SUBMICROSOMAL LOCALIZATION OF HEPATIC 3-HYDROXY-3-METHYLGLUTARYL COENZYME A (HMG-COA) REDUCTASE

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1. Introduction

Hepatic HMG CoA reductase (mevalonate:NADP oxidoreductase (acylating CoA), EC 1.1.1.34), the rate controlling enzyme for cholesterol synthesis, is known to be located almost entirely in the microsomal fraction of the liver [1, 2]. Two previous reports in the literature have suggested that 95% of the microsomal enzyme activity is located in the rough endoplasmic reticulum [3, 4]. We wish to report the results of our own cell fractionation studies which indicate that, quite to the contrary, over 80% of the activity is found in the fractions of smooth membranes composed of smooth endoplasmic reticulum, Golgi apparatus and plasma membrane.

2. Materials and methods

For our studies, 120–140 g male Holtzman rats were cyclically fed a semi-synthetic diet containing 2% cholestyramine and 20% corn oil that is known to induce a high level of HMG CoA reductase activity [7]. The rats were fed for 8 hr a day between 8 a.m. and 4 p.m. and the lights were automatically controlled to go off at 8 a.m. and on at 8 p.m. daily. Rats were sacrificed at 8 a.m. on the 10th day of feeding, when previous studies had indicated that enzyme activity was about 1/2 maximal obtainable activity [8]. Livers were removed and homogenized in 2 vol of a pH 7.3 solution containing 0.25 M sucrose, 0.5 M Tris and 0.001 M $MgCl_2$ (0.25 M STM). We employed a discontinuous gradient method [5], based on Rothschild's observation [6] that smooth membranes float about 1.30 M sucrose whereas rough endoplasmic reticulum sediments. Homogenates were centrifuged for 10 min at 18,000 *g* and the microsome rich supernatant carefully decanted. Part of the supernatant was diluted with 1.5 vol of 2.0 M STM to give a microsomal suspension in 1.31 M STM. Fifteen ml of this diluted supernatant were overlaid on 5 ml of 2.0 M STM in a 40 ml centrifuge tube. Ten ml of 1.23 M STM were next overlaid and a final 5 ml of 0.8 M STM were carefully layered on. For comparison and in order to approximate the condition of centrifugation in the separated fractions, control "whole microsomes" were prepared by layering 15 ml of the original 0.25 M STM supernatant on 20 ml of 2.0 M STM. These two types of discontinuous gradients were centrifuged at 50,000 rpm for 3 hr in a 50.1 rotor (Beckman Instruments). The tubes were then cut and 4 fractions were collected from the completely separated microsomes and two fractions from the "whole microsome" discontinuous gradients. The four part gradients contained a "smooth" smooth fraction at the interface between 0.8 M and 1.23 M STM, previously shown in this laboratory to contain Golgi vesicles and cell membranes; a fraction of smooth membranes containing predominantly derivatives of smooth endoplasmic reticulum at the interface between 1.23 M and 1.31 M STM; and a pellet at the bottom of the tube containing free polysomes [5]. In the "control" tubes, whole microsomes were collected from the interface region between the 2.0 M and 0.25 M STM and a small pellet of polysomes was also collected. All fractions were separated into centrifuge tubes, diluted with pH 7.3 buffer containing 0.25 M sucrose, 0.02 M K_2HPO_4, 0.07 M NaCl, 0.02 M Na_2EDTA, 0.001 M dithiothreitol and centrifuged for 1 hr at 30,000 rpm in a

30 rotor. The pellets were then resuspended in the same buffer and centrifuged for an additional hour at 30,000 rpm. Pellets were finally resuspended in a pH 7.3 buffer containing 0.02 M K_2HPO_4, 0.07 M NaCl, 0.02 M Na_2 EDTA and 0.001 M dithiothreitol. Proteins were determined by the Lowry method [9] and assays for HMG CoA reductase were carried out at 0.25 and 0.5 mg microsomal protein using our own double label assay procedure [10].

3. Results and discussion

In a typical fractionation (table 1), the smooth membrane and "smooth" smooth fractions together accounted for 81% of the total enzyme activity and the rough microsomes accounted for only 14% of the total activity. Although this was in part a result of the separation which yielded 72% of the protein in the smooth fractions, more significantly the specific activity of the smooth fractions was at least 2 times greater than that in the rough endoplasmic reticulum. Furthermore, even if the initial 10 min centrifugation of the whole homogenate at 18,000 *g* resulted in a preferential loss of as much as 2/3 of the rough endoplasmic reticulum, then this fraction's activity would still only account for about 1/3 of the total enzyme activity. The finding of moderate enzyme specific activity in the free polysomes is also noteworthy, since this fraction might contain HMG CoA reductase, which is synthesized here and then transferred to the microsomal membranes. Alternatively, the enzyme might be synthesized on both free and bound polysomes. Also of interest is our finding that the sum of the HMG CoA reductase activities in the washed "control" whole microsomes and polysomes (table 2) is only 8% less than the sum of fraction activities in the more complete separations. This indicates that the physical separation of the microsomal fractions did not appreciably alter the enzyme activity. In contrast, separation reduced the protein content by almost 1/3, probably by removing some intravesicular or trapped soluble proteins.

We cannot account for the striking discrepancy

Table 1
Complete fractionation.

	mg protein/g liver	HMG CoA reductase activity*/mg protein	HMG CoA reductase activity*/g liver
Smooth membranes	4.5	36.6	164.7
"Smooth" smooth membranes	3.0	40.7	122.0
Rough microsomes	2.7	18.3	49.4
Free polysomes	0.7	26.8	18.8
Total	10.4	–	354.9

* mμmoles mevalonate/hr.

Table 2
Whole microsomes preparation.

	mg protein/g liver	HMG CoA reductase activity*/mg protein	HMG CoA reductase activity*/g liver
Whole microsomes	14.1	23.1	325.7
Free polysomes	0.3	17.2	5.2
Total	14.4	–	330.9

* mμmoles mevalonate/hr.

between our localization of HMG CoA reductase in smooth membranes and the earlier descriptions of preponderant enzyme activity in the rough endoplasmic reticulum, since the exact details of the method [11] used in those separations are not available. In view of the striking diurnal rhythmicity of this enzyme, which in most cases follows the feeding pattern, [12] considerable caution is justified in relating the localization of the enzyme to previous reports in the literature, where the feeding was not carefully controlled. However, it may be significant that effects known to increase the amounts of smooth endoplasmic reticulum including ethanol feeding, [13], bile duct ligation, [14], and phenobarbital treatment [15] have all been reported to be associated with increased levels of hepatic cholesterogenesis [16–18].

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