Subcellular localization of 3α,7α-dihydroxy- and 3α,7α,12α-trihydroxy-5β-cholestanoyl-coenzyme A ligase(s) in rat liver

Kristian Prydz, Bengt Frode Kase, Ingemar Björkhem,* and Jan I. Pedersen[1]

Institute for Nutrition Research, School of Medicine, University of Oslo, Oslo, 3, Norway, and Karolinska Institutet at Huddinge University Hospital,* Stockholm, Sweden

Abstract Liver peroxisomes from both rat and humans have previously been shown to contain enzymes that catalyze the oxidative cleavage of the C_{27}-steroid side chain in the formation of bile acids. It has not been clear, however, whether the initial step, formation of the CoA-esters of the 5β-cholestanoic acids, also occurs in these organelles. In the present work the subcellular localization of 3α,7α,12α-trihydroxy-5β-cholestanoyl-CoA (THCA-CoA) ligase (THCA-CoA synthetase) and of 3α,7α-dihydroxy-5β-cholestanoyl-CoA (DHCA-CoA) ligase in rat liver has been investigated. Main subcellular fractions and peroxisome-rich density gradient fractions from rat liver were incubated with THCA or DHCA, CoA, ATP, and Mg^{2+}. Formation of THCA-CoA and DHCA-CoA was determined after high pressure liquid chromatography of the incubation extracts. The microsomal fraction contained the highest specific (and also relative specific) activity both for the formation of THCA-CoA and DHCA-CoA. The rates of THCA-CoA formation were further increased from 124–159 nmol/mg · hr^{-1} in crude microsomal fractions to 184–220 nmol/mg · hr^{-1} when studied in purified rough endoplasmic reticulum fractions. Formation of THCA-CoA in peroxisomal fractions prepared in Nycodenz density gradients could be accounted for by a small contamination (3–7%) by microsomal protein. The distribution of THCA-CoA ligase was different from that of palmitoyl-CoA ligase that was found to be localized also to the peroxisomal fractions. —**Prydz, K., B. F. Kase, I. Björkhem, and J. I. Pedersen.** Subcellular localization of 3α,7α-dihydroxy- and 3α,7α,12α-trihydroxy-5β-cholestanoyl-coenzyme A ligase(s) in rat liver. *J. Lipid Res.* 1988. **29:** 997–1004.

Supplementary key words bile acids • cholic acid • chenodeoxycholic acid

It is now well established that formation of cholic acid and chenodeoxycholic acid involves intermediate formation of 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA) and 3α,7α-dihydroxy-5β-cholestanoic acid (DHCA), respectively (1–3). Conversion of THCA into cholic acid (and of DHCA into chenodeoxycholic acid) is most efficiently catalyzed by peroxisome-rich fractions from both rat and human liver (4–7) and requires the presence of CoA, NAD, ATP, and Mg^{2+} in the incubation mixture (4–7). The reaction sequence is similar to the peroxisomal β-oxidation of fatty acids, and involves the initial activation to THCA-CoA (or to DHCA-CoA) by a ligase (synthetase). The fatty acyl-CoA ligase (fatty acid:CoA ligase) activities in rat liver are distributed among mitochondria, peroxisomes, and microsomes, the relative activity depending on chain length of the fatty acids (8). About 6–12% of the palmitoyl-CoA ligase activity was found to be associated with the peroxisomes (8).

The distribution of THCA-CoA and DHCA-CoA ligase activities among liver organelles has not been investigated previously and it is not known whether it is similar to that of the fatty acid:CoA ligase. In the present work we have studied the subcellular distribution of THCA-CoA and DHCA-CoA ligase in rat liver. It was found that these enzyme activities are localized almost entirely, if not exclusively, to the endoplasmic reticulum. By the subcellular fractionation methods employed, we could not obtain evidence for the presence of enzymes activating THCA or DHCA in peroxisomes.

MATERIALS AND METHODS

Chemicals

[7β-^{3}H]3α,7α,12α-Trihydroxy-5β-cholestanoic acid (THCA, 200 Ci/mol), [7β-^{3}H]3α,7β-dihydroxy-5β-cholestanoic acid (DHCA, 200 Ci/mol), and the corresponding unlabeled compounds were prepared as previously described (9). [7β-^{3}H]THCA-CoA (1.3 Ci/mol) was chemically synthesized as described (10). The substrates were purified

Abbreviations: THCA, 3α,7α,12α-trihydroxy-5β-cholestanoic acid; DHCA, 3α,7α-dihydroxy-5β-cholestanoic acid; HPLC, high pressure liquid chromatography; EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

[1]To whom correspondence should be addressed.

before use by high pressure liquid chromatography (7). [^{14}C]cholic acid and [^{14}C]chenodeoxycholic acid used as standards were from the Radiochemical Centre, Amersham, England. Nycodenz and Maxidenz were from Nycomed A/S, Oslo, Norway. Other chemicals were from Sigma Chemical Co., St. Louis, MO or E. Merck, Darmstadt, F.R.G. All solvents were of analytical or HPLC grade.

Preparation of liver subcellular fractions

Male Wistar rats (170–240 g) given a commercial pellet diet and water ad libitum were used. The animals were fasted overnight and killed between 8 and 9 AM. The liver was finely minced and homogenized in ice-cold 0.25 M sucrose, 1 mM EDTA, pH 6.5, with Trizma base (6.5 vol, v/w), by one stroke in a Potter-Elvehjem homogenizer with a loose-fitting pestle. The homogenate was centrifuged for 10 min at 2,200 rpm (600 g_{av}) in the HB 4 rotor in a Sorvall RC2-B centrifuge. The pellet was rehomogenized (3.5 vol, v/w) and the suspension was recentrifuged. The combined postnuclear supernatants were centrifuged at 6,500 rpm (4,900 g_{av}) for 10 min in the same rotor, giving the heavy mitochondrial (M) fraction as a pellet. The pellet was washed once in the homogenization medium. The combined 4,900 g_{av} supernatant was centrifuged at 16,200 rpm (24,000 g_{av}) for 10 min in the SS-34 rotor. The resulting pellet (light mitochondrial (L) fraction) was washed once, and the combined supernatants were centrifuged at 30,000 rpm (60,000 g_{av}) for 1 hr in the Ti 60 rotor using a Sorvall OTD 55B ultracentrifuge to obtain the microsomal (P) pellet. The supernatant was used as the cytosolic fraction. All pellets were resuspended in 0.25 M sucrose, 15 mM HEPES, pH 7.4.

Subfractionation of the light mitochondrial (L) fraction (7)

The light mitochondrial fraction (50–100 mg of protein) was layered on top of linear Nycodenz gradients ranging from either 10% or 15% (w/v) in 0.25 M sucrose, 1 mM HEPES, pH 7.4, and 1 mM EDTA to 48% or 45% in 1 mM HEPES and 1 mM EDTA. The gradient tubes contained a prelayered 3 ml Maxidenz cushion and were centrifuged at 20,000 rpm (35,000 g_{av}) for 75 min in a Sorvall OTD 55 B centrifuge using a Beckman VTi 50 vertical rotor. In some experiments (cf. Fig. 4) the centrifugation conditions were altered to 30,000 rpm (74,000 g_{av}) for 20 min. By this procedure a somewhat better separation between peroxisomes and microsomes was obtained. Fractions of 2.5 ml were collected.

The following marker enzymes were used: monoamine oxidase (11), glutamate dehydrogenase (12), rotenone-insensitive NADPH-cytochrome *c* reductase (13), esterase (14), catalase (15), D-amino acid oxidase (15), β-acetyl glucosaminidase (16), succinate dehydrogenase (17). Palmitoyl-CoA ligase was measured as described by Krisans, Mortenson, and Lazarow (8). This enzyme activity was inhibited by Nycodenz which had to be removed by centrifugation (100,000 *g* for 1 hr) from the gradient fractions. This procedure reduced the recovery of protein from the gradient from 95–100% to about 70%. Other enzyme activities were not inhibited by Nycodenz. Protein was measured by the method of Lowry et al. (18) or by the modified method of Bensadoun and Weinstein (19) in fractions containing Nycodenz.

Subfractionation of the microsomal (P) fraction

The microsomal fraction was further fractionated into three subfractions (rough and smooth endoplasmic reticulum and Golgi apparatus) on a discontinuous CsCl-sucrose gradient as previously described (20). The gradient was centrifuged at 25,000 rpm for 5 hr in a Beckman SW 27 swing-out rotor.

Incubation, extraction, and chromatographic procedures

All incubations were performed in duplicate. Under standard conditions the incubation mixture contained the following in 0.75 ml of 0.1 M Tris-HCl buffer, pH 8.0: 2 mM CoA, 7.5 mM ATP, 10 mM $MgCl_2$, 12 mM DTT, and 0.67 mg/ml bovine serum albumin. The amount of protein was usually 100 μg per incubation. The reaction was started by the addition of [^{3}H]THCA or [^{3}H]DHCA (100,000–200,000 cpm) in less than 6 μl of ethanol, to a final concentration of 23 μM, or, when indicated, 44 μM. The control incubations were either in the absence of enzyme protein, or the enzyme protein was replaced by an equivalent amount of bovine serum albumin, or in the absence of either ATP/Mg or CoA. After 30 min of incubation at 37°C under air, the reaction was terminated by the addition of 100 μl of 2 M HCl and 2 ml of butanol. One ml of water was added and the aqueous phase was extracted 6 times with 2 ml of butanol. The combined butanol phase was evaporated under reduced pressure in a Rotavapor (Buchi, Switzerland), and the dry residue was dissolved in methanol, concentrated to dryness under nitrogen, and redissolved in 150 μl of the HPLC eluent.

Aliquots of the incubation extracts were analyzed by HPLC using a Zorbax ODS column (4.6 × 250 mm) and assayed for radioactivity as previously described (5). The extracts of the incubations with DHCA were eluted with 38.5% 2-propanol in 50 mM phosphate buffer, pH 7.00 (21), giving the elution volumes of DHCA and DHCA-CoA equal to 35 ml and 19 ml, respectively. The extracts of the incubations with THCA were eluted with 33.5% 2-propanol in 50 mM phosphate buffer, pH 7.00. The elution volumes of THCA and THCA-CoA were 34 ml and 19 ml, respectively. Recovery of radioactivity from incubation to scintillation counting was above 95%.

RESULTS

Assay conditions for the measurement of THCA-CoA formation

After incubation of rat liver subcellular fractions with THCA in the presence of ATP, CoA, and $MgCl_2$, one product was obtained as shown by HPLC of the incubation extract (**Fig. 1**). The elution volume of the product peak was identical to that of chemically synthesized THCA-CoA (10). The content of the product peak could be completely reconverted to THCA after mild alkaline hydrolysis (50°C, 30 min) and no radioactivity corresponding to hydroxylated derivatives of THCA appeared in the HPLC profile after this treatment.

In preliminary experiments we incubated subcellular fractions (equal amount of protein) with concentrations of substrate, CoA, ATP, and $MgCl_2$ found to be optimal for the overall conversion of THCA into cholic acid (5). Under these conditions the highest specific activity of THCA-CoA formation was found in the microsomal (P) fraction (45 nmol/mg of protein $\cdot$ hr^{-1}) and the formation was linear with time for at least 45 min. The conversion of THCA into THCA-CoA catalyzed by the microsomal fraction was examined with respect to dependence on substrate, protein, and different cofactors. Addition of bovine serum albumin to the incubation mixture stimulated the reaction (**Fig. 2A**), and in its presence the reaction was linear with microsomal protein up to about 0.2 mg. The presence of dithiothreitol stimulated the reaction even more and markedly affected the CoA dependency of the reaction (Fig. 2B). In the presence of dithiothreitol, the saturating concentration of CoA was lowered to 0.1 mM (Fig. 2B), presumably due to a protective effect of dithiothreitol on CoA. The dependence on ATP/Mg^{2+} is shown in Fig. 2C. The saturating concentration was about 10 mM. The pH optimum of the reaction was around 7.7 (Fig. 2D). Triton X-100 stimulated the reaction about 50% in the concentration range 0.05–0.1 mg/ml but was inhibitory above 0.2 mg/ml.

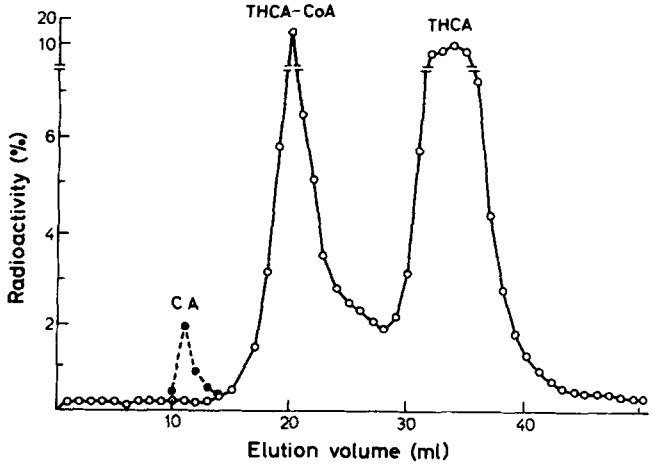

Fig. 1. Reversed phase HPLC of butanol extract of incubation with THCA and rat liver microsomes. The incubation conditions, extraction, and chromatographic procedures were as described in Materials and Methods. The chromatogram also shows the elution volume of ^{14}C cholic acid standard (CA). The eluent was 33.5% 2-propanol in 50 mM phosphate buffer, pH 7.00.

In the presence of bovine serum albumin and dithiothreitol, substrate saturation was obtained at 44 μmol/l which was about three times higher than in the absence of these stimulatory agents (Fig. 2E). From the reciprocal plotting of the data an apparent K'_m of 30 μM was found.

Subcellular distribution of THCA-CoA (and DHCA-CoA) ligase activity

Main subcellular fractions were incubated with THCA and DHCA under standard conditions as described under Materials and Methods. The results are shown in **Table 1.** The microsomal fraction most efficiently catalyzed formation both of THCA-CoA and of DHCA-CoA. In fact, when compared to the distribution of marker enzymes (**Table 2**) it is seen that both activities were distributed almost identically to those of esterase and NADPH-cytochrome *c* reductase, markers for the endoplasmic reticulum. No enrichment in relative specific activity was observed in the mitochondrial or the light mitochondrial fraction (Table 1) which would be expected if some ligase activity was located to the mitochondrial or the peroxisomal fractions.

In the experiment reported in Table 1, the specific activities for THCA-CoA and DHCA-CoA formation in a pooled peroxisomal fraction (prepared from the L-fraction on a Nycodenz gradient, cf. Materials and Methods) were 14 and 23 nmol/mg $\cdot$ hr^{-1}, i.e., slightly higher than would be expected from the microsomal contamination which in this particular preparation was found to be 6.9%. In other peroxisomal preparations this contamination was generally below 5% and the rate of THCA-CoA formation was always lower than could be accounted for by this contamination.

In order to better answer the question of a possible peroxisomal localization of a THCA-CoA ligase, the L-fraction was separated on a Nycodenz gradient and all fractions were tested for their THCA-CoA forming activity. As shown in **Fig. 3** the highest activities were found in the fractions with the highest esterase activity, i.e., corresponding to the distribution of the microsomes. The small activity detected in the peroxisome-rich fractions (2 and 3) could be entirely accounted for by the microsomal contamination as detected by the esterase activity.

In rat liver long-chain acyl-CoA ligase is localized in peroxisomes in addition to mitochondria and microsomes (8). The distribution of THCA-CoA ligase was therefore compared to that of palmitoyl-CoA ligase in an L-fraction separated on a Nycodenz gradient. The results disclosed a different distribution for the two enzyme activities

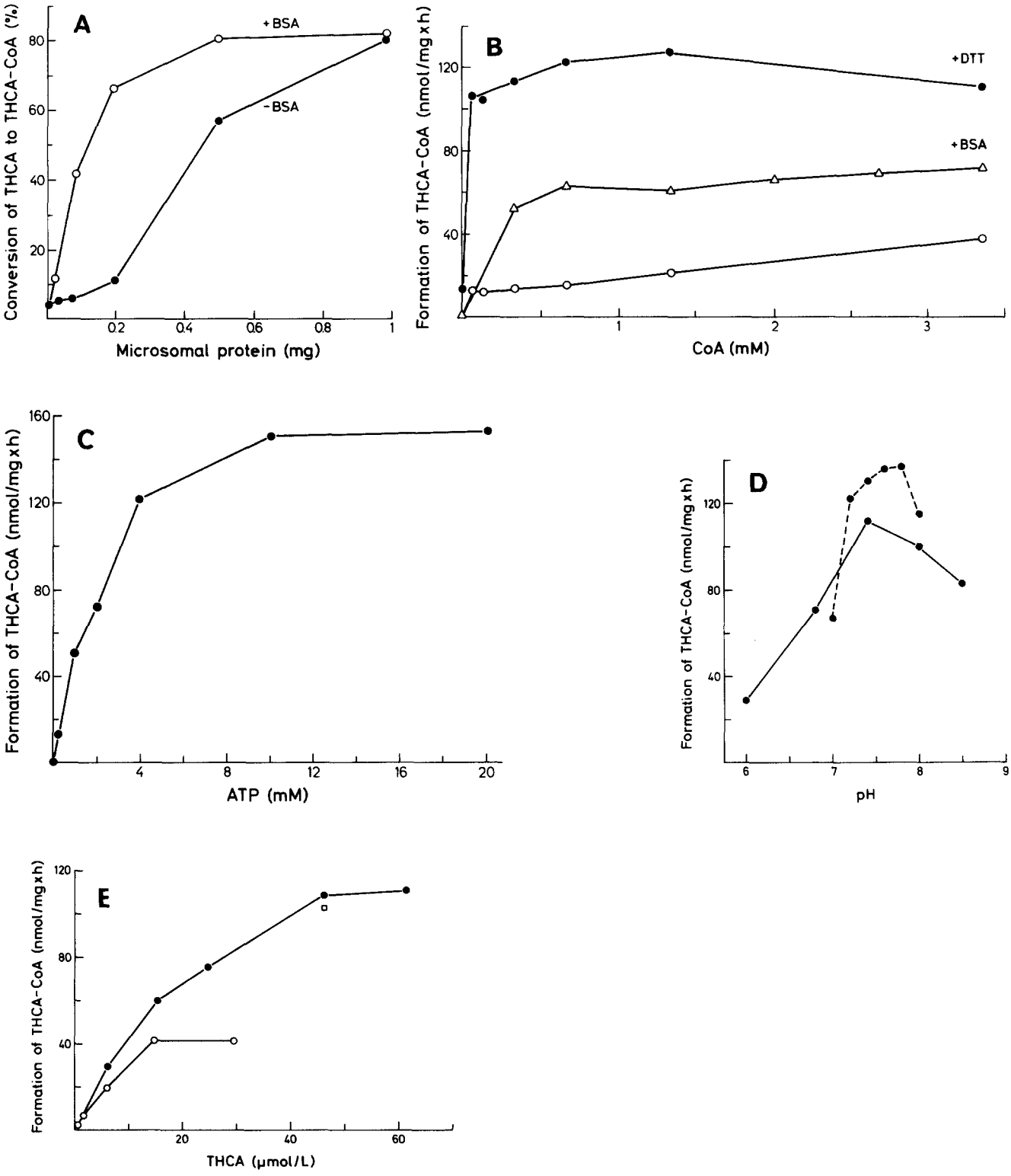

Fig. 2. Effect of concentration of microsomal protein (A) in the absence (−BSA) and in the presence (+BSA) of bovine serum albumin; of CoA (B) in the absence of bovine serum albumin and dithiothreitol (○—○), in the presence of bovine serum albumin (+BSA) and in the presence of dithiothreitol (+DTT); of ATP/$MgCl_2$ (C) (molar ratio 1 : 0.8); of pH (results of two separate experiments) (D); and of substrate (E) in the absence (○—○) and presence (●—●) of dithiothreitol and bovine serum albumin on the formation of THCA-CoA. Standard incubation conditions were used except for the variations as shown. The results are means of duplicate determinations.

TABLE 1. Formation of 3α,7α,12α-trihydroxy-5β-cholestanoyl-CoA (THCA-CoA) from 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA) and 3α,7α-dihydroxy-5β-cholestanoyl-CoA (DHCA-CoA) from 3α,7α-dihydroxy-5β-cholestanoic acid (DHCA) in subcellular fractions of rat liver

	Protein		Formation of THCA-CoA				Formation of DHCA-CoA			
Fraction	mg	%	nmol/hr	%	nmol/mg · hr^{-1}	(RSA)[a]	nmol/hr	%	nmol/mg · hr^{-1}	(RSA)[a]
E + N	3174	100	129174	100			122050	100		
N	954	30	23945	19	25.1	(0.6)	19748	16	20.7	(0.5)
M	730	23	9782	8	13.4	(0.3)	9636	8	13.2	(0.3)
L	311	10	9765	8	31.4	(0.8)	8957	7	28.8	(0.7)
P	468	15	49280	38	105.3	(2.5)	40903	34	87.4	(2.3)
S	920	29	6348	5	6.9	(0.2)	8740	7	9.5	(0.2)
Recovery		107		78				72		

The data represent the results of one of two sets of experiments with essentially the same distribution of enzyme activity and protein. E, postnuclear supernatant; N, crude nuclear fraction; M, heavy mitochondrial fraction; L, light mitochondrial fraction; P, microsomal fraction; S, cytosolic fraction.
[a]Relative specific activity, % activity/% protein.

(**Fig. 4**). Thus, the four fractions containing the highest catalase and D-amino acid oxidase activities (fraction numbers 1–4) contained 21.1% of the total palmitoyl-CoA ligase activity recovered from the gradient but only 4.7% of the recovered THCA-CoA ligase activity. The same fractions contained 2.4% of the total esterase activity (Fig. 4).

While the distribution of THCA-CoA ligase activity among subcellular fractions was essentially the same in three different preparations, the rate of microsomal THCA-CoA formation differed to some extent from one preparation to another (range 100–186 nmol/mg · hr^{-1} at a substrate concentration of 23 μM). Freezing and thawing also reduced the activity to some extent but long-term freezing did not result in any appreciable loss of activity.

Two microsomal (P) fractions catalyzing formation of THCA-CoA at rates of 124 and 159 nmol/mg of protein · hr^{-1} were further subfractionated as described in Materials and Methods into three subfractions, rough endoplasmic reticulum, smooth endoplasmic reticulum, and Golgi apparatus. The highest specific activities of THCA-CoA formation were found in the rough endoplasmic reticulum subfractions (184 and 220 nmol/mg of protein · hr^{-1}, only slightly higher than those found in the smooth endoplasmic reticulum (177 and 208 nmol/mg of protein · hr^{-1}. The Golgi apparatus catalyzed formation of THCA-CoA at significantly lower rates, <2 (detection limit) and 40 nmol/mg protein · hr^{-1}. No activity of microsomal marker enzymes could be detected in the Golgi apparatus fractions (12, 13). The finding of THCA-CoA formation in one of the Golgi fractions while no esterase or NADPH-cytochrome *c* reductase could be demonstrated does not exclude contamination of endoplasmic reticulum protein in the Golgi fraction since these enzymes may be inactivated during preparation and storage. A different degree of inactivation of endoplasmic reticulum marker enzymes in Golgi fractions has, in fact, been demonstrated (22).

TABLE 2. Subcellular distribution and relative specific activity[a] (in parentheses) of marker enzymes in rat liver

Fraction	Protein	Esterase	Rotenone-Insensitive NADPH-Cytochrome c Reductase	Monoamine Oxidase	Catalase
			%		
E + N	100	100 (1.0)	100 (1.0)	100 (1.0)	100 (1.0)
N	30	25 (0.8)	20 (0.7)	23 (0.8)	25 (0.8)
M	23	5 (0.2)	7 (0.3)	52 (2.3)	10 (0.4)
L	10	9 (0.9)	8 (0.8)	10 (1.0)	23 (2.3)
P	15	53 (3.5)	46 (3.1)	7 (0.5)	1 (0.1)
S	29	8 (0.3)	9 (0.3)	0	27 (0.9)
Recovery	107	100	90	92	86

The enzyme activities were measured as referred to in Material and Methods. Fractions are abbreviated as in Table 1.
[a]Relative specific activity, % activity/% protein.

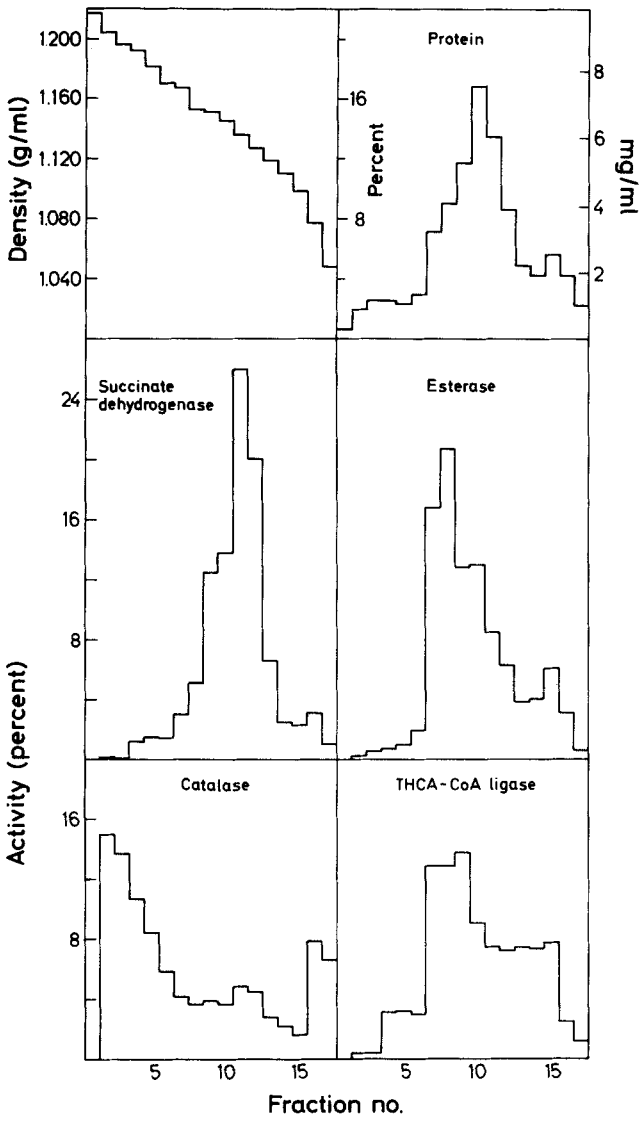

Fig. 3. Density profile and protein distribution (upper panel) and enzyme activities in fractions after centrifugation of the light mitochondrial fraction of rat liver on a Nycodenz density gradient (15–48%). The experimental procedures are described in Materials and Methods except that THCA-CoA formation was determined in single incubations and with a substrate concentration of 44 μM.

DISCUSSION

In previous studies we have shown that the conversion of THCA into cholic acid and that of DHCA into chenodeoxycholic acid are catalyzed most efficiently by the peroxisomal fraction of rat liver (4, 5, 7). A lag phase was observed in the conversion of THCA into cholic acid (5) and the possibility that activation of THCA to THCA-CoA was a rate-limiting step was discussed. Furthermore, we could not exclude the possibility that the activation reaction was catalyzed by microsomal protein contaminating the peroxisomal fraction (5, 7). From the present results we can conclude that activation of THCA and of DHCA to the corresponding CoA esters is primarily, if not exclusively, catalyzed by ligase(s) localized to the endoplasmic reticulum. The small amounts of THCA-CoA and DHCA-CoA formed in the peroxisomal fractions can be explained by contamination by microsomal protein. Based on microsomal marker enzyme activities, this contamination was of the order of 3–7%. Detection of a peroxisome-specific THCA-CoA ligase activity amounting to only a few percent of that in the microsomal fraction would require other and more specific methods than those used in the present work.

Fatty acid activation has been reported to occur in mitochondria, endoplasmic reticulum, and peroxisomes in rat liver (8) with 6–12% of the total palmitoyl-CoA ligase activity in the latter organelles. We could confirm the presence of palmitoyl-CoA ligase activity in peroxisomal fractions, and the distribution of this activity was clearly different from that of THCA-CoA ligase activity (Fig. 4). We may thus conclude that the localization of ligase(s) active on THCA and DHCA appears to be different from that of fatty acids.

The requirement for cofactors (CoA, Mg^{2+}, and ATP) for THCA-CoA formation is in agreement with that for most activation reactions studied earlier, while the substrate specificity of the activating enzyme(s) is not known. Activation of bile acids has previously been shown to take place in the microsomal fraction of rat liver (23–25). Whether one or more ligases are involved in the activation of bile acids and of 5β-cholestanoic acids is not known. The pH optimum for the choloyl-CoA ligase was reported to be 7.3 and the apparent K'_m for cholate to be 6 μM (25). These values are lower than we report here for THCA-CoA formation and may indicate that different enzymes are involved.

We have previously shown that peroxisome-rich fractions of rat liver are able to catalyze oxidation of 5β-cholestanoic acids into bile acids in the presence of CoA, ATP, Mg^{2+}, and NAD (4, 5, 7). It appeared that activation could be a rate-limiting step in these experiments and the addition of microsomal protein stimulated the reaction (5). From the results presented here we must conclude that the activation step most probably was catalyzed by microsomes contaminating the peroxisome-enriched fractions. The specific rates of activation of THCA by microsomal fractions observed in this work (about 200 nmol/mg · hr^{-1}) are several times higher than the rates of peroxisomal oxidation of the steroid side chain previously observed (4, 5, 7). A rather small contamination of the peroxisomal fraction by microsomes may thus be sufficient to provide enough THCA-CoA for the oxidase reaction. The rates of activation of THCA and of DHCA in pooled peroxisomal fractions were generally somewhat lower (14–23 nmol/mg protein · hr^{-1}) than the maximal rates of

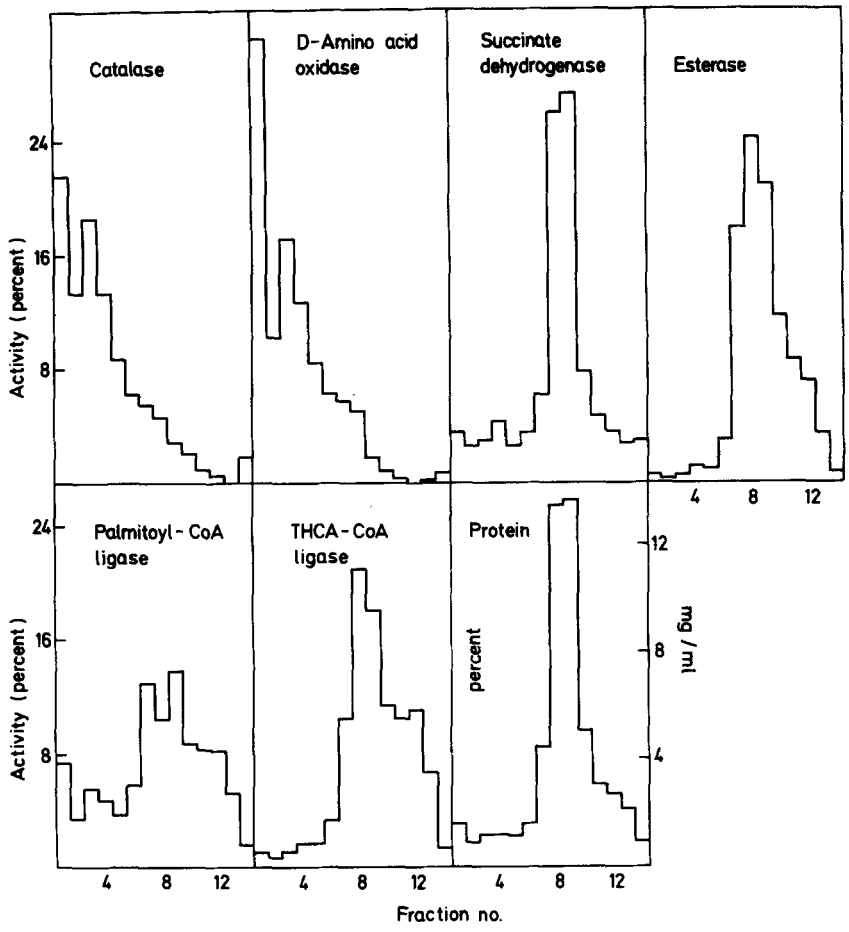

Fig. 4. Enzyme activities in fractions after centrifugation of a light mitochondrial fraction of rat liver on a Nycodenz density gradient (15–45%). The gradient was centrifuged at 30,000 rpm for 20 min. Fractions were collected as described in Materials and Methods and the Nycodenz was subsequently removed by centrifugation of the fractions at 30,000 rpm in the Beckman TFT 45.5 rotor. The pellets were resuspended in 0.25 M sucrose, 15 mM HEPES, pH 7.4. Recovery of protein was 69%. THCA-CoA ligase and palmitoyl-CoA ligase were assayed in duplicate as described in Materials and Methods except that the concentration of THCA was 44 μM.

bile acid formation observed in vitro in the previous work (approx. 30 nmol/mg protein · hr^{-1}) (7). There may be several explanations for this discrepancy. In the previous experiments on the oxidation of THCA (and of DHCA) we have always preincubated for 15 min in the absence of NAD in order to allow a build up of substrate for the oxidase. It is also conceivable that the activation reaction may proceed at higher rates under conditions where the product (THCA-CoA) is continuously removed by the oxidase reaction. Finally, in our previous studies the microsomal contamination of the peroxisomal fractions may have reached up to 10% (7) compared to 3–7% in this work.

Fatty acids can be activated by a peroxisomal ligase with a capacity sufficient to maintain peroxisomal β-oxidation of fatty acids (8). This enzyme is reported to be localized on the outside of the peroxisomal membrane (26). Thus, both activated fatty acids and activated 5β-cholestanoic acids must be transported into the peroxisomes. No information on the nature of such transport mechanism is reported, but peroxisomes are permeable to small water-soluble molecules (27). A separate CoA pool is reported to exist within the peroxisomes (27). Since no activation reactions are known to take place inside the peroxisomes, this CoA pool may be utilized for thiolytic cleavage reactions.

In conclusion, we have shown that the microsomal fraction of rat liver can efficiently catalyze the activation of both DHCA and THCA to the corresponding CoA esters. No activation of THCA was detected that could be explained by activating enzymes located in other organelles.

The skillful technical assistance of Eva Torma Grabner and Torhild Morsund is appreciated. The synthesis of coprostanoic acids and THCA-CoA by Manfred Held is appreciated. The work was supported by the Norwegian Research Council for Science and the Humanities, the Anders Jahre's Foundation, Nordisk Insulinfond, and the Swedish Medical Research Council (03X-3141).

Manuscript received 8 April 1987 and in revised form 7 January 1988.

REFERENCES

1. Danielsson, H. 1973. Mechanism 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. Björkhem, I. 1985. Mechanism of bile acid biosynthesis in mammalian liver. *In* Sterols and Bile Acids. H. Danielsson and J. Sjövall, editors. New Comprehensive Biochemistry, Vol. 12. Elsevier Scientific Publishing Co., Amsterdam. 231–278.
4. 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.
5. Kase, B. F., I. Björkhem, and J. I. Pedersen. 1983. Formation of cholic acid from 3α,7α,12α-trihydroxy-5β-cholestanoic acid by rat liver peroxisomes. *J. Lipid Res.* **24:** 1560–1567.
6. Kase, B. F., K. Prydz, I. Björkhem, and J. I. Pedersen. 1986. In vitro formation of bile acids from di- and trihydroxy-5β-cholestanoic acid in human liver peroxisomes. *Biochim. Biophys. Acta.* **877:** 37–42.
7. Prydz, K., B. F. Kase, I. Björkhem, and J. I. Pedersen. 1986. Formation of chenodeoxycholic acid from 3α,7α-dihydroxy-5β-cholestanoic acid by rat liver peroxisomes. *J. Lipid Res.* **27:** 622–628.
8. Krisans, S., R. M. Mortensen, and P. B. Lazarow. 1980. Acyl-CoA synthetase in rat liver peroxisomes. *J. Biol. Chem.* **255:** 9599–9607.
9. 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.
10. Shah, P. P., and E. Staple. 1968. Synthesis of coenzyme A esters of some bile acids. *Steroids.* **12:** 571–576.
11. Aas, M. 1971. Organ and cellular distribution of fatty acid activating enzymes in the rat. *Biochim. Biophys. Acta.* **231:** 32–48.
12. Schmidt, E. 1974. Glutamate dehydrogenase. UV-assay. *In* Methods of Enzymatic Analysis. 2nd edition. Vol. 2. H. U. Bergmeyer, editor. Academic Press, New York. 650–655.
13. Strobel, H. W., and J. D. Dignam. 1978. Purification and properties of NADPH-cytochrome P-450 reductase. *Methods Enzymol.* **52:** 89–96.
14. Beaufay, H., A. Amar-Costesec, E. Feytmans, D. Thines-Sempoux, M. Wibo, M. Robbi, and J. Berthet. 1974. Analytical studies of microsomes and isolated membranes from rat liver. *J. Cell Biol.* **61:** 188–200.
15. Baudhuin, P., H. Beaufay, Y. Rahman-Li, O. Z. Sellinger, R. Wattiaux, P. Jacques, and C. DeDuve. 1964. Tissue fractionation studies. *Biochem. J.* **92:** 179–184.
16. Barret, A. J. 1972. *In* Lysosomes—A Laboratory Handbook. J. T. Dingle, editor. North-Holland Publishing Co., Amsterdam. 111–125.
17. Pennington, R. J. 1961. Biochemistry of dystrophic muscle. Mitochondrial succinate-tetrazolium reductase and adenosine triphosphatase. *Biochem. J.* **80:** 649–654.
18. 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.
19. Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. *Anal. Biochem.* **70:** 241–250.
20. Sandberg, P-O., L. Marzella, and H. Glaumann. 1980. A method for rapid isolation of rough and smooth microsomes and Golgi apparatus from rat liver in the same sucrose gradient. *Exp. Cell Res.* **130:** 393–400.
21. Abbot, D. A., E. Schlarman, P. H. Patrick, D. M. Tal, and W. H. Elliott. 1985. High-performance liquid chromatographic analysis of bile acid coenzyme A derivatives. *Anal. Biochem.* **146:** 437–441.
22. Howell, K. E., A. Ito, and G. E. Palade. 1978. Endoplasmic reticulum marker enzymes in Golgi fractions. What does this mean? *J. Cell. Biol.* **79:** 581–589.
23. Bremer, J. 1956. Cholyl-S-CoA as an intermediate in the conjugation of cholic acid with taurine by rat liver microsomes. *Acta Chem. Scand.* **10:** 56–71.
24. Elliott, W. H. 1962. Activation and conjugation of bile acids. *Methods Enzymol.* **5:** 473–475.
25. Vessey, D. A., and D. Zakim. 1977. Characterization of microsomal choloyl-coenzyme A synthetase. *Biochem. J.* **163:** 357–362.
26. Mannaerts, G. P., P. Van Veldhoven, A. Van Broekhoven, G. Vandebroek, and L. J. Debeer. 1982. Evidence that peroxisomal acyl-CoA synthetase is located at the cytoplasmic side of the peroxisomal membrane. *Biochem. J.* **204:** 17–23.
27. Van Veldhoven, P. P., and G. P. Mannaerts. 1986. Coenzyme A in purified peroxisomes is not freely soluble in the matrix but firmly bound to a matrix protein. *Biochem. Biophys. Res. Commun.* **139:** 1195–1201.