RECONSTRUCTION OF AN ENZYMIC SYSTEM OF LIPID PEROXIDATION WITH PROPERTIES OF AN INTACT MICROSOMAL SYSTEM

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1. Introduction

Success in reconstruction of microsome enzyme systems depends on many factors, including the procedures employed to solubilize components from the microsomal membrane. In fact, if reconstruction of a hydroxylation system has been achieved with the help of NADPH-specific flavoprotein, isolated from microsomes treated with detergents (deoxycholate or Triton X-100), a system comprising NADPH-specific flavoprotein, solubilized with trypsin or bromelain, was incapable of metabolizing xenobiotics [1,2].

Encouraging is the fact that NADPH-specific flavoprotein, isolated with proteases, actively supports peroxidation in liposomes. Nevertheless, we can not state that the intact microsomal system of lipid peroxidation (LP) is similar to the reconstructed one, because the latter functions only in the presence of an additional component, namely, the iron ions chelated by ethylenediaminetetraacetate (EDTA·Fe) [3].

The purpose of this study was to assess the efficiency of NADPH-specific flavoprotein from microsomes solubilized with Triton X-100 in the reconstruction of LP system. The enzyme we prepared was found to be capable of supporting LP in reconstructed system in the presence of the ADP·Fe complex alone in contrast with the enzyme prepared by protease treatment.

2. Methods

Microsomes were isolated from the livers of Wistar male rats and random-bred male rabbits by the method of differential centrifugation. NADPH-Specific flavoprotein was separated from rat liver microsomes (LM) by the treatment with trypsin or Triton X-100, followed by DEAE-cellulose chromatography and gel-filtration [4,5]. Lipids were extracted from microsomes by the method of Bligh and Dyer [6] performing all operations under nitrogen at 0–4°C. Liposomes and lipoprotein complexes were prepared by the method of Racker et al. [7]. The content of total lipid phosphorus was measured by the method of Bartlett [8].

The activity if NADPH–cytochrome *c* reductase was determined as described earlier [9]. LP was determined by measuring the malondialdehyde production [10] and was expressed as nmol malondialdehyde/μmol lipid phosphorus/10 min. LP was conducted at 37°C for 10 min in reaction mixtures containing 125 mM KCl + 20 mM Tris, pH 7.5, 0.5 mM NADPH or 1.0 mM ascorbate and iron complexes. EDTA·Fe Complex was used as $C_{10}H_{12}FeN_2NaO_8$ salt. Protein content was assayed as outlined by Lowry et al. [11].

3. Results and discussion

Tables 1 and 2 summarize the results of LP measurements in the model systems reconstructed from purified NADPH-specific flavoprotein prepared either by trypsin or by Triton X-100 solubilization and lipids extracted from rat LM. In the presence of ADP·Fe, the activity of the system containing NADPH-specific flavoprotein, solubilized with protease, is 3.5 times lower than the activity of the system con-

Table 1
The activity of enzymic LP system reconstructed from rat LM components

	NADPH-specific flavoprotein isolation[a]	
	Trypsin	Triton X-100
ADP(2.0 mM)+Fe^{3+}(0.012 mM)	1.87	6.60
EDTA·Fe(0.1 mM)	6.05	7.04
EDTA·Fe(0.1 mM) + ADP(2.0 mM)+Fe^{3+}(0.012 mM)	5.50	9.13

[a] The reaction mixture contained liposomes (0.25 μmol lipid phosphorus in 1 ml) which were preincubated with purified NADPH-specific flavoprotein (of equal activity determined by the reduction of cytochrome *c*) at 37°C for 30 min.

taining the detergent-solubilized NADPH-specific flavoprotein (table 1). Both systems were equally active in the presence of another iron complex, EDTA·Fe. Thus, in contrast with the LP system reconstructed by Pederson et al. [3], in our experiment the functioning of the detergent-solubilized flavoprotein did not require the simultaneous presence of ADP– and EDTA–iron complexes. So, the interaction of the purified flavoprotein either with EDTA·Fe complex only or both iron complexes depends on the kind of the solubilization procedure.

It should be noted that in the system achieved by the self-assemblage of lipoprotein complexes from enzymic proteins and lipids after detergent-dialysis procedure [7], malondialdehyde was produced in amounts twice exceeding those generated in the system reconstructed by simple mixing of the components [3], under otherwise identical conditions (table 1 and 2).

It is known that LP reactions proceed 3–4-times slower in intact LM of the rabbit than of the rat. The reason for this difference is unclear. We have suggested earlier that the low activity of the LP system in rabbit microsomes may either be due to the increased content of natural antioxidants in membranes or to some structural features of the membrane itself [12]. We reconstructed the system of enzymic LP from rat LM NADPH-specific flavoprotein and the lipids extracted from rabbit LM. Having compared the activity of this LP system with that reconstructed from the components of rat LM (see table 2), we found that the lipids from rabbit LM may be peroxidized just as efficiently as rat LM lipids (table 3). These facts contradict to our earlier suggestions [12].

It may be inferred that the LP system reconstructed from NADPH-specific flavoprotein isolated by Triton X-100 and microsomal lipids resembles the intact microsomal enzymic system of LP because both systems are activated by the iron complex, ADP·Fe alone.

Table 2
Effect of different iron concentrations on the activity of the enzymic LP system reconstructed from rat LM Triton X-100-isolated flavoprotein and lipids

	Fe^{3+}(mM)			EDTA·Fe	Fe^{3+}(0.012 mM)[a] +
	0.012[a]	0.024[b]	0.1[b]	(0.1 mM)	EDTA·Fe(0.1 mM)
NADPH	9.8	12.5	16.4	17.9	15.9
Ascorbate	10.0	–	–	–	–

[a] Plus 2. 0 mM ADP
[b] Plus 4.0 mM ADP. The incubation medium, passed through a Chelex-100 resin column, contained lipoprotein complexes prepared by the method of Racker et al. [7]

Table 3
Peroxidation activity of lipids extracted from rabbit liver microsomes in a system containing NADPH-specific flavoprotein isolated from rat liver microsomes with Triton X-100

System	Liver microsome lipids	
	Rat	Rabbit
ADP·Fe	9.9	11.9
EDTA·Fe	11.4	13.9
ADP·Fe+EDTA·Fe	14.1	17.2

The reaction system was the same as for Table 2 [7]

Hogberg et al. [13] have shown that NADPH-dependent cytochrome *c* reductase is released from isolated rat LM into the medium during LP. When added to mitochondrial suspension, this enzyme supports the peroxidation of mitochondrial lipids in the sole presence of ADP·Fe, which is also needed by LP systems in intact microsomes. The LP system reconstructed by Hogberg et al. is hardly comparable with the system we have reconstructed with the help of lipids and NADPH–cytochrome *c* reductase isolated with Triton X-100. What is certain is that both systems are active in the presence of iron chelated by ADP.

Further insight into the mechanisms of enzymic LP would be rather provided by comparisons of the other two systems of LP reconstructed from NADPH-specific flavoprotein and liposomes. One system comprises NADPH-specific flavoprotein solubilized with proteases, the other is catalyzed by NADPH-specific flavoprotein isolated with detergent Triton X-100. These flavoproteins differ in molecular weight [2,5] and in the capacity of activating LP in the presence of ADP·Fe. In this connection, it is important to note that NADPH-specific flavoprotein solubilized with Triton X-100 was also efficient in the reconstruction of a hydroxylation system.

It seems quite possible that hydrolytic solubilization frees the catalytically active fragment of NADPH-specific flavoprotein while the hydrophobic part of the enzyme remains in the membrane. This may be the reason why after incubation of microsomes with trypsin or bromelain a flavoprotein of lower molecular weight is isolated as shown for many other microsomal components [14].

It has been reported that Triton X-100 solubilized membrane proteins without altering their conformation and loss of their biological activities [15]. The more likely possibility is that in the presence of Triton X-100, NADPH-specific flavoprotein retains a structure, similar to the enzyme in intact microsomal membrane, and its capacity to reduce Fe^{3+} in ADP·Fe complex.

Based on the data obtained, it may be concluded that different mechanisms underlie NADPH–ADP·Fe-dependent and NADPH–EDTA·Fe-dependent LP. It is assumed that ADP·Fe is required, firstly, to support iron in the solution and, secondly, to provide adequate interaction of iron with the microsomal membrane. Inasmuch as the trypsin-solubilized enzyme is incapable of reducing ADP·Fe, it is possible that the iron which is complexed with ADP binds precisely with the hydrophobic region of NADPH-specific flavoprotein. However, trypsin solubilized enzyme retains the capacity to reduce cytochrome *c* 2,6-dichlorophenol-indophenol and other acceptors and also supports LP in the presence of EDTA·Fe. The reduction of EDTA·Fe complex is probably accomplished by a mechanism reducing the above-mentioned acceptors and this process does not seem to require any interaction between EDTA·Fe and the hydrophobic region of flavoprotein.

References

[1] Coon, M. J., Strobel, H. W. and Boyer, R. F. (1973) Drug Metab. Disp. 1, 92–97.
[2] Lu, A. Y. H. and Levin, W. (1974) Biochim. Biophys. Acta 344, 205–240.
[3] Pederson, T. C., Buege, J. A. and Aust, S. D. (1973) J. Biol. Chem. 248, 7134–7141.
[4] Iyanagi, T. and Mason, H. S. (1973) Biochemistry 12, 2297–2308.
[5] Ichihara, K., Kusunose, E. and Kusunose, M. (1972) FEBS Lett. 20, 105–107.
[6] Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[7] Kagawa, Y., Kandrach, A. and Racker, E. (1973) J. Biol. Chem. 248, 676–684.
[8] Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–468.
[9] Phillips, A. H. and Langdon, R. G. (1962) J. Biol. Chem. 237, 2652–2660.
[10] Stancliff, R. C., Williams, M. A., Utsumi, K. and Packer, L. (1969) Arch. Biochem. Biophys. 131, 629–642.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.

[12] Mishin, V. M., Pospelova, L. N., Pokrovsky, A. G. and Lyakhovich, V. V. (1976) FEBS Lett. 62, 136–138.
[13] Hogberg, J., Larson, R. E., Kristoferson, A. and Orrenius, S. (1974) Biochem. Biophys. Res. Commun. 56, 836–842.
[14] DePierre, J. W. and Dallner, G. (1975) Biochim. Biophys. Acta 415, 411–472.
[15] Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79.