The role of sterol carrier protein₂ and other hepatic lipid-binding proteins in bile-acid biosynthesis

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The ability of different lipid-binding proteins in liver cytosol to affect enzyme activities in bile-acid biosynthesis was studied in whole microsomes (microsomal fractions) and mitochondria and in purified enzyme systems. Sterol carrier protein₂ stimulated the 7 α -hydroxylation of cholesterol and the 12 α -hydroxylation of 5 β -cholestane-3 α ,7 α -diol in microsomes and the 26-hydroxylation of cholesterol in mitochondria 2–3-fold. It also stimulated the oxidation of 5-cholestene-3 β ,7 α -diol into 7 α -hydroxy-4-cholesten-3-one in microsomes. The stimulatory effect of sterol carrier protein₂ was much less with purified cholesterol 7 α - and 26-hydroxylase systems than with microsomes and mitochondria. No stimulatory effect of sterol carrier protein₂ was observed with purified 12 α -hydroxylase and 3 β -hydroxy- Δ^5 -C₂₇-steroid oxidoreductase. Sterol carrier protein (fatty-acid-binding protein), 'DEAE-peak I protein' [Dempsey, McCoy, Baker, Dimitriadou-Vafiadou, Lorschach & Howards (1981) *J. Biol. Chem.* **256**, 1867–1873], ligandin (glutathione transferase B) and serum albumin had no marked stimulatory effects in either crude or in purified systems. The results suggest that sterol carrier protein₂ facilitates the introduction of the less-polar substrates in bile-acid biosynthesis to the membrane-bound enzymes in crude systems *in vitro*. The broad substrate specificity appears, however, not to be consistent with a specific regulatory function for sterol carrier protein₂ in bile-acid biosynthesis.

INTRODUCTION

The biosynthesis of bile acids from cholesterol in the liver involves hydroxylations catalysed by multiple species of microsomal cytochrome *P*-450 and a mitochondrial cytochrome *P*-450 (for a recent review, see Björkhem, 1985).

There are several reports on the possibility that cytosolic proteins might regulate bile-acid biosynthesis by modulating the rate of the hydroxylation reactions. Special interest has been focused on the cholesterol 7 α -hydroxylation, which is the rate-limiting step (Spence & Gaylor, 1977; Craig *et al.*, 1979; Danielsson *et al.*, 1980, 1983, 1984; Kwok *et al.*, 1981; Sanghvi *et al.*, 1981).

Sterol carrier proteins are known to be important for certain reactions in cholesterol biosynthesis (for a recent review, see Scallen & Vahouny, 1985). Recently, Seltman *et al.* (1985) reported that sterol carrier protein₂ stimulates microsomal 7 α -hydroxylation of cholesterol and suggested that it might play a regulatory role in bile-acid biosynthesis. Dempsey and associates have reported that a sterol carrier protein prepared by their procedures, as well as serum albumin, stimulates microsomal 12 α -hydroxylation (Grabowski *et al.*, 1976; Dempsey *et al.*, 1981). The two sterol carrier proteins are not identical (Scallen *et al.*, 1985). There is no information concerning the substrate specificity of the two sterol carrier proteins in bile-acid biosynthesis.

Work in this laboratory has provided evidence for the existence of specific protein fractions in the liver cell that selectively stimulate and inhibit purified and reconstituted cholesterol 7 α -hydroxylase and 12 α -hydroxylase systems (Danielsson *et al.*, 1980, 1983, 1984). It cannot be completely excluded that sterol carrier proteins or other lipid-binding proteins, present in liver cytosol, might also show these effects.

The present study examines the effects of various hepatic lipid-binding proteins on reactions in bile-acid biosynthesis in microsomes and mitochondria and in purified and reconstituted systems.

EXPERIMENTAL PROCEDURES

Materials

[4-¹⁴C]Cholesterol (61 Ci/mol) and [4-¹⁴C]testosterone (50 Ci/mol) were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). 5 β -[7 β -³H]cholestane-3 α ,7 α -diol (500 Ci/mol), 5 β -[7 β -³H]cholestane-3 α ,7 α ,12 α -triol (500 Ci/mol) and [7 β -³H]5-cholestene-3 β ,7 α -diol (3 Ci/mol) were prepared as described previously (Hansson & Wikvall, 1979; Wikvall, 1981).

Methods

The microsomal and mitochondrial fractions were prepared from livers of untreated rats of the Sprague-Dawley strain as described by Andersson *et al.* (1985).

A cytochrome *P*-450 fraction, active in 7 α -hydroxylation of cholesterol and 12 α -hydroxylation of 5 β -cholestane-3 α ,7 α -diol, was prepared from liver microsomes (microsomal fractions) of cholestyramine-treated rats as described by Andersson *et al.* (1985), with the exception that the DEAE-Sepharose chromatography step was omitted. Cytochrome *P*-450, active in 25-hydroxylation of 5 β -cholestane-3 α ,7 α ,12 α -triol, was prepared from liver microsomes of cholestyramine-treated rats as described previously (Andersson *et al.*, 1985). The preparations contained 3 and 16 nmol of cytochrome *P*-450/mg of protein respectively.

NADPH:cytochrome *P*-450 reductase was prepared from phenobarbital-treated rats as described by Yasukochi & Masters (1976) and had a specific activity of 50 units/mg of protein.

Cytochrome *P*-450 from rat liver mitochondria, active in 26-hydroxylation of cholesterol and 5 β -cholestane-3 α ,7 α ,12 α -triol, was purified up to the hydroxyapatite step by the procedures described by Wikvall (1984) for preparation of apparently homogeneous cytochrome *P*-450 from rabbit liver mitochondria. The preparation contained 2 nmol of cytochrome *P*-450/mg of protein.

Ferredoxin and ferredoxin reductase were purified as described by Wikvall (1984).

Cytochrome *P*-450 was determined as described by Omura & Sato (1964).

3 β -Hydroxy- Δ^5 -C₂₇-steroid oxidoreductase catalysing the conversion of 5-cholestene-3 β ,7 α -diol into 7 α -hydroxy-4-cholesten-3-one was purified as described by Wikvall (1981).

Sterol carrier protein (fatty-acid-binding protein) and 'DEAE-peak I protein' were purified from rat liver cytosol as described by Dempsey *et al.* (1981). The preparation of sterol carrier protein showed only one protein band with an apparent M_r of 16000 upon SDS/polyacrylamide-gel electrophoresis. The preparation exhibited the same spectral properties, indicating the absence of tryptophan, as described by Dempsey *et al.* (1981). The specific activity (the increase of 5,7-cholestadien-3 β -ol formation over that of the microsomal fraction without sterol carrier protein in nmol/45 min per mg of protein) was 1.7 in the starting cytosol and 18.5 in the purified protein. The purification factor is the same as that reported by Dempsey *et al.* (1981), but the initial activity in the cytosol is lower. The activity is, however, several times higher than that reported by Ishibashi & Bloch (1981) for a preparation of sterol carrier protein purified as described by Dempsey *et al.* (1981). DEAE-peak I protein gave a major protein band with an apparent M_r of 14000 and a minor band with an apparent M_r of 16000 on gel electrophoresis. The preparation differed in spectral properties from those of the sterol carrier protein described by Dempsey *et al.* (1981).

Sterol carrier protein₂ was purified from rat liver cytosol as described by Scallen and associates (Noland *et al.*, 1980; Scallen *et al.*, 1985). The preparation showed only one protein band, with an apparent M_r of 14000, on gel electrophoresis. The specific activity, expressed as nmol of cholesterol formed/2 h per mg of protein, was 9500, a value that is essentially the same as that reported by Noland *et al.* (1980).

Ligandin (glutathione transferase B) was purified from rat liver cytosol as described by Bass *et al.* (1977). The preparation had a specific glutathione transferase activity of 11 μ mol of product formed/min per mg of protein when determined by measuring the rate of conjugation of 1-chloro-2,4-dinitrobenzene with glutathione as described by Habig *et al.* (1974).

Protein was determined by the method of Bradford (1976).

Gel electrophoresis was performed in the presence of SDS as described by Laemmli (1970), 20% (w/v) polyacrylamide gels being used. The gels were silver-stained as described by Wray *et al.* (1981).

Microsomes or reconstituted hydroxylase systems were preincubated for 30 min with substrate and 5 nmol of lipid-binding protein in 0.75 ml of buffer (including the buffer in which the particular protein was dissolved and the buffer used for the particular assay) before assay of enzyme activity. Control incubations, without binding

protein, contained the corresponding amount of buffer in which the particular protein was dissolved. Sterol carrier protein (fatty-acid-binding protein) was in 30 mM-Tris/HCl buffer, pH 9.0, containing 64 mM-NaCl, DEAE-peak I protein was in 30 mM-Tris/HCl buffer, pH 9.0, sterol carrier protein₂ was in 300 mM-potassium phosphate buffer, pH 6.8, ligandin was in 10 mM-Tris/HCl buffer, pH 8.8, and albumin was in the buffer used for the particular assay. Microsomes corresponding to 0.3–1 mg of protein were used. Reconstituted systems contained 0.05–0.1 nmol of the appropriate cytochrome *P*-450, 0.5–1.5 units of NADPH:cytochrome *P*-450 reductase and 15 μ g of dodecanoylglycero-3-phosphocholine. Labelled cholesterol, 25 nmol in 15 μ l of acetone, 5 β -cholestane-3 α ,7 α -diol, 125 nmol in 25 μ l of acetone, 5 β -cholestane-3 α ,7 α ,12 α -triol, 125 nmol in 25 μ l of acetone, and testosterone, 200 nmol in 25 μ l of acetone, were used as substrates.

Cholesterol 7 α -hydroxylase activity was assayed by addition of 0.3 mg of Triton X-100, 2.5 μ mol of dithiothreitol and 1.2 μ mol of NADPH. Incubations were performed for 20 min in a total volume of 1.0 ml of 50 mM-Tris/acetate buffer, pH 7.4, containing 20% (v/v) glycerol. Incubations with microsomes did not contain dithiothreitol.

5 β -Cholestane-3 α ,7 α -diol 12 α -hydroxylase, 5 β -cholestane-3 α ,7 α ,12 α -triol 25-hydroxylase and testosterone hydroxylase activities were assayed as described for cholesterol 7 α -hydroxylase, except that Triton X-100 and dithiothreitol were omitted and that 0.15 M-potassium phosphate buffer, pH 7.4, containing 20% (v/v) glycerol and 0.1 mM-EDTA was used instead of the Tris/acetate buffer.

In experiments on the microsomal conversion of 5-cholestene-3 β ,7 α -diol into 7 α -hydroxy-4-cholesten-3-one, microsomes or purified 3 β -hydroxy- Δ^5 -C₂₇-steroid oxidoreductase were preincubated with labelled 5-cholestene-3 β ,7 α -diol, 125 nmol in 25 μ l of acetone, and lipid-binding protein or corresponding buffer as described above for microsomal hydroxylations. The oxidoreductase activity was assayed by the addition of 1.2 μ mol of NAD. Incubations were performed for 15 min in 1.0 ml of 0.1 M-potassium phosphate buffer, pH 7.4.

In experiments on the mitochondrial 26-hydroxylation, mitochondria or reconstituted 26-hydroxylase system were incubated with substrate, 5 nmol of lipid-binding protein or the corresponding buffer and 3.5 μ mol of NADPH in a total volume of 3 ml of 50 mM-Tris/acetate buffer, pH 7.4. Mitochondria corresponding to 5 mg of protein were used. The reconstituted system contained 0.1 nmol of mitochondrial cytochrome *P*-450, 2 nmol of ferredoxin and 0.2 nmol of ferredoxin reductase. Labelled cholesterol, 25 nmol in 25 μ l of acetone, and 5 β -cholestane-3 α ,7 α ,12 α -triol, 125 nmol in 25 μ l of acetone, were used. Incubations were performed for 40 min.

Analyses of incubation mixtures were performed by using t.l.c. as described previously for cholesterol, 5 β -cholestane-3 α ,7 α -diol and 5 β -cholestane-3 α ,7 α ,12 α -triol (Andersson *et al.*, 1985), for testosterone (Boström *et al.*, 1981) and 5-cholestene-3 β ,7 α -diol (Wikvall, 1981).

RESULTS

The effect of lipid-binding proteins on various reactions in bile-acid biosynthesis was studied in crude and purified systems. A concentration of 5 μ M of each

Table 1. Effects of different lipid-binding proteins on the 7 α -hydroxylation of cholesterol in microsomes and in the reconstituted system

Microsomes or reconstituted system, containing cytochrome *P*-450, NADPH:cytochrome *P*-450 reductase and didodecanoylglycerol-3-phosphocholine, were preincubated with cholesterol and 5 nmol of each lipid-binding protein for 30 min at 37 °C before assay of enzyme activity. Control incubations without lipid-binding protein were performed in all cases. These incubations contained the specific buffer in which the lipid-binding protein was dissolved (see the Experimental procedures section). The hydroxylase activity in the control incubations varied with the buffer added. A cholesterol 7 α -hydroxylase activity of 100% corresponds to 8–20 pmol of 7 α -hydroxycholesterol formed/min per nmol of cytochrome *P*-450 in microsomes and 450–625 pmol of 7 α -hydroxycholesterol formed/min per nmol of cytochrome *P*-450 in the reconstituted system.

Lipid-binding protein	7 α -Hydroxylase activity (% of control)	
	Microsomes	Reconstituted system
None (control)	100	100
+ Sterol carrier protein (fatty-acid-binding protein)	50	105
+ DEAE-peak I protein	145	125
+ Sterol carrier protein ₂	265	150
+ Ligandin (glutathione transferase B)	90	110
+ Serum albumin	100	105

protein was used for microsomal reactions (Seltman *et al.*, 1985) and a concentration of 1.7 μ M was used for mitochondrial reactions (Vahouny *et al.*, 1983). The experiments were performed twice giving essentially the same results. Table 1 shows that 7 α -hydroxylation of cholesterol was enhanced about 3-fold when sterol carrier protein₂ was preincubated with cholesterol and crude microsomes. When sterol carrier protein₂ and substrate were preincubated with a reconstituted cholesterol 7 α -hydroxylase system, only a 50% increase in 7 α -hydroxylase activity was observed. The sterol carrier protein described by Dempsey *et al.* (1981) decreased the 7 α -hydroxylase activity in microsomes by 50%. This effect was not observed with the reconstituted system. Neither DEAE-peak I protein, ligandin nor albumin had any marked effects on cholesterol 7 α -hydroxylation in microsomes or the reconstituted system.

In a separate series of experiments with the reconstituted system from rabbit liver microsomes, it was found that sterol carrier protein₂ did not increase cholesterol 7 α -hydroxylase activity present in cytochrome *P*-450 LM₄.

Table 2 shows that 12 α -hydroxylation of 5 β -cholestane-3 α ,7 α -diol was stimulated 2-fold when microsomes and substrate were preincubated with sterol carrier protein₂. The protein had no effect on 12 α -hydroxylation in the reconstituted system. Neither sterol carrier protein described by Dempsey *et al.* (1981), DEAE-peak I protein, ligandin nor albumin had any marked effects on 12 α -hydroxylation in microsomes or reconstituted system.

Table 3 shows that the mitochondrial 26-hydroxylation

Table 2. Effects of different lipid-binding proteins on the 12 α -hydroxylation of 5 β -cholestane-3 α ,7 α -diol in microsomes and in the reconstituted system

Microsomes or reconstituted system, containing cytochrome *P*-450, NADPH:cytochrome *P*-450 reductase and didodecanoylglycerol-3-phosphocholine, were preincubated with 5 β -cholestane-3 α ,7 α -diol and 5 nmol of each protein for 30 min at 37 °C before enzyme assay. Control incubations were performed as described in Table 1 (see the Experimental procedures section). The hydroxylase activity in the control incubations varied with the buffer added. A 12 α -hydroxylase activity of 100% corresponds to 95–218 pmol of 5 β -cholestane-3 α ,7 α ,12 α -triol formed/min per nmol of cytochrome *P*-450 in the microsomes and 250–320 pmol of 5 β -cholestane-3 α ,7 α ,12 α -triol formed/min per nmol of cytochrome *P*-450 in the reconstituted system.

Lipid-binding protein	12 α -Hydroxylase activity (% of control)	
	Microsomes	Reconstituted system
None (control)	100	100
+ Sterol carrier protein (fatty-acid-binding protein)	105	110
+ DEAE-peak I protein	145	80
+ Sterol carrier protein ₂	205	110
+ Ligandin (glutathione transferase B)	100	95
+ Serum albumin	85	75

Table 3. Effects of different lipid-binding proteins on the 26-hydroxylation of 5 β -cholestane-3 α ,7 α ,12 α -triol in mitochondria and in the reconstituted system

Mitochondria or reconstituted system, containing mitochondrial cytochrome *P*-450, ferredoxin and ferredoxin reductase, were incubated with 5 β -cholestane-3 α ,7 α ,12 α -triol and 5 nmol of each lipid-binding protein. Incubations were performed for 40 min at 37 °C. Control incubations without lipid-binding protein were performed in all cases. These incubations contained the specific buffer in which the lipid-binding protein was dissolved (see the Experimental procedures section). The hydroxylase activity in the control incubations varied with the buffer added. A 26-hydroxylase activity of 100% corresponds to 70–95 pmol of 5 β -cholestane-3 α ,7 α ,12 α ,26-tetrol formed/min per mg of protein in the mitochondria and 7500–13000 pmol of 5 β -cholestane-3 α ,7 α ,12 α ,26-tetrol formed/min per nmol of cytochrome *P*-450 in the reconstituted system.

Lipid-binding protein	26-Hydroxylase activity (% of control)	
	Mitochondria	Reconstituted system
None (control)	100	100
+ Sterol carrier protein (fatty-acid-binding protein)	80	100
+ DEAE-peak I protein	90	95
+ Sterol carrier protein ₂	90	135
+ Ligandin (glutathione transferase B)	55	90
+ Serum albumin	80	115

Table 4. Effects of different lipid-binding proteins on the 26-hydroxylation of cholesterol in mitochondria and in the reconstituted system

Incubations were performed as described in Table 3, but with cholesterol as substrate. The hydroxylase activity in the control incubations varied with the buffer added. A 26-hydroxylase activity of 100% corresponds to 4–7 pmol of 26-hydroxycholesterol formed/min per mg of protein in the mitochondria and 60–100 pmol of 26-hydroxycholesterol formed/min per nmol of cytochrome *P*-450 in the reconstituted system.

Lipid-binding protein	26-Hydroxylase activity (% of control)	
	Mitochondria	Reconstituted system
None (control)	100	100
+ Sterol carrier protein (fatty-acid-binding protein)	110	115
+ DEAE-peak I protein	85	75
+ Sterol carrier protein ₂	235	160
+ Ligandin (glutathione transferase B)	115	125
+ Serum albumin	125	130

Table 5. Substrate specificity of sterol carrier protein₂ when studied in microsomes and mitochondria or in purified enzyme systems

Incubations were performed as described in the Experimental procedures section. 26-Hydroxylation of cholesterol and 5 β -cholestane-3 α ,7 α ,12 α -triol was catalysed by mitochondria and reconstituted mitochondrial system. All other reactions were catalysed by microsomes and reconstituted microsomal systems. Abbreviation used: N.D., not determined.

Substrate (reaction)	Stimulation by sterol carrier protein ₂ (fold)	
	Crude system	Purified system
Cholesterol		
7 α -Hydroxylation	2.7	1.5
26-Hydroxylation	2.4	1.6
5-Cholestene-3 β ,7 α -diol		
Oxidation	2.3	1.2
5 β -Cholestane-3 β ,7 α ,12 α -triol		
12 α -Hydroxylation	2.0	1.1
5 β -Cholestane-3 α ,7 α ,12 α -triol		
25-Hydroxylation	1.1	N.D.
26-Hydroxylation	0.9	1.3
Testosterone		
2 α -Hydroxylation	1.0	N.D.
6 β -Hydroxylation	0.8	N.D.
16 α -Hydroxylation	1.0	N.D.

of 5 β -cholestane-3 α ,7 α ,12 α -triol in crude mitochondria or in a reconstituted mitochondrial system was not stimulated by any of the lipid-binding proteins examined. A 50% decrease in 26-hydroxylase activity was observed when ligandin was incubated with the mitochondrial

fraction. This effect was not observed in the reconstituted system.

Table 4 shows that mitochondrial 26-hydroxylation of cholesterol was increased 2–3-fold when sterol carrier protein₂ was incubated with the mitochondrial fraction, whereas only a 60% increase of the activity occurred with the reconstituted system. There were no effects of the other proteins in either crude or in the reconstituted system.

Table 5 summarizes the substrate specificity of sterol carrier protein₂ when studied with microsomes and mitochondria or purified and reconstituted systems. 7 α -Hydroxylation of cholesterol, 26-hydroxylation of cholesterol, oxidation of 5-cholestene-3 β ,7 α -diol and 12 α -hydroxylation of 5 β -cholestane-3 α ,7 α -diol were stimulated 2–3-fold by sterol carrier protein₂ in the crude systems. No marked stimulation was observed with purified and reconstituted systems. Sterol carrier protein₂ had no effect on the microsomal 25-hydroxylation or the mitochondrial 26-hydroxylation of 5 β -cholestane-3 α ,7 α ,12 α -triol. The protein had no effect on the microsomal 2 α -,6 β - and 16 α -hydroxylations of testosterone.

DISCUSSION

The cytosolic proteins included in the present study are known to bind sterols, fatty acids or bile acids (Chanderbhan *et al.* 1982; Dempsey *et al.*, 1981; Sugiyama *et al.*, 1983). Sterol carrier protein described by Dempsey *et al.* (1981) has been reported to stimulate conversion of lanosterol into cholesterol in microsomes (Song & Dempsey, 1981). It undergoes a marked diurnal variation in hepatic content (McGuire *et al.*, 1984). The protein is apparently identical with the hepatic fatty-acid-binding protein (Dempsey *et al.*, 1981; Ockner *et al.*, 1982; Scallen *et al.*, 1985). The 'DEAE-peak I protein' described by Dempsey *et al.* (1981) has the ability to bind fatty acids. Sterol carrier protein₂ described by Scallen and associates stimulates microsomal conversion of lanosterol into cholesterol and microsomal conversion of cholesterol into cholesterol ester in the liver (Noland *et al.*, 1980; Gavey *et al.*, 1981). It also enhances mitochondrial utilization of cholesterol for pregnenolone formation in the adrenals (Chanderbhan *et al.*, 1982). Ligandin, which is a glutathione transferase, is known to bind bile acids (Sugiyama *et al.*, 1983).

The present results show that sterol carrier protein₂ is able to stimulate several reactions in bile-acid biosynthesis when studied in crude systems *in vitro*. There is a correlation between degree of stimulation and polarity of substrates. Thus hydroxylations of cholesterol and 5 β -cholestane-3 α ,7 α -diol by microsomes and mitochondria were stimulated in addition to the conversion of 5-cholestene-3 β ,7 α -diol into 7 α -hydroxy-4-cholesten-3-one. However, there was no effect on hydroxylations of the more polar substrate, 5 β -cholestane-3 α ,7 α ,12 α -triol.

Boström (1983) has shown that the product, 5-cholestene-3 β ,7 α -diol, inhibits competitively the binding of cholesterol to cholesterol 7 α -hydroxylase present in cytochrome *P*-450 LM₄ from rabbit liver. The product also inhibits by more than 50% the 7 α -hydroxylation of cholesterol. Similar findings have been reported earlier in studies with rat liver microsomes (Van Cantfort, 1972; Schwartz & Margolis, 1983). It could therefore not be excluded that the stimulatory effect of sterol carrier protein₂ on cholesterol 7 α -hydroxylation in microsomes

is due to removal of product. If the stimulatory effect is due to a reversed product inhibition, the degree of stimulation would be at least as marked with a solubilized and reconstituted system as with a crude system. The present results, showing no marked stimulatory effect with reconstituted systems from rat as well as rabbit liver, suggest that the protein acts by facilitating introduction of the substrate to the membrane-bound enzyme rather than by product binding.

Since sterol carrier protein₂ was not specific for cholesterol 7 α -hydroxylation but also stimulated reactions in bile-acid biosynthesis that are not rate-limiting, it appears less likely that the protein has a specific regulatory function in this process. However, it cannot be excluded, from the present results, that sterol carrier protein₂ might play a physiological role in bile-acid biosynthesis *in vivo*.

DEAE-peak I protein described by Dempsey *et al.* (1981) and albumin did not show any effects on reactions in bile-acid biosynthesis either in crude or in purified systems. Sterol carrier protein (fatty-acid-binding protein) described by Dempsey *et al.* (1981) inhibited microsomal 7 α -hydroxylation of cholesterol, and ligandin inhibited mitochondrial 26-hydroxylation of 5 β -cholestane-3 α ,7 α ,12 α -triol. No explanation for the inhibitory effects can be offered at present.

Grabowski *et al.* (1976) reported that squalene and sterol carrier protein stimulated 12 α -hydroxylation in microsomes. The preparation of sterol carrier protein (fatty-acid-binding protein) isolated as described by Dempsey *et al.* (1981) used in the present study did not stimulate the reaction. The reason for this discrepancy is not known. It is possible that the preparation used by Grabowski *et al.* (1976) contained some sterol carrier protein₂, which stimulates 12 α -hydroxylation in microsomes.

Our laboratory has for several years been studying the effects of intracellular proteins on reconstituted hydroxylase activities in bile-acid biosynthesis. In these studies, only effects of proteins resulting in at least a 2-fold increase or at least a 2-fold decrease in hydroxylase activity have been considered to be of interest. Two protein fractions have been isolated that respectively stimulate and inhibit reconstituted cholesterol 7 α -hydroxylase activity (Danielsson *et al.*, 1980, 1984) and two protein fractions have been isolated that respectively stimulate and inhibit reconstituted 12 α -hydroxylase activity (Danielsson *et al.*, 1983). In contrast with sterol carrier protein₂, which showed a broad substrate specificity with microsomes, these proteins appear to be specific for cholesterol 7 α -hydroxylation and 12 α -hydroxylation respectively in reconstituted systems. The fact that none of the lipid-binding proteins, including sterol carrier protein₂ and serum albumin, had any marked effects on the reconstituted hydroxylase systems indicates that the stimulatory protein fractions described by Danielsson *et al.* (1980, 1983, 1984) exert their effects by mechanisms other than introduction of substrate to the enzyme. It is also evident that the effects are not due to non-specific protein effects. The cytosolic cholesterol 7 α -hydroxylase stimulatory protein, which has been purified to apparent homogeneity, requires glutathione or thioredoxin for activity (Danielsson *et al.*, 1984). The stimulation of cholesterol 7 α -hydroxylation by this protein is apparently mediated by specific effects in the 7 α -hydroxylating cytochrome P-450 molecule.

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