ISOLATION OF RIBOSE-POOR ROUGH MICROSOMES FROM RAT LIVER

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1. Introduction

The rough microsomal fraction of the rat liver is the site of synthesis of newly appearing enzymes and membrane components in the newborn, phenobarbital- and alloxan-treated rats [1, 2]. It also synthesizes enzymes for other subcellular particles [3] and performs the complete or partial biosynthesis of most of the blood proteins [4–6]. During membrane biosynthesis, an increasing "spacing" among ribosomal groups is apparent on electronmicroscopical investigation, which raises the possibility that membrane growth, including the incorporation of constitutive microsomal enzymes, occurs in specific parts of the endoplasmic reticulum [7]. In order to approach this problem, we elaborated a method which enabled us to separate rough microsomal vesicles with only a few attached ribosomes. The procedure is based on the decreased sensitivity of these vesicles to monovalent cations which specifically aggregate rough microsomes.

2. Materials and methods

Livers of starved rats were homogenized in 0.44 M sucrose at a tissue concentration of 20% (w/v). Non-microsomal large particles were removed by centrifugation at 10,000 *g* for 20 min. The supernate was mixed with 1 M CsCl to give a final concentration of 15 mM. Total microsomes were obtained by centrifugation at 105,000 *g* for 90 min. For subfractionation, 3.5 ml CsCl-containing supernate was layered over 3 ml 1.3 M sucrose–15 mM CsCl. After centrifugation in a No. 40.2 rotor at 102,000 *g* for 90 min (Beckman Spinco, model L2-65B centrifuge), the content of the centrifuge tube was subdivided by using a bent needle connected to a syringe. The centrifuge tube was placed on a variable stand so that it could be raised to the exact height required. The clear upper phase was discarded, and the fluffy double layer at the gradient boundary was removed and designated *smooth microsomes* (Sm). The 1.3 M sucrose layer down to the upper edge of the pellet was sucked off (*rough III microsomes*, R III). The remaining sucrose solution was taken up with a Pasteur pipette (*rough II microsomes*, R II), and the pellet was suspended in 0.25 M sucrose (*rough I microsomes*, R I). Smooth, R II and R III microsomes were supplemented with water or sucrose to obtain a final concentration of 0.25 M sucrose, and after centrifugation at 105,000 *g* for 90 min the pellets were resuspended in 0.25 M sucrose.

The washing of subfractions and the chemical and enzyme assays were performed as described previously [7–11].

3. Results and discussion

The four microsomal fractions, R I, R II, R III and Sm contain about 47, 10, 18 and 25%, respectively, of the total protein (table 1). A sizable amount of protein can be removed by washing, which detaches mainly adsorbed and luminal nonmembranous proteins. The ribonucleic acid (RNA) to phospholipid (PLP) ratio is of decreasing order from R I to R III, indicating decreasing numbers of bound ribosomes. Preliminary electron microscopical investigations also demonstrate the paucity of ribosomes on R III microsomes. The RNA in Sm can be explained by the presence of free ribosomes, not affected by Cs^+. The protein to

Table 1
Chemical composition of rough microsomal subfractions.

	Total	R I	R II	R III	Sm
Protein*	23.5	10.1	2.2	3.9	5.2
Protein* (washed)**	13.3	5.1	1.5	2.8	2.7
PLP*	7.0	2.5	0.62	1.5	1.8
RNA*	2.5	1.6	0.19	0.30	0.20
Cholesterol*	0.58	0.19	0.047	0.095	0.20
RNA / PLP	0.36	0.62	0.31	0.20	0.11
Protein / PLP	3.4	3.4	3.4	2.4	2.8
Protein / PLP (washed)**	1.9	2.1	2.0	1.8	1.6
Cholesterol / PLP	0.083	0.076	0.076	0.062	0.11

* mg/g liver.
** Microsomes were washed with 0.15 M Tris-HCl buffer, pH 8.0 and incubated in water as described previously [12]. The results are the means of 5 experiments.

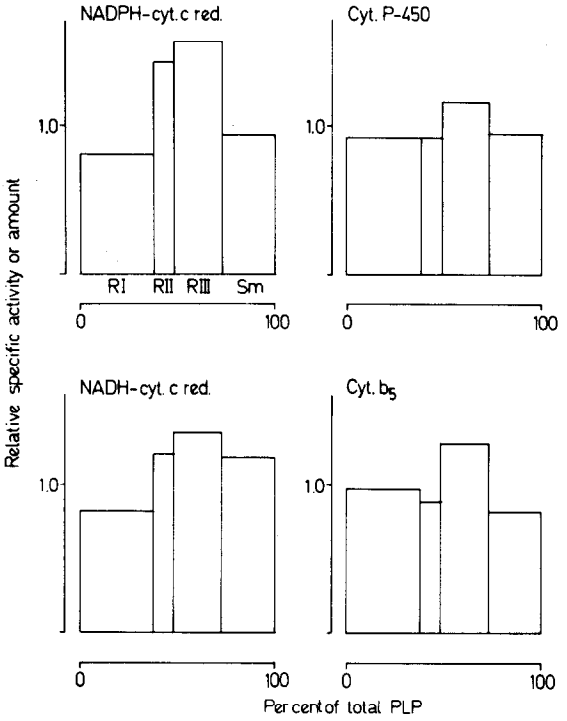

Fig. 1. Electron transport enzymes in rough microsomal subfractions. The plot is made in the manner adopted by de Duve et al. [13]. The results are the means of 9 experiments.

PLP ratio in washed microsomes shows a slight decrease from R I to Sm. All three rough subfractions exhibit a lower cholesterol to PLP ratio in comparison with smooth microsomes, which is in agreement with previous data [12].

Both NADPH- and NADH-cytochrome *c* reductase activities are enriched in R II but particularly in R III microsomes (fig. 1). Interestingly, the enrichments of cytochromes *P*-450 and b_5 are less pronounced. Commonly, specific activities are based on the protein content of isolated, non-washed subcellular particles, which in our case would give an even higher specific activity in R III microsomes. In this subfractionation procedure, PLP measurement seems to be more reliable. R II microsomes do not seem to represent a separate entity enzymatically but were treated separately in order to maintain a high level of purity in the R I and R III subfractions.

All four phosphates measured – glucose-6-phosphatase (G6Pase), inosine diphosphatase (IDPase), adenosine triphosphatase (ATPase), and p-nitrophenyl phosphatase (p-NPPase) – as well as uridine diphosphoglucuronic acid transferase (UDPGA-transferase) display a lower specific activity in R III than in R I

Table 2
Distribution of some enzymes in rough microsomal subfractions.

	Total	R I	R II	R III	Sm
G6Pase[1]	20.6	27.2	25.1	23.4	13.4
IDPase[1]	12.6	21.4	11.3	13.8	7.6
ATPase[1]	7.4	10.4	9.1	6.4	5.3
p-NPPase[2]	15.3	20.2	13.7	11.3	17.8
UDPGA--transferase[3]	4.3	5.6	4.5	4.5	3.1

[1] μmoles P_i/20 min/mg PLP.
[2] μmoles p-NPP hydrolyzed/min/mg PLP.
[3] μmoles p-nitrophenyl conjugated/min/mg PLP. The results are the means of 5 experiments.

microsomes (table 2). This distribution pattern is in contrast to that of the electron transport enzymes which are enriched in the former subfraction.

The presence of bound ribosomes on microsomal membranes introduces properties which are absent in ribosome-free vesicles, such as high affinity for monovalent cations and, consequently, aggregation. Since rough vesicles with few ribosomes are clearly less sensitive to Cs^+, they remain in an intermediate position on a cation-containing discontinuous sucrose gradient and for this reason can be easily separated with a simple one-step centrifugation procedure. A specific role for rough III microsomes is suggested by the enrichment of the electron transport enzymes in this subfraction.

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