THE RECONSTRUCTION OF ENZYMIC LIPID PEROXIDATION SYSTEMS FROM MICROSOMES OF VARIOUS ORIGIN

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1. Introduction

The components required for enzymic lipid peroxidation (LP) in liver microsomes are known to be ferric ions, membranous phospholipids and NADPH-specific flavo-protein generating anion radicals of oxygen ($O_2^{-\cdot}$) during NADPH oxidation [1–3]. Regardless of the essentially common organization of the electron transport chain in microsomes of various origin, the activity of enzymic LP varies considerably, representing, for example, 0.2 μatoms O_2/min/mg protein in rat liver microsomes and 0.03 μatoms O_2/min/mg protein in rabbit liver microsomes [4–6]. The reasons for such differences, of fundamental significance for the regulation of LP systems, are unknown and may be in either the properties of NADPH-specific flavoprotein or in the properties of the endoplasmic reticulum membranes. Elucidation of these problems is the object of this work.

2. Methods

Preparation of microsomes from rat and rabbit livers; assay of LP activity and NADPH-cytochrome *c* reductase activity and determination of cytochrome *P*-450, cytochrome b_5 and protein concentration were as previously described by us [7]. Activities of adrenaline oxidase and Nitro Blue Tetrazolium (NBT) reductase were estimated as described in [8,9]. Separation of NADPH-dependent flavoprotein and submicrosomal particles was by the method of Ichikawa and Yamano [10]. Partially purified flavoprotein was obtained by salting it out with ammonium sulfate as reported in [11] with subsequent dialysis against 0.1 M potassium phosphate buffer (pH 7.4) for 12 h at 4°C. Preparation of liposomes from soya-bean phospholipids mixture (azolectin, 'Sigma') was done as in [12].

3. Results

In accordance with earlier data we have found that the amount of the most vital components – cytochrome *P*-450 and cytochrome b_5 – and enzymic activity of NADPH-specific flavoprotein (NADPH-cytochrome *c* reductase, NADPH-NBT reductase, NADPH-adrenaline oxidase, NADPH-cytochrome *P*-450 reductase) in rat and rabbit liver microsomes are virtually the same, that is, no differences were detected between the two tissues. Of especial interest is the identity of the rates of adrenaline oxidation, which is indicative of the same rate of generation of $O_2^{-\cdot}$ radicals that initiate microsomal LP. At the same time, the activity of LP in the preparations under study differed considerably (table 1). This distinction was observed in the ascorbate-dependent LP reaction, whose microsomal components are membranous phospholipids only. The submicrosomal particles obtained by us were practically devoid of NADPH-specific flavoprotein (the activity of NADPH-dependent reactions was less than 2% of initial), but retained the content of native cytochrome *P*-450 and cytochrome b_5. As can be seen from table 1 the difference also remained, though less pronounced, in the ascorbate-

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Table 1
Lipid peroxidation activity in microsomes and submicrosomal particles from rat and rabbit livers[a]

	LP (μatoms O_2/min/mg protein)	
	NADPH-dependent	Ascorbate-dependent
Rat liver microsomes	0.18	0.16
Rat liver submicrosomal particles	–	0.25
Rabbit liver microsomes	0.05	0.03
Rabbit liver submicrosomal particles	–	0.1

[a] The incubation system contained: 125 mM KCl + 20 mM Tris–HCl, pH 7.4; 4 mM ADP, 0.012 mM Fe^{3+} (as $FeCl_3$); 0.3 mM NADPH or 1 mM ascorbate; 1.5 mg protein from microsomes or submicrosomal particles; total volume 1.4 ml, at 22°C.

dependent LP. The high activity of this reaction in rat liver submicrosomal particles showed that unsaturated fatty acids can be peroxidated after tripsin treatment of the microsomal membrane.

Partially purified flavoproteins of rat and rabbit liver microsomes (4–5-fold purification was determined by cytochrome *c* reduction in nmoles/min/mg protein) were capable of catalyzing adrenaline oxidation, completely dependent on $O_2^{-\cdot}$, and reducing such acceptors as cytochrome *c* and NBT. The activity of these enzymes (per mg protein of preparation estimated) was also identical.

We attempted to determine, by reconstructing the enzymic LP system, which of two components, NADPH-specific flavoprotein or membranous

Table 2
Activity of lipid peroxidation activated by NADPH-specific flavoprotein[a]

		LP (μatoms O_2/min/mg protein)
Rat liver submicrosomal particles	+flavoprotein from rat liver microsomes	0.20
	+flavoprotein from rabbit liver microsomes	0.19
Rabbit liver submicrosomal particles	+flavoprotein from rat liver microsomes	0.1
	+flavoprotein from rabbit liver microsomes	0.095
Liposomes prepared from azolektine	+flavoprotein from rat liver microsomes	0.17 (86)[b]
	+flavoprotein from rabbit liver microsomes	0.17 (89)[b]

[a] The incubation system contained: 125 mM KCl + 20 mM Tris–HCl, pH 7.4; 4 mM ADP, 0.012 mM Fe^{3+} (as $FeCl_3$), 0.1 mM EDTA-Fe^{3+} (sodium salt); 0.3 mM NADPH; 1.5 mg protein from submicrosomal particles and amount of NADPH-specific flavoprotein, which reduces 70 nmol cytochrome *c* /min; total volume 1.4 ml.

[b] In brackets - MDA production in nmoles/mg protein/30 min.

phospholipids, is responsible for the low activity of LP in rabbit liver microsomes. For this purpose we added to the submicrosomal particles obtained from rabbit liver flavoprotein from rat liver microsomes and vice versa. Serving as control were the reconstructed systems prepared from the same source. As table 2 demonstrates, both NADPH-specific flavoproteins effectively catalyze LP reaction in submicrosomal particles from rat liver, moreover, the activity of the reaction is analogous to the one in whole rat liver microsomes. In contrast to this, rabbit liver submicrosomal particles display low LP activity regardless of the origin of NADPH-specific flavoprotein. It is to be noted that in the latter case the rate of LP did not exceed the rate obtained during activation of the non-enzymic (ascorbate-dependent) reaction (see table 1). Table 2 also shows that NADPH-specific flavoproteins, whatever their source catalyzed LP in the same manner and sufficiently well in proteoliposomes prepared from azolectin with the help of cholate and ultrasonication. This fact was confirmed by two independent methods for measuring the activity of LP, O_2 uptake and MDA formation, which is the product of unsaturated fatty acid cleavage.

4. Discussion

It is apparent that both purified NADPH-specific flavoproteins can effectively support enzymic LP when recombining them with submicrosomal particles from rat liver and azolectine liposomes. These flavoproteins show low activity as regards LP, since they are bound with the submicrosomal particles prepared from the endoplasmic reticulum membrane of the rabbit liver cells. Therefore, we assume that the low activity of the LP system in rabbit liver microsomes is not connected with any distinctive properties of the microsomal flavoprotein, which in its functional characteristics, is similar to the flavoprotein from rat liver microsomes, but is most probably due to either the increased content of endogenous antioxidant in rabbit liver microsomes or to the structural pecularities of the rabbit microsomal membrane.

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