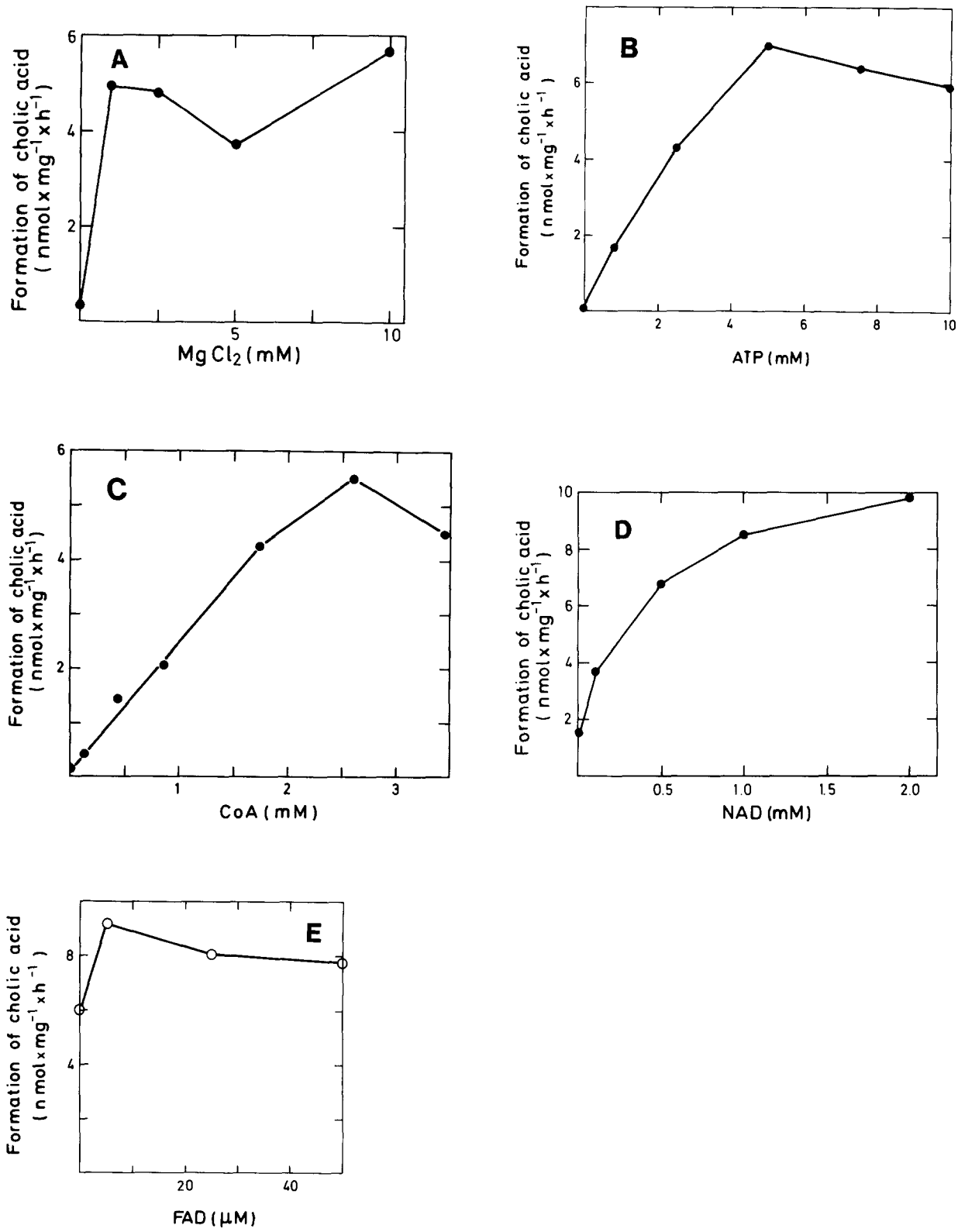

Fig. 4. Effect of concentration of $MgCl_2$ (A), ATP (B), CoA (C), NAD^+ (D), and FAD (E) on the formation of cholic acid from THCA by rat liver peroxisomes. Standard incubation conditions were used with the following alterations: NAD^+, 1 mM and pH, 8.5 in A, B, and C; ATP, 2.5 mM in A and C; CoA, 0.9 mM in A; incubation time 30 min in D and E; peroxisomal protein, 0.4 mg in A and 0.25 mg in E; bovine serum albumin 6.7 mg/ml in E. Otherwise the conditions were varied as shown in the figures.

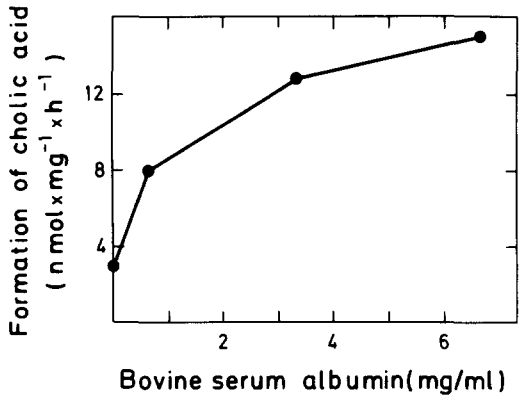

Fig. 5. Effect of concentration of bovine serum albumin on the formation of cholic acid from THCA by rat liver peroxisomes. Peroxisomal protein, 0.2 mg, was incubated for 30 min under conditions as described in Experimental Procedures.

Apparently, THCA is first activated to THCA-CoA by CoA and ATP. The lag phase observed in the formation of cholic acid suggests that, under the specific in vitro conditions used here, the activation may be a rate-limiting step in the overall reaction.

No systematic study has previously been published on the compartments involved in the activation of THCA. It is well established that rat liver peroxisomes contain a fatty acyl-CoA synthetase with a capacity sufficient to maintain maximal peroxisomal β-oxidation (23). Most likely, the activation of THCA in our in vitro system takes place in the peroxisomes and not in contaminating microsomes. A contribution of microsomal activation cannot be excluded, however. The ratio of the specific NADPH-cytochrome *c* reductase activity in the purified peroxisomal fraction to that in the microsomal fraction was 0.34. It can thus be estimated that up to one-third of the protein in the peroxisomal fraction may be contaminating microsomal protein.

If activation of THCA to THCA-CoA is rate-limiting in our in vitro system, the stimulation of cholic acid formation by addition of microsomal fraction to the peroxisomes could be explained by microsomal activation of THCA. However, the stimulatory effect could equally well be similar to that observed with added bovine serum albumin or cytosolic protein (Figs. 5 and 6).

The stimulatory effect of bovine serum albumin and cytosolic protein on cholic acid formation may be due to binding of substrate or product. A fatty acid binding protein has been proposed to function as a carrier in the transfer of acyl-CoA derivatives through the peroxisomal membrane (24). It is possible that a similar protein is involved in the peroxisomal oxidation of THCA. Binding of the substrate to albumin may also increase the critical micellar concentration and thus increase the concentration of THCA-monomeres over that in a protein-free aqueous medium.

The identification of 24-OH-THCA as a reaction product indicates that this steroid may be an intermediate in cholic acid formation as suggested by previous studies (6). Formation of 24-OH-THCA from THCA by a combination of the microsomal and cytosolic fractions of a liver homogenate has been considered to be the result of the combined action of a desaturase and a hydratase (5). These part-reactions may be similar to those catalyzed by the FAD-containing fatty acyl-CoA oxidase and the enoyl-CoA hydratase identified as part of the peroxisomal fatty acid β-oxidation system (for review, see ref. 25). Our finding of a stimulatory effect of FAD on the conversion of THCA to cholic acid indicates that the THCA-CoA oxidase is a FAD-containing flavoprotein.

The requirement of NAD^+ suggests that the further oxidation of 24-OH-THCA-CoA to cholic acid is similar to the NAD^+-dependent β-OH-acyl-CoA dehydrogenase reaction and the thiolytic cleavage reaction in peroxisomal fatty acid β-oxidation (25).

The part-reactions in the peroxisomal formation of

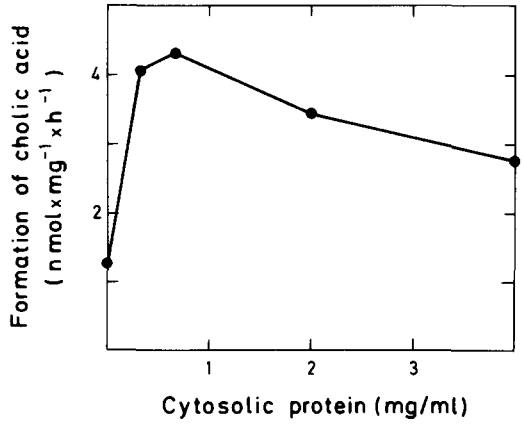

Fig. 6. Effect of cytosolic protein on the formation of cholic acid from THCA by rat liver peroxisomes. Peroxisomal protein, 0.25 mg, was incubated with cytosolic protein (75,000 g_{av} supernatant) as shown in the figure for 30 min under conditions as described in Experimental Procedures.

TABLE 1. Effect of microsomal protein on the formation of cholic acid from THCA by rat liver peroxisomes

	Experiment Number					
	1	2	3	4	5	6
Peroxisomal protein (mg)	0.3	0.3	0.3	0	0	0
Microsomal protein (mg)	0	0.3	0.6	0.3	0.6	0
Formation of cholic acid (nmol × hr^{-1})	2.1	4.2	3.5	0.3	0.5	0

Peroxisomal and microsomal protein in amounts as given in the table were incubated as described in Experimental Procedures, except that the concentration of ATP was 2.5 mM and of CoA was 0.9 mM.

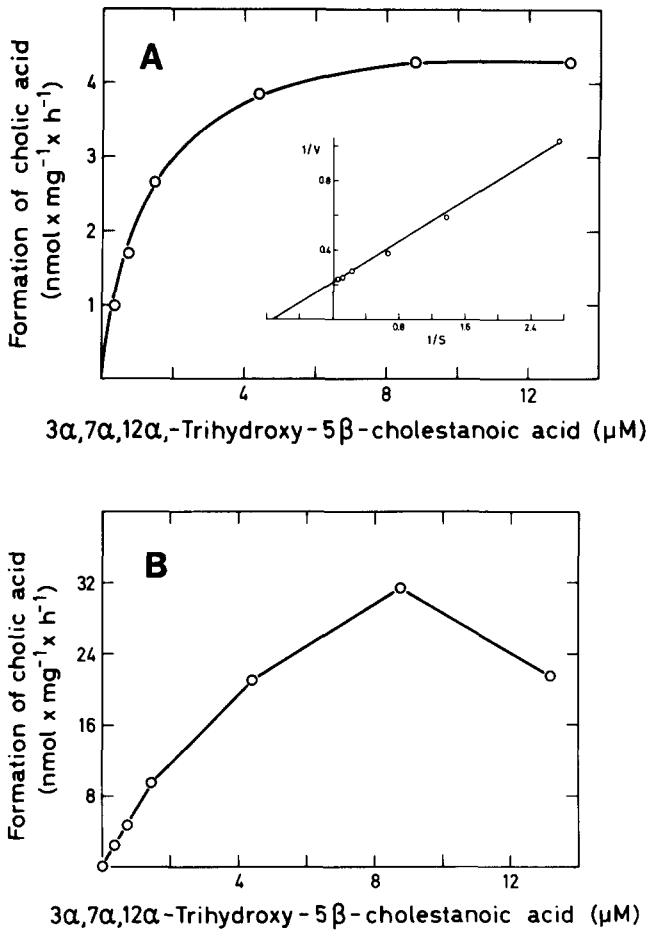

Fig. 7. Effect of THCA concentration on the formation of cholic acid by rat liver peroxisomes in the absence (A) and presence (B) of bovine serum albumin. Peroxisomal protein, 0.35 mg, was incubated as described in Experimental Procedures with the addition of 5 μM FAD. Bovine serum albumin (B) was added at a concentration of 6.7 mg/ml. The inset in A represents the reciprocal plotting of the data.

cholic acid from THCA may thus all be similar to those of the peroxisomal fatty acid oxidizing system. For the moment we cannot decide whether the same enzymes are active on both groups of substrates or if separate enzyme systems are involved. A possible interplay between long chain fatty acids and bile acid intermediates raises interesting regulatory aspects in this regard. The maximum rate of THCA oxidation to cholic acid (approx. 0.5 nmol × mg^{-1} × min^{-1}) is lower than the reported rate of peroxisomal oxidation of palmitoyl-CoA (100 nmol × mg^{-1} × min^{-1} measured as reduction of NAD) (10). These numbers cannot be directly compared, however, because of the different assay systems used and because we do not know to what extent activation of THCA to THCA-CoA is rate-limiting in the overall conversion to cholic acid.

The peroxisomal acyl-CoA synthetase involved in β-oxidation of fatty acids, is a firmly bound membrane enzyme (26). The other enzymes of the peroxisomal β-oxidation system may be more or less easily released from the particle (26, 27). The peroxisomal membranes are known to be very fragile under various in vitro conditions (28). In previous studies on the subcellular distribution of enzymes involved in the oxidation of THCA to cholic acid, the contamination of the fractions by peroxisomal protein was never evaluated. The variable results obtained (3–8), may at least partly be explained by release and redistribution of enzyme activities during subcellular preparation and contamination of the different subcellular fractions by intact or fragmented peroxisomal membranes.

The maximal specific rates of conversion of THCA to cholic acid that we have observed (ca. 30 nmol × mg^{-1} × hr^{-1}) are considerably higher than previously observed with mitochondrial or microsomal fractions (8). Taken together, the results of the present work and those of the previous study (9) clearly indicate that the conversion of THCA to cholic acid mainly takes place in the peroxisomal fraction of rat liver.

The similar oxidation of C_{27}-steroid carboxylic acids other than THCA by the peroxisomes has not yet been investigated. Of interest in this regard is the recent report that the cleavage of the side chain of cholesterol and formation of propionic acid is efficiently catalyzed by the peroxisomal fraction of rat liver (29). The mechanism of the ω-oxidation of the cholesterol side chain that must precede the oxidative cleavage was not explained, however, in that study (29).

In conclusion, we have shown that the peroxisomal fraction of rat liver can efficiently catalyze the conversion of THCA into cholic acid. Our findings indicate that the oxidation of the steroid side chain in the formation of bile acids is an important function of liver peroxisomes.

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REFERENCES

1. Danielsson, H. 1973. Mechanisms of bile acid biosynthesis. *In* The Bile Acids: Chemistry, Physiology and Metabolism. Vol. 2: Physiology and Metabolism. P. P. Nair and D. Kritchevsky, editors. Plenum Publishing Corporation, New York. 1–32.
2. Danielsson, H., and J. Sjövall. 1975. Bile acid metabolism. *Annu. Rev. Biochem.* **44:** 233–253.

3. Inai, Y., Y. Tanaka, S. Betsuki, and T. Katzuno. 1964. Stero-bile acids and bile sterols. LXVI. Synthesis of 3α,7α,12α,24ε-tetrahydroxy-coprostanic acid. *J. Biochem. (Tokyo).* **56:** 591–593.
4. Masui, T., and E. Staple. 1965. The formation of cholic acid from 3α,7α,12α,24β-tetrahydroxycoprostanic acid by rat liver. *Biochim. Biophys. Acta.* **104:** 305–307.
5. Gustafsson, J. 1975. Biosynthesis of cholic acid in rat liver. 24-Hydroxylation of 3α,7α,12α-trihydroxy-5β-cholestanoic acid. *J. Biol. Chem.* **250:** 8243–8247.
6. Masui, T., and E. Staple. 1966. The formation of bile acids from cholesterol. *J. Biol. Chem.* **241:** 3889–3893.
7. Suld, H. M., E. Staple, and S. Gurin. 1962. Mechanism of formation of bile acids from cholesterol: oxidation of 5β-cholestane-3α,7α,12α-triol and formation of propionic acid from the side chain by rat liver mitochondria. *J. Biol. Chem.* **237:** 338–344.
8. Gustafsson, J. 1980. Biosynthesis of cholic acid in rat liver: formation of cholic acid from 3α,7α,12α-trihydroxy- and 3α,7α,12α,24-tetrahydroxy-5β-cholestanoic acids. *Lipids.* **15:** 113–121.
9. Pedersen, J. I., and J. Gustafsson. 1980. Conversion of 3α,7α,12α-trihydroxy-5β-cholestanoic acid into cholic acid by rat liver peroxisomes. *FEBS Lett.* **121:** 345–348.
10. Lazarow, P. B., and C. DeDuve. 1976. A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad. Sci. USA.* **73:** 2043–2046.
11. Lazarow, P. B. 1978. Rat liver peroxisomes catalyze the β-oxidation of fatty acids. *J. Biol. Chem.* **253:** 1522–1528.
12. Bronfman, M., N. C. Inestrosa, and F. Leighton. 1979. Fatty acid oxidation by human liver peroxisomes. *Biochem. Biophys. Res. Commun.* **88:** 1030–1036.
13. Baudhuin, P. 1974. Isolation of rat liver peroxisomes. *Methods Enzymol.* **31:** 356–368.
14. Flatmark, T., E. Christiansen, and H. Kryvi. 1981. Polydispersity of rat liver peroxisomes induced by the hypolipidemic and carcinogenic agent clofibrate. *Eur. J. Cell Biol.* **24:** 62–69.
15. Slinde, E., and T. Flatmark. 1973. Determination of sedimentation coefficients of subcellular particles of rat liver homogenates. *Anal. Biochem.* **56:** 324–340.
16. Aas, M. 1971. Organ and subcellular distribution of fatty acid activating enzymes in the rat. *Biochim. Biophys. Acta.* **231:** 32–48.
17. Strobel, H. W., and J. D. Dignam. 1978. Purification and properties of NADPH-cytochrome P-450 reductase. *Methods Enzymol.* **52:** 89–96.
18. Schneider, W. C., and G. H. Hogeboom. 1952. Intracellular distribution of enzymes. IX. Certain purine-metabolizing enzymes. *J. Biol. Chem.* **195:** 161–166.
19. Chance, B., and A. C. Maehly. 1955. Catalase assay by disappearance of peroxide. *Methods Enzymol.* **2:** 764–768.
20. Barrett, A. J. 1972. α-Glucosidase, β-glucosidase, β-galactosidase, α-mannosidase, β-acetylglucosaminidase, β-glucuronidase, β-xylosidase and α-L-fucosidase. *In* Lysosomes. A Laboratory Handbook. J. T. Dingle, editor. Elsevier/North-Holland, Amsterdam, New York. 119.
21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
22. Makita, M., and W. W. Wells. 1963. Quantitative analysis of fecal bile acids by gas–liquid chromatography. *Anal. Biochem.* **5:** 523–530.
23. Krisans, S. K., R. M. Mortensen, and P. B. Lazarow. 1980. Acyl-CoA synthetase in rat liver peroxisomes. *J. Biol. Chem.* **255:** 9599–9607.
24. Appelkvist, E. L., and G. Dallner. 1980. Possible involvement of fatty acid binding protein in peroxisomal β-oxidation of fatty acids. *Biochim. Biophys. Acta.* **617:** 156–160.
25. Lazarow, P. B. 1982. Compartmentation of β-oxidation of fatty acids in peroxisomes. *In* Metabolic Compartmentation. H. Sies, editor. Academic Press, New York. 317–329.
26. Alexson, S., H. Shio, and P. B. Lazarow. 1982. Intraperoxisomal localization of β-oxidation enzymes in rat liver. *Hoppe-Seyler's Z. Physiol. Chem.* **363:** 968–969.
27. Hayashi, H., S. Hino, and F. Yamasaki. 1981. Intraparticulate localization of some peroxisomal enzymes related to fatty acid β-oxidation. *Eur. J. Biochem.* **120:** 47–51.
28. Bronfman, M., and H. Beaufay. 1973. Alteration of subcellular organelles induced by compression. *FEBS Lett.* **36:** 163–168.
29. Hagey, L. R., and S. K. Krisans. 1982. Degradation of cholesterol to propionic acid by rat liver peroxisomes. *Biochem. Biophys. Res. Commun.* **107:** 834–841.