The Role of a Carrier Protein in Cholesterol and Steroid Hormone Synthesis by Adrenal Enzymes, [1,2]

Kenneth W. Kan, Mary C. Ritter, Frank Ungar, and Mary E. Dempsey

Department of Biochemistry
University of Minnesota Medical School
Minneapolis, Minnesota 55455

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Squalene and sterol carrier protein (SCP) of liver, known to be required for cholesterol synthesis by liver microsomal enzymes, functions with adrenal microsomal enzymes (Δ^7-sterol Δ^5-dehydrogenase and $\Delta^{5,7}$-sterol Δ^7-reductase) catalyzing cholesterol synthesis from sterol precursors. Adrenal preparations contain a heat-stable protein which functions similarly to liver-SCP with adrenal and liver enzymes. Liver-SCP also can participate with an adrenal enzyme (solubilized from an acetone powder of mitochondria) in the conversion of cholesterol to pregnenolone. These findings indicate that an SCP-like protein not only participates in cholesterol synthesis and transport in the adrenal, but also in the initial steps of cholesterol metabolism to steroid hormones.

Previous studies demonstrated the molecular properties and function in cholesterol synthesis of a squalene and sterol carrier protein (SCP), e.g. (1-4). SCP occurs in the soluble fraction of liver homogenates; has a high affinity for cholesterol and its water insoluble precursors; and is required for cholesterol synthesis by liver microsomal enzymes. These and related findings indicate that a cholesterol precursor·SCP complex forms part of the active site of each enzyme. Furthermore, one of the human plasma lipoprotein peptides will substitute for and may have some structural similarity to human liver-SCP (5).

In this report we demonstrate that liver-SCP functions in the synthesis of cholesterol from sterol precursors by adrenal enzymes and that there is an SCP-like protein in adrenal preparations. Liver-SCP also can participate with adrenal mitochondrial enzymes in the conversion of cholesterol to pregnenolone (3β-hydroxy-pregn-5-en-20-one), a precursor of adrenal steroid hormones.

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1. This is article IV in the series "Squalene and Sterol Carrier Protein"; for articles I-III, see References (2-4).
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Table I

STEROL SYNTHESIS BY LIVER AND ADRENAL ENZYME SYSTEMS

Preparation[a]	Protein Ratio, SCP/Enzyme	Δ^5-Dehydrogenase Activity [b]	Δ^7-Reductase Activity [c]
		μM sterol formed/mg enzyme	
L-SCP	---	0.0	0.0
L-Enz	---	1.1	0.3
L-SCP + L-Enz	4.8	5.2	2.5
A-SCP + L-Enz	3.0	3.6	0.9
A-SCP	---	0.0	0.0
A-Enz	---	0.9	2.1
L-SCP + A-Enz	7.8	4.6	8.8
A-SCP + A-Enz	4.7	4.0	5.9

(a) L-SCP (22 mg/ml) and A-SCP (8.6 mg/ml) are the unpurified soluble fractions of homogenates prepared from liver and adrenal tissue respectively. L-Enz (13.7 mg/ml) and A-Enz (8.6 mg/ml) are the washed liver and adrenal microsomal fractions, respectively (see Methods and (6)).

(b) Conversion of Δ^7-cholesten-3β-ol to $\Delta^{5,7}$-cholestadien-3β-ol; incubation conditions (total volume 1.06 ml): 80 μM Δ^7-cholestenol, 1 mM NAD, 113 mμM AY-9944, 0.1 M phosphate, pH 7.4, 30 minutes under O_2 (1,6).

(c) Conversion of $\Delta^{5,7}$-cholestadien-3β-ol to cholesterol; incubation conditions (total volume 1.06 ml): 31 μM $\Delta^{5,7}$-cholestadienol-4-^{14}C, 1 mM NADPH, 0.1 M phosphate, pH 7.4, 30 minutes under N_2 at 37° (1,6).

METHODS

Rat liver microsomal and soluble (unpurified SCP) fractions (Table I) were prepared as described in detail previously (6). Rat adrenal gland microsomal and soluble (unpurified SCP) fractions (Table I) were prepared by the same techniques (6), except that the buffer was 0.25 M sucrose-50mM phosphate, pH 7.0. Δ^7-Sterol Δ^5-dehydrogenase (Table I) was assayed by the ultraviolet technique and $\Delta^{5,7}$-sterol Δ^7-reductase by the dibromide method (6). The data of Figure 1 were obtained with a liver-SCP prepara-

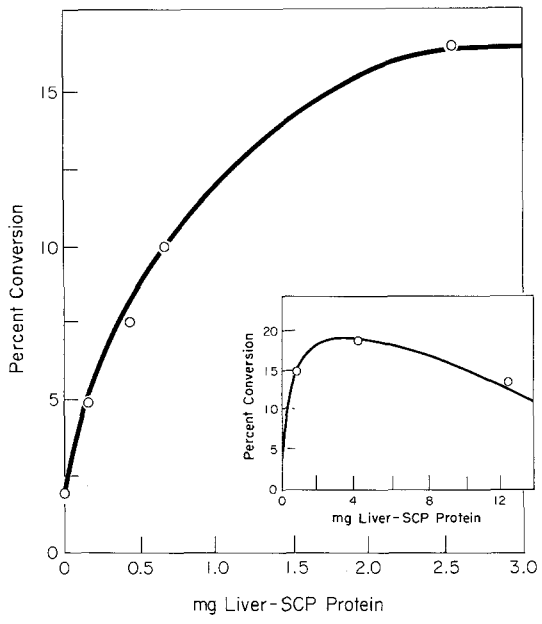

Figure 1. Stimulation by Liver-SCP of the Cholesterol Side-Chain Cleavage Enzyme System of the Adrenal.

Incubation medium (5.0 ml) contained 1.0 µmole cholesterol-1,2-^{3}H (40 Ci/mmole), 0.5 mM NADPH and 0.02 M potassium phosphate, pH 7.4. Partially purified liver-SCP (see Methods) was incubated with 1.0 mg equivalent bovine adrenal mitochondrial acetone powder extract at 37° under 95% O_2:5% CO_2 for 30 minutes.

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tion partially purified by heat treatment, $(NH_4)_2SO_4$ fractionation, and gel filtration (Sephadex G-25) as given in the conditions with Figure 1A of reference (3).

Highly purified liver-SCP (Table II) was prepared by aggregation of 20 mg of the heat stable protomer form of SCP (16,000 daltons) to the oligomer form (>150,000 daltons) in the presence of cholesterol-1,2-^{3}H (180 pmoles, 40 Ci/mmole) or the same level of cholesterol-1,2-^{3}H and lecithin (46 µmoles) (3,4). The oligomer (lipid·SCP complex) was separated from contaminating proteins by gel filtration (Sephadex G-75) (3,4). The latter procedure yields SCP purified >720-fold over its level in the soluble fraction of liver homogenates and probably of maximum purity.

The soluble adrenal enzyme catalyzing cholesterol side-chain cleavage

Table II

CONVERSION OF CHOLESTEROL-^{3}H TO PREGNENOLONE:
STIMULATION BY PURIFIED LIVER-SCP AND ADRENAL ACTIVATOR; INHIBITION BY LECITHIN

Experiment	Components of Incubation Mixture[a]	Pregnenolone Formed in 30 min.	
		pmoles	per cent
I[b]	A-Enz + cholesterol-^{3}H[c]	0.7	10
	A-Enz + cholesterol-^{3}H·L-SCP[d]		
	10µg	0.6	20
	25µg	1.2	16
	50µg	2.0	13
	A-Enz + cholesterol-^{3}H[c] + A-Activator[e]	1.0	15
II[b]	A-Enz + cholesterol-^{3}H[c]	0.4	18
	A-Enz + cholesterol-^{3}H·Lecithin·L-SCP[d]		
	15µg	0.2	13
	40µg	<0.1	< 1
	A-Enz + cholesterol-^{3}H[c] + A-Activator[e]	0.7	32

(a) Incubation medium (5.0 ml) contained 0.5 mM NADPH and 0.02 M potassium phosphate -1 mM $MgSO_4$, pH 7.4; other conditions as stated with Figure 1 and in Methods.

(b) A-Enz (cholesterol side-chain cleavage enzyme system) obtained from an extract equivalent to 1 mg (Expt. I) and 1.5 mg (Expt II), respectively, of rat adrenal mitochondrial acetone powder.

(c) The amount of cholesterol-1,2-^{3}H (40 Ci/mmole), in 30 µl methanol-propylene glycol (4:1, v:v), added to incubation flasks with 1.0 mg A-Enz in Expt. I=6.8 pmoles; with 1.5 mg A-Enz in Expt. II=2.2 pmoles.

(d) The oligomer of cholesterol-^{3}H·L-SCP in Expt. I contained 0.3 pmoles bound sterol per µg carrier protein; the oligomer of cholesterol-^{3}H·lecithin·L-SCP in Expt. II contained 0.1 pmoles bound sterol and 0.3 mmoles bound lecithin per µg carrier protein (see Methods).

(e) A-Activator is the heat-stable extract equivalent to 4 mg adrenal mitochondrial acetone powder (see Methods and (8)).

(Figure 1 and Table II) was obtained by extraction (with 0.02 M phosphate buffer, pH 7.4, containing 1 mM $MgSO_4$) of an acetone powder made from bovine adrenal mitochondria according to the procedure of Halkerston et al. (7).

The adrenal activator (Table II) was prepared by heat treatment (100°, 2 min.) of the soluble cleavage enzyme preparation, followed by removal and discard of coagulated protein (8). Cleavage enzyme activity was destroyed by the heating. Isolation and identification of the conversion product of cleavage enzyme activity, pregnenolone, was similar in detail to that described by Koritz and Hall (9).

RESULTS AND DISCUSSION

The data of Table I show that it is possible to isolate from adrenal glands two enzymes catalyzing final steps in cholesterol synthesis, i.e. Δ^7-sterol Δ^5-dehydrogenase and $\Delta^{5,7}$-sterol Δ^7-reductase (1,6). The activities, cellular location, and properties of the adrenal enzymes are strikingly similar to those of the liver enzymes. In particular, both the adrenal and liver enzymes require the soluble cell fraction for maximum activity. The liver soluble fraction (i.e. unpurified SCP) will substitute for the adrenal soluble fraction and vice versa, yielding a three-fold or greater activation of each enzyme. Earlier reports showed that the degree of activation by liver-SCP of liver enzymes is dependent on the protein ratio (Table 1, Column 2) of SCP to enzyme (2,3); infinite activation or absolute requirement for added SCP is observed when residual SCP is removed from the liver enzymes (4). Further studies are required to determine whether the molecular properties of the activating material present in the adrenal soluble fraction are similar to those of liver-SCP, as is suggested by these functional properties.

In prior work the affinity of liver-SCP for pregnenolone was shown to be approximately one-third that for cholesterol (3). On the basis of these bindings studies and related findings, the suggestion was made by Ritter and Dempsey (2) that a cholesterol·SCP complex participates in the initial stages of steroid hormone biogenesis. More recently a similar suggestion was made by other authors (10). Support for this proposal now has been obtained using liver-SCP preparations with a soluble enzyme, isolated from adrenal mitochondria,

which catalyzes the conversion of cholesterol to pregnenolone. A partially purified liver-SCP preparation activated (>4-fold) the enzymic side-chain cleavage of cholesterol (Figure 1). With increasing levels of liver-SCP the degree of activation reached a maximum and then decreased slightly, similar to the effects of liver-SCP on liver enzymes catalyzing cholesterol synthesis (2). The same effects on the cleavage enzyme system were also observed with a highly purified complex of labeled cholesterol and liver-SCP (Table II). Bound cholesterol was readily converted to pregnenolone by the enzyme system. Control studies showed that liver-SCP preparations do not contain cleavage enzyme activity. Neither bovine serum albumin nor rat plasma could substitute for liver-SCP. However, when a labeled cholesterol·lecithin·SCP complex was used as substrate for the cleavage enzyme system, marked inhibition of pregnenolone synthesis was observed (Table II). The latter observation is in accord with the report of Mason and Boyd (11), showing that the cleavage enzyme of human term placenta can be inhibited by phospholipid. In contrast, liver enzymes catalyzing cholesterol synthesis are not affected by the presence of phospholipids in a cholesterol precursor·SCP complex (4).

In related studies the occurrence of an activator of the cleavage enzyme system was detected in adrenal preparations (Table II). Further work (described in detail elsewhere (12)) showed that, similarily to liver-SCP, the adrenal activator is a heat-stable protein with high affinity for cholesterol. Identity of the physical and functional properties of the adrenal activator with those of liver-SCP must await further purification and characterization of the activator. In addition, it is probable that purification of the cleavage enzyme system will result in greater levels of activation by SCP and the adrenal activator than those reported here (Figure 1 and Table II).

Demonstration of the occurrence of an SCP-like protein in the adrenal and the function of liver-SCP with adrenal enzymes is in accord with the suggestion that an SCP-type protein is a general biological requirement for synthesis, metabolism, and transport of certain water insoluble compounds, in addition to

cholesterol. Further evidence for this proposal was provided by Rilling (13) who showed that liver-SCP participates in the synthesis of squalene from farnesyl- and presqualene- pyrophosphate by liver and yeast enzymes. He also detected an SCP-like protein in yeast. Related evidence is the presence of an SCP-like protein in protozoa and the participation of liver-SCP with protozoan enzymes catalyzing sterol interconversions (14).

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