THE EFFECT OF STEROL CARRIER PROTEIN ON SQUALENE SYNTHESIS

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SUMMARY

Sterol carrier protein from liver has been shown to bind the squalene precursor presqualene pyrophosphate but not farnesyl pyrophosphate. This protein fraction also stimulated the enzymatic conversion of farnesyl pyrophosphate to both presqualene pyrophosphate and squalene. A heated, high-speed supernatant fraction from yeast also was shown to bind presqualene pyrophosphate and to enhance the conversion of farnesyl pyrophosphate to presqualene pyrophosphate and squalene.

Sterol carrier protein (SCP), a protein fraction from liver, binds water-insoluble cholesterol precursors and enhances their metabolism in vitro[1, 2]. This protein fraction does not bind to or enhance the metabolism of the water-soluble sterol precursors[2]. The transition from water solubility to insolubility for cholesterol precursors occurs during the conversion of farnesyl pyrophosphate to presqualene pyrophosphate and squalene, a sequence of reactions that has not been examined for sensitivity to SCP. In this paper the stimulation of the synthesis of presqualene pyrophosphate and squalene from farnesyl pyrophosphate is demonstrated. Also, presqualene pyrophosphate, but not farnesyl pyrophosphate, is shown to be bound by SCP.

MATERIALS AND METHODS

The preparation of trans 1-$^{3}H_2$-farnesyl pyrophosphate of yeast microsomes and the assays for the biosynthesis of presqualene pyrophosphate and squalene have been described[3, 4, 5]. Tritiated presqualene pyrophosphate was obtained by pyrophosphorylating chemically prepared radioactive presqualene alcohol (samples of which were

furnished by Drs. L. J. Altman and R. M. Coates). Liver microsomes and SCP were prepared by the method of Ritter and Dempsey[1]; protein was determined by the biuret method[6].

RESULTS

Initial experiments showed squalene synthesis from farnesyl pyrophosphate was enhanced as much as several fold when SCP was included in incubation mixtures containing liver microsomes (Table 1). The stimulation was consistently greater at low enzyme concentrations and disappeared at the higher concentrations, although at the higher concentration only about 10% of substrate was converted to squalene. Lower amounts of SCP were without effect on squalene synthesis by liver microsomes. In control experiments, equivalent amounts of phosphate buffer did not stimulate squalene synthesis significantly. Since farnesyl pyrophosphate and presqualene pyrophosphate are rapidly hydrolyzed by liver microsomes, these microsomes are a poor enzyme source for studying SCP effects on squalene synthetase[5].

Yeast microsomes, which are a better source of the synthetase, since they are relatively free of phosphatase activity, were tested for stimulation of squalene synthesis by liver SCP. The data presented in Table 2 show that relatively low levels of SCP from

TABLE 1

EFFECT OF SCP ON SQUALENE SYNTHESIS BY LIVER MICROSOMES

Microsomes µg	Squalene synthesized (pM)			
	- SCP	+ SCP 200 µg	- SCP	+ SCP 500 µg
0	- -	11	- -	13
11	29	71	26	68
55	230	320	230	295
220	510	690	850	820

Incubation mixtures contained K phosphate, 10 mM, pH 7.4; KF, 5 mM; $MgCl_2$, 5 mM; NADPH, 0.5 mM; 1-$^3H_2$-farnesyl pyrophosphate, 8 nmoles (specific activity 9 mc/mmole); in a total volume of 0.2 ml. Incubations were for 15 min at 30°.

TABLE 2

EFFECT OF SCP ON SQUALENE SYNTHESIS BY YEAST MICROSOMES

SCP added, μg	Squalene synthesized (pM)
0	285
10	455
50	710
200	760
500	870
500+	10

Conditions were the same as given in Table 1 except that 20 μg of yeast microsomes were added per tube.

+ Incubated minus microsomes.

liver enhanced squalene synthesis by yeast microsomes. In experiments not reported, the most dramatic stimulation of squalene synthesis by SCP was found at the lowest yeast enzyme concentration assayed.

The synthesis of presqualene pyrophosphate from 1-$^3H_2$-farnesyl pyrophosphate is most conveniently determined by measuring the radioactive proton ejected during the condensation reaction[5]. With this assay, an enhancement of presqualene pyrophosphate synthesis by liver SCP was observed (Table 3), although this stimulation was never as great as that with squalene synthesis. If NADPH was added to the incubation mixtures so that presqualene pyrophosphate would not accumulate, the stimulation of presqualene pyrophosphate synthesis by SCP was still observed. Here again, with increased enzyme concentration, the stimulatory effect of SCP decreased and finally disappeared.

The stimulation of yeast microsomal squalene synthesis by liver SCP suggested the possibility that yeast might also contain SCP activity. Initial experiments showed sufficient squalene synthetase activity was present in the high-speed supernatant fraction from yeast to obscure any SCP activity. After the squalene synthetase was inactivated by brief heating, the high-speed supernatant fraction stimulated the conversion of farnesyl pyrophosphate to squalene with either yeast or liver microsomes as the enzyme source. The degree of stimulation was comparable to that obtained with liver SCP.

TABLE 3

EFFECT OF SCP ON PRESQUALENE PYROPHOSPHATE SYNTHESIS

SCP added, μg	Presqualene pyrophosphate synthesized (pM)	
	- NADPH	+ NADPH
0	47	47
20	58	139
100	85	143
500	122	153
500+	0	7

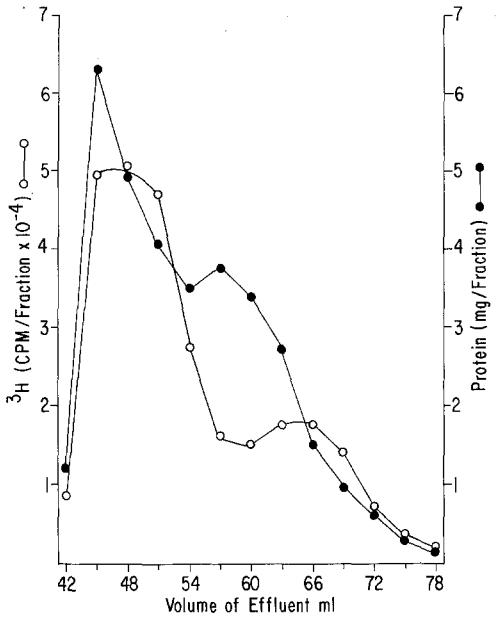
Conditions are the same as Table 1 except that 9 nmole farnesyl pyrophosphate of specific activity of 50 mc/mmole was added per tube.

+ Incubated minus microsomes.

In addition to stimulating sterol synthesis, SCP has been shown to bind water-insoluble cholesterol precursors. Following the method of Ritter and Dempsey[1], a dioxane solution of presqualene pyrophosphate was added to an SCP fraction from pig liver. After precipitation by ammonium sulfate, the protein was chromatographed on Sephadex G-75. As shown in the figure, the radioactivity remained associated with the protein during this fractionation procedure. The radioactive material that eluted with protein was shown by thin-layer chromatography to be presqualene pyrophosphate. In similar experiments, both rat liver SCP and a heated yeast high-speed supernatant fraction were shown to bind presqualene pyrophosphate. When these experiments were repeated with radioactive farnesyl pyrophosphate, no significant binding to protein was observed. If SCP was omitted from this procedure, presqualene pyrophosphate was not eluted in the excluded volume from the sephadex column.

DISCUSSION

The function proposed for SCP is to bind water-insoluble cholesterol precursors, thus solubilizing them and facilitating their diffusion from enzyme to enzyme. In this series of experiments, we have examined the reactants and the reactions between the



Binding of presqualene pyrophosphate to SCP from pig liver. The procedure described in the legend to Figure 1, Reference 1, was used. Approximately 1.4 µmoles presqualene pyrophosphate (50 mC/mmole) were combined with 55 mg of SCP and chromatographed on a 1.5- by 80-cm column of Sephadex G-75. The volume of the fractions was three ml.

water-soluble precursor farnesyl pyrophosphate and squalene, a water-insoluble precursor, which has been shown by others [1] to bind to SCP. In this instance we found the squalene precursor presqualene pyrophosphate was bound by SCP while its precursor farnesyl pyrophosphate was not.

A SCP-mediated acceleration of squalene synthesis from farnesyl pyrophosphate also was found. This can be interpreted in terms of the proposed role for SCP. Presqualene pyrophosphate, the water-insoluble product of the condensation of farnesyl pyrophosphate, would be bound by SCP for transport to the active site of the next enzyme or to a different site in the same enzyme complex for reduction to squalene by NADPH. The activation of the synthesis of presqualene pyrophosphate by SCP is less readily explained, since the substrate for this reaction is water soluble and is not bound by SCP. A possible explanation for this comes from the observation that this reaction was stimulated by NADPH, which by conversion of the product to squalene may well relieve product inhibition[7]. Thus, SCP could also activate the synthesis of presqualene pyro-

phosphate by binding (removing) the product with concomitant elimination of product inhibition. An alternate explanation would be that SCP was activating this reaction simply by detergency. This would be consistent with the observation that this reaction[7], like others[8], can be stimulated by a variety of detergents.

This paper also reports SCP activity in preparations from yeast.

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