DISTRIBUTION OF GLYCEROLIPID-SYNTHESIZING ENZYMES IN THE SUBFRACTIONS OF RAT LIVER MICROSOMES

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Received 14 December 1972

1. Introduction

Several lines of evidence indicate that endoplasmic reticulum is the main location of lipid synthesis in the cell [1–10]. This vacuolar system is differentiated into rough-surfaced reticulum containing ribosomes and smooth-surfaced reticulum without ribosomes. Certain findings point to the smooth membrane system as the primary subcellular site of lipid synthesis in the liver cell [6–10].

The present investigation concerns the distribution of some enzymes involved in glycerolipid synthesis in the subfractions of rat liver microsomes. We have found that glycerophosphate acyltransferase, which catalyzes the initial step of glycerolipid formation, is located predominantly in the smooth microsomal fraction. On the other hand, monoacylglycerophosphate acyltransferase, phosphatidate cytidyltransferase, cholinephosphotransferase and diglyceride acyltransferase have been shown to occur in both types of microsomes.

2. Materials and methods

Male Wistar rats (180–220 g) fed ad libitum a balanced diet (Clea, Tokyo, Japan) were used in all experiments. Liver microsomes were fractionated by the method of Glaumann and Dallner [11] with some modifications. The postmitochondrial supernatant (2.7 ml) in 0.25 M sucrose containing 15 mM CsCl was placed upon a discontinuous gradient which consisted of 2 ml of 1.3 M sucrose containing 15 mM CsCl and 0.5 ml of 2.0 M sucrose. After centrifugation at 650,000 rpm for 2 hr in an SW 65L rotor (Beckman Instruments, Palo Alto, USA), the rough microsomal fraction was obtained as a layer at the interface between 1.3 M and 2.0 M sucrose, and the smooth microsomal fraction between 0.25 M and 1.3 M sucrose. Total microsomes were prepared as described [11].

All enzymes were assayed at 37° unless otherwise stated. One enzyme unit is defined as the amount of enzyme catalyzing transformation of 1 μmole of substrate per min. Specific activity is expressed as units per milligram of protein. Glycerophosphate acyltransferase was assayed as described previously [12], except that incubation was carried out at 37° for 2 min. Monoacylglycerophosphate acyltransferase was assayed according to the principle described by Lands and Hart [13]; the reaction mixture (total volume, 1.0 ml) contained 100 μmoles Tris-HCl buffer pH 7.4, 1 μmole 5,5′-dithiobis (2-nitrobenzoic acid), 20 nmoles palmityl-CoA, 50 nmoles 1-acylglycerol 3-phosphate derived from egg lecithin and 100–200 μg microsomal protein. Phosphatidate cytidyltransferase was assayed by the method of Carter and Kennedy [14]; incubation was carried out for 20 min. Cholinephosphotransferase was assayed according to Young and Lynen [15], except that the concentration of diglyceride was 5 mM, cysteine was replaced by 1 mM dithiothreitol, and Tris-HCl buffer pH 7.8 was used. Diglyceride acyltransferase was assayed by the method of Weiss et al. [16] with the following modifications: The reaction mixture (total volume, 0.25 ml) contained 7.5 μmoles Tris-HCl buffer pH 7.6, 1.5 μmoles $MgCl_2$, 0.5 μmole dithiothreitol, 140 μg bovine serum albumin, 75 nmoles [9, 10-$^3H_2$] palmityl-CoA, 1 μmole diglyceride derived from egg lecithin and 30–50 μg microsomal protein. After shaking for 15 min, the reaction was

Table 1

Submicrosomal distribution of glycerophosphate acyltransferase, cholinephosphotransferase and diglyceride acyltransferase.

Microsomal Subfraction	Protein	RNA/protein	Glycerophosphate acyltransferase		Cholinephospho-transferase		Diglyceride acyltransferase	
			Specific activity	Total activity	Specific activity	Total activity	Specific activity	Total activity
	(%)	(mg/mg)	(m-units/mg)	(%)	(m-units/mg)	(%)	(m-units/mg)	(%)
Total	100	0.13	2.20	100	20.8	100	3.93	100
Rough	31	0.16	0.58	8	22.0	33	4.66	37
Smooth	36	0.04	3.15	51	12.5	21	2.95	27
Recovery	67			59		54		64

terminated by the addition of 5 ml of a mixture containing petroleum ether (b. p., 40–45°), isopropanol and 1 N H_2SO_4 (10 : 40 : 1 by vol). Two phases were then separated by adding 4 ml petroleum ether and 2 ml water. The upper phase, which contained the radioactive lipid product, was washed successively with 0.2 M $NaHCO_3$ and with 0.2 M KCl, and was then chromatographed on a 10 mM-Na_2CO_3-impregnated silica gel H plate with ether–*n*-hexane–acetic acid (25 : 75 : 1 by vol). The area containing triglyceride was scraped and counted in Bray's solution [17].

Other materials and methods were as described previously [12].

3. Results

In table 1 is shown the distribution of glycerophosphate acyltransferase, cholinephosphotransferase and diglyceride acyltransferase between rough and smooth microsomes. The most outstanding feature was the predominant location of glycerophosphate acyltransferase in smooth microsomes. On the other hand, cholinephosphotransferase and diglyceride acyltransferase, which catalyze the essential steps in the synthesis of phosphatidylcholine and triglyceride, respectively, were divided between the two microsomal fractions. The localization of monoacylglycerophosphate acyltransferase and phosphatidate cytidyltransferase was also bimodal as documented in table 2. Table 3 presents the results of an experiment in which glycerophosphate acyltransferase was assayed with various acyl-CoA thioesters as acyl donors. The data again indicated the preferential location of this enzyme in the smooth microsomal fraction. In order to examine a possible effect of the nutritional state on the distribution of the enzymes, analogous studies were performed with rats fasted for 16–22 hr. However, similar distribution patterns were observed.

4. Discussion

In view of our recent finding that the phosphatidate-synthesizing system of rat liver microsomes can be resolved into and reconstituted from glycerophosphate acyltransferase and 1-acylglycerophosphate acyltransferase, the conversion of *sn*-glycerol 3-phosphate to monoacylglycerol 3-phosphate represents the initial step of glycerolipid synthesis [12, 18]. Therefore, the results of the present investigation indicate that rough microsomes are deficient in the enzyme responsible for the initial step of the synthetic pathway, and that smooth microsomes, in contrast, contain all tested enzymes necessary for glycerolipid formation. This seems to favor the view that smooth-surfaced endoplasmic reticulum is the primary subcellular site of lipid synthesis in the liver cell [6–10].

It has been suggested that enzymes in liver microsomal membranes are synthesized by membrane-bound ribosomes and then incorporated into membranes [19, 20]. The enzyme distribution pattern observed in the present investigation might suggest that newly synthesized glycerophosphate acyltransferase molecules are inserted preferentially into the smooth region of endoplasmic reticulum. An alternative interpretation of the present results might be that the synthesis of glycerophosphate acyltransferase molecules on bound ribo-

Table 2

Submicrosomal distribution of monoacylglycerophosphate acyltransferase and phosphatidate cytidyltransferase.

Microsomal subfraction	Protein	RNA/protein	Monoacylglycerophosphate acyltransferase		Phosphatidate cytidyltransferase	
			Specific activity	Total activity	Specific activity	Total activity
	(%)	(mg/mg)	(m-units/mg)	(%)	(m-units/mg)	(%)
Total	100	0.12	27	100	0.96	100
Rough	28	0.18	23	24	0.77	23
Smooth	46	0.03	31	53	1.04	50
Recovery	74			77		73

Table 3

Submicrosomal distribution of glycerophosphate acyltransferase as assayed with various acyl donors.

Microsomal subfraction	RNA/protein	Glycerophosphate acyltransferase*				
		Palmityl-CoA	Stearyl-CoA	Oleyl-CoA	Linoleyl-CoA	Arachidonyl-CoA
	(mg/mg)			(m-units/mg)		
Total	0.13	1.03	0.33	0.72	1.03	0.30
Rough	0.20	0.33	0.11	0.20	0.30	0.10
Smooth	0.04	1.73	0.90	1.59	1.60	0.50

* Incubation was conducted at 20° for 5 min.

somes proceeds very slowly and is accomplished only shortly before the detachment of ribosomes from the membrane; such a detachment of ribosomes has been proposed as a possible mechanism for the biogenesis of smooth-surfaced endoplasmic reticulum [21].

Acknowledgements

This work was supported in part by research grants from the Ministry of Education of Japan, the Toray Science Foundation, the Japanese Foundation of Metabolism and Diseases, and the Tanabe Amino Acid Research Foundation.

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