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THE DISTINCT ENZYMIC LIPID PEROXIDATION SYSTEMS FROM LIVER MICROSOMES IN THE PRESENCE OF ADP– OR EDTA–IRON COMPLEXES

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1. Introduction

It is known that the ADP–Fe complex is the most effective and widely used cofactor for lipid peroxidation in rat liver microsomes [1,2]. Many authors have considered the iron ions as initiators of this free-radical reaction [3,4] and ADP as a component which prevents the precipitation of iron at neutral pH [4]. However, the latest data on the reconstruction of the microsomal lipid peroxidation system indicate that besides ADP–Fe the presence of the EDTA–Fe complex is also necessary [5].

In this report data are presented about the interchange ability of ADP–Fe and EDTA–Fe in NADPH-dependent lipid peroxidation in intact rat liver microsomes.

2. Methods

Microsomes were prepared from livers of albino male rats (Wistar strain) as previously described [6]. The isolation and incubation media were passed through a Chelex-100 resin column to remove iron ions. Oxygen uptake was measured polarographically with a Clark-type electrode [6] and lipid peroxidation products were determined by the thiobarbituric acid method [7]. The activity of lipid peroxidation was assayed in a reaction mixture containing 125 mM KCl + 20 mM Tris–HCl, pH 7.4; 0.012 mM Fe^{3+} as $Fe(NO_3)$; 2.0 mM ADP; 0.5 mM NADPH or NADH. The EDTA–Fe complex was used as the salt $C_{10}H_{12}N_2O_8FeNa$. Separations of NADPH-specific flavoprotein and cytochrome b_5 were carried out by the method of Ichikawa and Yamano [8]. The cytochrome c and ferricyanide reductase activities were measured as described in [9]. Protein concentration was determined by the method of Lowry et al. [10]. Details of the experiments are described in the legends to the tables and the figure.

3. Results and discussion

The data presented in table 1 indicate that the EDTA–Fe complex effectively promotes lipid peroxidation in intact microsomes in the presence of both NADPH and NADH. This conclusion is based on determinations of malonic-dialdehyde (MDA), one of the end products of unsaturated fatty acids peroxidation. Only the NADPH-dependent lipid peroxidation can be promoted by the ADP–Fe complex.

Thus, it becomes evident that NADPH-specific flavoprotein and some component of the NADH-dependent electron transport chain in intact microsomes may catalyze lipid peroxidation in the presence of EDTA–Fe. This conclusion differs from (but does not contradict) the findings of Pederson et al. [5] who showed the necessity of the presence of both ADP–Fe and EDTA–Fe in promoting the NADH-dependent microsomal lipid peroxidation.

We did an experiment to identify the NADH-specific component which promoted lipid peroxidation in the presence of EDTA–Fe. When microsomes are treated with trypsin NADPH-cytochrome c

Table 1
NADPH- and NADH-dependent lipid peroxidation activities in intact microsomes in the presence of ADP–Fe or EDTA–Fe

Reaction mixture	O_2 Uptake[a]	MDA Production[b]
Microsomes in the presence of ADP–Fe plus		
NADPH	250.0	40.5
NADH	8.0	7.0
Microsomes in the presence of EDTA–Fe (0.1 mM) plus		
NADPH	60.0	44.0
NADH	70.0	35.2
Microsomes in the presence of EDTA–Fe (0.5 mM) plus		
NADPH	65.0	38.0
NADH	270.0	42.0

[a]natoms oxygen/mg protein/min, at 22–23°C
[b]nmol MDA formed/mg protein/10 min, at 37°C

reductase is removed first then cytochrome b_5 [8], while NADH-cytochrome b_5 reductase is not solubilized. Table 2 shows a 6-fold decrease in cytochrome b_5 content and a more pronounced decrease in the NADH-cytochrome *c* reductase activity in submicrosomal II particles. Despite this, the NADH-dependent lipid peroxidation in the presence of EDTA–Fe and the NADH-ferricyanide reductase activity remained high enough to characterise cytochrome b_5 reductase. From these data one may conclude that it is the

Table 2
Comparison of NADPH- and NADH-dependent reductase activities and lipid peroxidation in microsomes and submicrosomal particles

	Microsomes	Submicrosomal particles	
		I[d]	II[e]
Cytochrome *c* reductase[a]			
NADPH	72.0	3.5	2.5
NADH	140.0	140.0	16.7
NADH-ferricyanide reductase[a]	720.0	700.0	640.0
Cytochrome b_5[b]	0.58	0.56	0.095
Lipid peroxidation[c] in the presence of			
ADP–Fe and NADPH	40.6	7.7	0–2
ADP–Fe and NADH	5.6	9.7	4.0
ADP–Fe and ascorbate	35.7	39.2	38.8
EDTA–Fe and NADH	44.2	37.7	33.8

[a]nmol of acceptor reduced/mg protein/min
[b]nmol/mg protein
[c]nmol MDA formed/mg protein/10 min, at 37°C
[d]Trypsin treatment 1 h, at 10°C
[e]Trypsin treatment 10 h, at 10°C

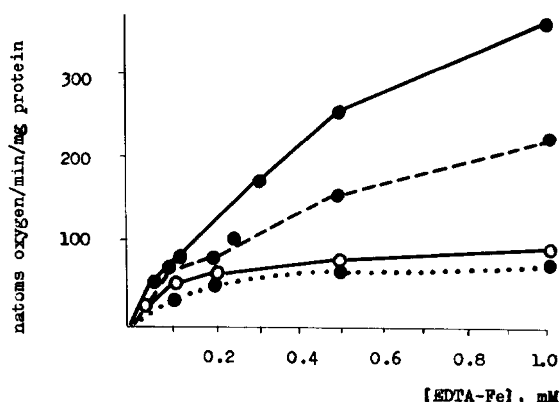

Fig.1. Effect of various EDTA–Fe concentrations on O_2 uptake in microsomes and submicrosomal particles. Medium, conditions same as in Methods except for ADP and $Fe(NO_3)_3$. (●—●) microsomes + NADH (0.5 mM), (●- - -●) submicrosomal I particles + NADH, (●· · · ·●) submicrosomal II particles + NADH, (○—○) microsomes + NADPH (0.5 mM).

NADH-cytochrome b_5 reductase that reduces iron chelated by EDTA. At the same time, in intact microsomes and in submicrosomal particles, in the presence of ADP–Fe, lipid peroxidation is not promoted by NADH.

A significant difference was found between the rates of O_2 uptake and MDA production during NADH-dependent lipid peroxidation by intact microsomes. At higher concentrations of EDTA–Fe the rate of O_2 uptake increased while the production of malondialdehyde did not change. This phenomenon was not observed if lipid peroxidation was initiated either by NADPH in intact microsomes or by NADH in submicrosomal II particles, in which the cytochrome b_5 content was sharply decreased. It has been shown by Bilimoria and Kamin [11] that the reduced cytochrome b_5 can be reoxidized in the presence of EDTA–Fe. In our experiments the rate of O_2 uptake, in the presence of both NADH and EDTA–Fe, also correlated with the cytochrome b_5 content of the preparations studied. This supports the conclusion that the uptake of 'superfluous' oxygen in the system containing NADH and EDTA–Fe is due to the reoxidation of the reduced cytochrome b_5 by EDTA–Fe. Thus, it should be noted that cytochrome b_5 does not participate in the NADH-dependent lipid peroxidation because removal of cytochrome b_5 has no influence on the accumulation of malonic-dialdehyde in submicrosomal particles. In this respect our conclusions correspond to those of Pederson et al. [5].

Thus, it is evident that both NADPH- and NADH-specific flavoproteins can promote the peroxidation of liver microsomal lipid in the presence of iron ions chelated by EDTA. Only the NADPH-specific flavoprotein can be involved in microsomal lipid peroxidation in the presence of the ADP–Fe complex. The results presented in this paper suggest that there is some additional component which is included in NADPH-specific microsomal lipid peroxidation when the ADP–Fe complex is added to the reaction mixture.

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