THE RECONSTITUTION OF MICROSOMAL MONOOXYGENASE DURING SEPHADEX LH-20 GEL FILTRATION

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1. Introduction

The microsomal 'drug-metabolizing system' has not until recently been the subject of such intense study by self-assembly methods as have some other membranous enzyme systems, e.g., electron transport and oxidative phosphorylation in mitochondria [1]. At the same time, as noted by Razin [1], the self-assembly method is a very promising one for obtaining exact knowledge of molecular architecture and function of proteins and lipids in biomembranes. A dialysis procedure resulting in the self-assembling of microsomal enzyme system has been described [2,3]. This method enabled one to obtain the drug metabolizing system without any conversion of cytochrome *P*-450 to cytochrome *P*-420 [3].

In this report we describe a new procedure for carrying out the self-assembly of microsomes disintegrated by detergent. The method proposed, based on Sephadex LH-20 gel-chromatography, is remarkable because of the following characteristics: (1) the formation of microsome-like particles possessing a metabolizing activity ≈ 80% of the level of intact microsomes; (2) the high yield of protein in microsomal particles after self-assembly (up to ≈ 50% of the microsomal protein initially used; (3) the rapidity of self-assembly.

2. Materials and methods

Isolation of microsomal fraction from liver of male Wistar rats was done as described previously [3]. The final suspension of the microsomal fraction was performed in 0.1 M Tris–HCl (pH 7.4); 20% glycerol (v/v) and 1 mM dithiothreitol. Sodium cholate (20% solution, w/v, pH 7.5) was added dropwise during gentle mixing of the microsomal suspension having a final protein concentration of 10 mg/ml. The final cholate concentration was 4% and the cholate/protein ratio was 4 : 1. The microsomal suspension clarified instantly after the addition of sodium cholate. The clarified solubilizate was centrifuged at 150 000 × *g* for 90 min. The supernatant obtained was applied to a Sephadex LH-20 column (2.6 × 30 cm) previously equilibrated in 0.1 M Tris–HCl (pH 7.4), 20% glycerol (w/v) and 1 mM dithiothreitol. The volume of the applied sample was ≈ 18 ml and total protein 150–160 mg. The column was then washed with the equilibration buffer. The main portion of microsomal protein was eluted in the void volume while the cholate remained bound to the gel. The protein fractions were combined and centrifuged at 150 000 × *g* for 90 min. These pellets were suspended as microsomes and considered as microsome-like particles formed from solubilized microsomal constituents by self-assembly.

Protein was determined by the method of Lowry et al. [4] and by the biuret method [5]. The cytochrome b_5, cytochrome *P*-450 and cytochrome *P*-420 contents were determined by the method of Omura and Sato [6]. NADPH-cytochrome *c* oxidoreductase (EC 1.6.99.2.) was assayed by the reduction of cytochrome *c* [7]. Metabolism of benzpyrene was assayed according to the method of Robie et al. [8].

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3. Results and discussion

To establish that there was true solubilization of microsomes under our conditions (cholate/microsomal protein ratio, the microsomal protein concentration at the cholate addition) we showed that the microsomal proteins and phospholipids were separated distinctly during gel chromatography on Bio-Gel P-30. No protein was eluted in the void volume when the microsomal solubilizate was exposed to gel chromatography on Bio-Gel A 1.5 m. The proteins were eluted in the range 6×10^5–5×10^4 daltons on Bio-Gel A 1.5 m. These experiments prove that the treatment of microsomes by cholate leads to true dissociation of the microsomal proteins and phospholipids and the microsomal proteins from each other.

After Sephadex LH-20 gel filtration of the microsomal solubilizate we obtained particles which sedimented at 150 000 × *g* and were eluted in the void volume from Sepharose 4B. From this we believe the formation of microsome-like particles during Sephadex LH-20 chromatography to be due to the self-assembly of membrane-organized enzyme complexes in the sense interpreted in detail by Razin [1].

Table 1 presents the main characteristics of the fractions studied. We would like to emphasize some characteristics. In microsome-like particles the activity of benzpyrene metabolism is 70% of that in the intact microsomes. It is important to stress that the solubilized microsomal fraction has a low activity toward benzpyrene metabolism (10% of the microsomal level). We consider this restoration of the main microsomal function – metabolism of xenobiotics – as a good result. The other characteristic is the rather high yield of microsomal protein after self-assembly. We obtained 40–60% proteins as microsomal-like particles. This parameter is important as far as further work is concerned and testifies to a high efficiency of self-assembling process.

The purified microsomal enzymes, namely cytochrome *P*-450 and NADPH-cytochrome *P*-450 reductase, on being mixed under certain conditions have been previously shown to form complexes with high metabolizing activity [9,10]. Autor et al. have

Table 1
The main characteristics of microsomal fractions under study

Fraction	Total protein[a]	Cytochromes content[b]		Activity of reductases[c]		Activity of benzpyrene metabolism[d]
		P-450	b_5	NADPH	NADH	
Intact microsomes	200	0.8	0.6	80	600	160
Solubilized microsomal fraction	170	0.6	0.6	85	700	15–20
Microsomal fraction after gel chromatography (not sedimented)	130	0.8	0.6	90	700	80
Microsome-like particles	85	0.8	0.7	100	700	100
Microsomal protein in supernatant	55	0.65	0.6	90	750	90

[a] In mg
[b] nmoles per mg of protein
[c] nmoles of acceptor reduced per mg of protein per min
[d] pmoles of 3OH-benzpyrene per nmole cytochrome *P*-450 per min

demonstrated that the molecular weight of these complexes does not exceed 6×10^5 and they do not sediment during prolonged high-speed centrifugation. In this respect the microsome-like particles we obtained differ from the well-known metabolizing system [9,10]. Moreover, the microsome-like particles possess a cytochrome *P*-450–NADPH-cytochrome *P*-450 ratio similar to that of intact microsomes in contrast to the system described previously [11], where the excess of NADPH-cytochrome P–450 reductase is of particular importance for active metabolism.

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