Short Communication

Intracellular Localization of Cholesterol Biosynthesis*

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Cholesterol is present in various amounts in different tissues and intracellular organelles and contributes to the structure and stability of membranes. Newly synthesized cholesterol is also a component of lipoproteins which are transported through the endoplasmic reticulum (ER)–Golgi system to the blood. Newly synthesized cholesterol is also transported to the lysosomes, followed by excretion in the bile. Cholesterol thus functions as a substrate for both bile acid and steroid synthesis.

Experiments based on subcellular fractionation lead to the conclusion that cholesterol is synthesized in the ER and is then redistributed to both intra- and extra-cellular compartments.[1,2] Recent investigations have demonstrated that a certain amount of cholesterol biosynthesis also occurs in the soluble luminal compartment of peroxisomes.[3,4] The contribution of this latter process to the total cholesterol synthesis under normal conditions is limited, but under certain experimental conditions, such as during cholestyramine treatment, the rate of peroxisomal cholesterol synthesis can be increased by a factor of 100.

The microsomal fraction isolated from hepatocytes catalyzes cholesterol synthesis at a high rate according to previous investigations.[1,2] The lipid in this fraction also displays a high initial level of labeling after administration of (^{3}H)mevalonate *in vivo*. Synthesis of cholesterol on the endoplasmic reticulum has, however, recently been questioned.[5,6] Based on subfractionation experiments it was claimed that in cultured human fibroblasts, incorporation of (^{3}H)acetate into cholesterol and its metabolites does not occur in the ER, but in structures recovered in the Golgi fraction.

This paper deals with the intracellular site of cholesterol biosynthesis as determined by following the (^{3}H)mevalonate incorporation into cholesterol in an *in vitro* system consisting of microsomal subfractions, supernantant, divalent cations, phosphate buffer, NAD, NADPH, FAD and an effective ATP-generating system. After alkaline hydrolysis, lipid extraction and purification, the lipid extracts were subjected to high performance liquid chromatography (HPLC) (Fig. 1). Cholesterol and two metabolites, lanosterol and squalene, were identified by the use of standards and found to elute at 7.29, 7.96 and 11.89 min, respectively. The radioactivity in these isolated peaks was subsequently determined by scintillation counting.

In order to characterize our microsomal fractions, we measured marker enzymes in the isolated subfractions and calculated the contamination of the microsomal fractions on a protein basis (Table 1). The contamination of rough microsomes with mitochondria, Golgi vesicles, lysosomes, peroxisomes and plasma membranes was altogether around 10 %. In smooth microsomes there was a somewhat higher content of Golgi vesicles and plasma membranes, but the purity of this fraction was still as high as approximately 85 %. Clearly, the microsomal fractions used in our experiments are of sufficiently high quality for the study of the localization of biosynthetic processes.

Upon incubation with (^{3}H)mevalonate, smooth I microsomes, the major smooth microsomal fraction from liver,[7] exhibited about twice as much labeling in cholesterol as did rough microsomes (Table 2). A high rate of incorporation into squalene was obtained in both subfractions, while the level of labeling in lanosterol was only a tenth of that observed in cholesterol. This pattern indicates that there is a rate-limiting step involved in the transformation of squalene into lanosterol. Cholesterol biosynthesis was also found in smooth II microsomes, the Mg^{++}-insensitive portion of the smooth microsomal fraction,[7] but the rate of (^{3}H)mevalonate incorporation into cholesterol, squalene and lanosterol was, in this case, significantly lower than that in the other microsomal subfractions. Upon incubation of mitochondria, Golgi vesicles, lysosomes and plasma membranes with (^{3}H)mevalonate, no labeling of cholesterol was observed, but with peroxisomes moderate labeling was seen.

The experiments described above demonstrate that cholesterol biosynthesis in fact occurs in both rough and smooth microsomes. The corresponding localization in the ER is supported by the fact that the contamination of microsomes by other cellular membranes is low. Also, the

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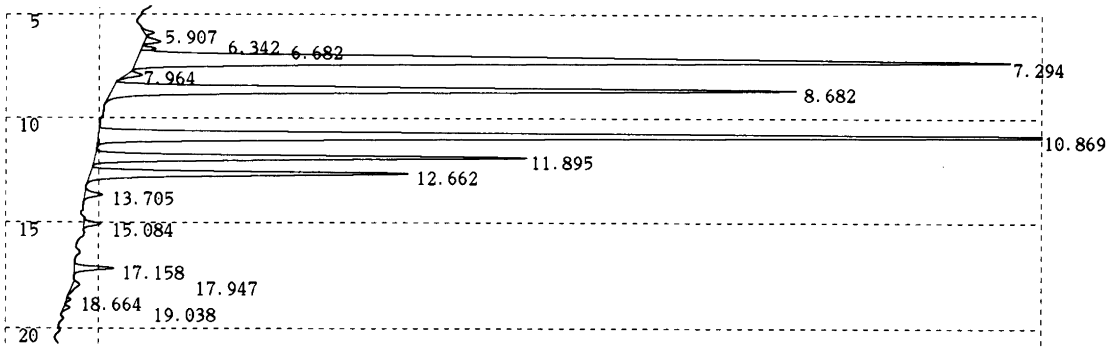

Fig. 1. HPLC pattern of mevalonate products after *in vitro* incubation of rat liver smooth I microsomal subfraction. The peaks eluting at 7.296, 7.964 and 11.895 min were identified as cholesterol, lanosterol and squalene, respectively.

rate of cholesterol biosynthesis by other isolated subcellular fractions was found to be neglible. The purity of the membrane fraction employed is of crucial importance when discussing localization of a synthetic process in the same membranes. In experiments where the ER localization of cholesterol biosynthesis was investigated, a simplified subfractionation of tissue culture homogenate using a one-step density gradient was employed.[4,5] It should also be taken into consideration that different types of tissues were used in these experiments, and that *in vivo* labeling may not be equivalent to labeling of an isolated subcellular fraction in an *in vitro* system.

The conclusion that the major site of cholesterol biosynthesis resides in the ER agrees well with a number of previous findings. The ER is the cellular compartment where initial assembly of the hepatic lipoproteins takes place and the enzyme which esterifies newly synthesized cholesterol, acyl-CoA-cholesterol acyltransferase, is found exclusively in this organelle. In addition, the initial and major portion of bile-acid and sterol synthesis takes place in the ER and in *in vivo* experiments a high level of initial labeling of cholesterol from labeled precursors was observed in microsomes. Thus, the major cellular site of cholesterol biosynthesis in liver cells is the rough and smooth endoplasmic reticulum. This, however, does not exclude the possibility that specialized membrane segments of this organelle are enriched in the synthetic enzymes involved, since the heterogeneity of these fractions is well documented.

Table 1. Purity of rough and total smooth microsomes isolated from rat liver. The percentage contamination was calculated as described in the Experimental section. The values are the means of 5 experiments.

	Contamination (%)	
	Rough microsomes	Smooth microsomes
Mitochondria	0.6	0.3
Golgi vesicles	3	6
Lysosomes	2	3
Peroxisomes	4	2
Plasma membranes	2	5

Table 2. In vitro labeling of squalene, lanosterol and cholesterol with (^{3}H)mevalonate by rat liver microsomal subfractions. The values are the means ± SEM of 5 experiments.

Subfractions	cpm/min/mg protein		
	Squalene	Lanosterol	Cholesterol
Microsomes			
Rough	11900±805	321±29	2510±175
Smooth I	12060±1320	476±46	4850±581
Smooth II	4305±486	86±10	120±153
Mitochondria	–	–	85
Golgi vesicles	–	–	204
Lysosomes	–	–	96
Peroxisomes	–	–	717
Plasma membranes	–	–	127

Experimental

Microsomal subfractions and other membrane fractions were prepared from livers of Sprague–Dawley rats as described previously.[7,8] The specific activities of various marker enzymes in the isolated fractions were determined and used to calculate the percentage contamination on a protein basis in the microsomal subfractions as described by Tollbom *et al.*[9] The incubation mixture contained, in a final volume of 1.4 ml, 7 mM phosphoenolpyruvate, 3.6 mM ATP, 25 units pyruvate kinase, 3.6 mM $MgCl_2$, 2.8 μM NAD, 0.05 μM FAD, 1.10 μM NADPH, 50 μCi (*RS*)-(5-^{3}H)mevalonolactone (13.8 Ci/mmol, New England Nuclear) and 6 mg protein. Incubation was performed at 37 °C for 10 min. After alkaline hydrolysis, ether extraction of lipids, purification on a C-18 cartridge and filtration, HPLC separation was performed on a Hewlett-Packard Hypersil ODS 3-μm reversed-phase column. A linear gradient was used from the initial methanol:water (9:1) in pump system

A to methanol:2-propanol (4:1) in pump system B. The absorbance of the eluate at 210 nm was monitored. The individual lipids were collected and the radioactivity was determined by scintillation counting.

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