A SIMPLE PROCEDURE FOR THE ISOLATION OF RAT LIVER MICROSOMES

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1. Introduction

Endoplasmic reticulum of rat liver undergoes extensive fragmentation upon homogenization [1]. The major component of endoplasmic reticulum which accounted for 10–13% of total liver was recognized as microsomes in early 1941 [2]. The rat liver microsomes were reported to have variable size from 50–300 nm [3], necessitating a high centrifugal force to isolate them in contrast to other subcellular fractions like nuclei and mitochondria. The standard procedure, thus requires a sophisticated instrument like an ultracentrifuge and, at the same time, is very time consuming. Taking advantage of one of the characteristic properties of rat liver microsomes and rat skeletal muscle microsomes [4, 5] to bind calcium ions, we report in this communication a relatively simple method for the isolation of rat liver microsomes.

2. Methods

A 20–25% liver homogenate of fasted adult rats of body weight 400–500 g was prepared in a Dounce homogenizer. The homogenizing medium consisted of isotonic sucrose containing 0.05 M Tris-HCl, pH 7.5, 0.005 M $MgCl_2$, 0.025 M KCl and 0.008 M $CaCl_2$. The pH of the salt solutions were also adjusted to 7.5. After clarifying the homogenate at 10,000 *g* for 15 min, 4 or 5 ml (equivalent to 1 g wet weight tissue) of the post-mitochondrial supernatant was diluted with 25 ml of 0.0125 M sucrose solution containing 0.008 M $CaCl_2$ and 0.005 M $MgCl_2$. The entire solution was stirred occasionally for a few minutes and then centrifuged at 1500 g_{max} for 10 min in a Sorvall GSA-rotor. The reddish pellet obtained was dispersed and recentrifuged twice at 1500 g_{max} for 10 min in dilute (0.0125 M) sucrose and finally homogenized in 0.05 M Tris-HCl buffer containing 0.005 $MgCl_2$ and 0.025 M KCl. Treating the rat liver under identical conditions described above with the exception of the addition of calcium chloride resulted in no pellet sedimenting at 1500 *g* for 10 min. On the other hand, normal microsomes which had been isolated at 105,000 *g* could be quantitatively sedimented at 1500 *g* in the presence of 0.008 M $CaCl_2$ under the similar experimental conditions described above. The normal rat liver microsomes were prepared from liver homogenates in isotonic sucrose containing 0.05 M Tris-HCl, pH 7.5, 0.005 M $MgCl_2$ and 0.025 M KCl [6]. The crude homogenate was centrifuged at 10,000 *g* for 10 min and the post-mitochondrial supernatant was carefully decanted. Microsomes were obtained by centrifuging at 105,000 *g* for 1 hr. The microsomal pellet was successively washed (rehomogenizing and sedimenting) twice in the same buffer and then finally suspended in the buffer. All operations for both preparations were carried out between 0–4°. Fresh samples were used in all enzymatic assays.

3. Results and discussion

In table 1 are summarized the studies of the "1500 *g* pellet" and the normal microsomes derived from rat liver. The main features of the "1500 *g* pellet" included a high glucose-6-phosphatase activity (14.3 ± 1.2) with the presence of other phosphatases namely Mg^{2+} –K^+ –Na^+-activated adenosine triphosphatase (6.3 ± 0.41), ionosine diphosphatase (8.1 ± 0.6) and

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Table 1
Total activities, lipid and RNA contents of "1500 *g* pellet" and normal microsomes.

	per g liver tissue	
	"1500 *g* pellet"	normal microsomes
Yield (protein)*	20.4 ± 0.8	17.8 ± 0.8
Glucose-6-phosphatase**	291.6	270.8
Mg^{2+}-K^+-Na^+-activated** adenosine triphosphatase	128.6	123.0
5'-Nucleotidase**	144.8	125.0
Ionosine diphosphatase**	185.6	166.3
succinic dehydrogenase***	0	0
Total lipids*	6.8	7.3
Phospholipids*	6.2	6.7
Cholesterol*	0.48	0.50
RNA*	4.2	3.9
Calcium*	0.56	0.13
Magnesium*	1.18	0.99

* Expressed in mg.
** μmoles Pi liberated per hr.
*** μmoles INT formazan per hr.
Results are mean of 4 or more preparations except for calcium and magnesium.

5'-nucleotidase (7.1 ± 0.5) and with practically no succinic dehydrogenase. All the enzyme activities referred to above are expressed in terms of μmole of inorganic phosphate released per mg protein per hr except succinic dehydrogenase which is given in terms of μmoles of INT formazan per mg protein per hr. Lipid composition showed that this pellet was comprised of 90–94% of phospholipid and 5–7% of cholesterol as percentage of total lipids which in turn was about 0.37–0.42 mg per mg protein. The RNA to protein ratio was about 0.22. Calcium and magnesium bound to "1500 *g* pellet" was 27 and 59 μg per mg protein respectively. On the basis of these results and on comparison with normal microsomes and those reported in the literature [7, 8], it was concluded that the preparation of "1500 *g* pellet" was indeed microsomes derived from endoplasmic reticulum.

As the microsomes are well known to be the major site of protein synthesis [9, 10], an important property like the incorporation of amino acids into microsomal proteins was investigated [11]. Here again, we have obtained evidence that there was no significant difference between the "1500 *g* pellet" and the normal microsomal preparation.

Preliminary studies [11] on the nature of Ca^{2+} binding indicate that it was specific for the aggregation of microsomes even in presence of other cations like Mg^{2+} and K^+. However, the addition of Mg^{2+} and K^+ was found to be necessary in obtaining the microsomes quantitatively and was perhaps necessary to maintain their structure [12, 13]. Data on the site of Ca^{2+} binding showed that it was largely bound to the membranous portion containing lipoproteins rather than to the ribosomal part (consisting mainly of RNA) of microsomes. Furthermore, more of the Ca^{2+} bound to microsomes was present in the smooth microsomal particles rather than in the rough microsomal particles, both separated according to the method of Dallner [7].

On the basis of these results it can be conclusively said that Ca^{2+} has the capacity to aggregate the microsomes leading to their precipitation. A critical comparison of the present as well as the classical method indicates that the present method requires relatively less time, uses simple equipment, is far more convenient and apparently results in a more quantitative separation of microsomes. The present procedure was used in the preparation of microsomes of spleen, heart and kidney of rat, chicken liver and a flagellated green protozoan, *Euglena var. bacillaris*, and was found to be highly satisfactory.

As microsomes have diverse functions, in lipid and protein synthesis, in oxidative demethylation, in detoxification, in electron transport systems, in the regulation of hepatic enzymes, in control of drug metabolizing enzymes and in the hydroxylation of drugs, carcinogen compounds and steroid hormones, an improved method for their preparation may find wide application.

4. Conclusions

A simple method has been developed for the isolation of rat liver microsomes. The yield as well as the

enzyme activities, lipid composition and RNA content agree with values reported in the literature. The method involves the use of calcium ions and eliminates the need for ultracentrifugation at 105,000 *g*. The simplicity, rapidity and convenience makes the method suitable for application in diverse areas.

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