INTENSIFICATION OF PROTEOLYTIC DEGRADATION OF CYTOCHROME P-450 DURING LIPID PEROXIDATION IN RAT LIVER MICROSOMES

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The terminal component of the mixed function oxygenase system, namely cytochrome P-450 undergoes degradation in membranes of the endoplasmic reticulum of the liver both under normal physiological conditions (in the course of circadian rhythms, after the action of monooxygenase inducers), and during the development of various pathological states (avitaminosis E, ischemia and reoxygenation, radiation injury, the action of halogen derivatives of alkanes, etc.) [1, 4, 6, 8, 9, 11].

It has recently been shown that a process of lipid peroxidation (LPO) participates in this degradation [3-5, 12]. However, it is still not clear whether LPO is the trigger mechanism of disassembly of cytochrome P-450 or whether it is directly responsible for its destruction. Accordingly, in the investigation described below, the connection between processes of proteolytic degradation of cytochrome P-450 and LPO was studied.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 130-150 g were used. For induction a solution of phenobarbital in water in a dose of 150 mg/kg or a solution of 20-methylcholanthrene in olive oil (80 mg/kg) was injected intraperitoneally into an animal. The microsomal fraction of rat liver was isolated by differential centrifugation [2] on Yanezki-21 (East Germany) and Beckman L-5 (USA) centrifuges. The content of cytochrome P-450 was determined by the method in [10] on an Aminco dW-uV-vis spectrophotometer (USA), using a coefficient of molar extinction of 91 $mM^{-1} \cdot cm^{-1}$. Trypsin was used for proteolysis in concentrations of 1×10^{-6} M and 2×10^{-5} M. This process was stopped by the proteolysis inhibitor phenylmethylsulfonyl fluoride. Microsomes were incubated in medium containing 0.5 mM NADPH (NADH, ascorbate), 0.1 M NaCl, 50 mM Tris-HCl (pH 7.4, 37°C), and 10 μM $FeSO_4$. The reaction was triggered by addition of protein in a concentration of 1 mg/ml. All components of the medium except NADPH (NADH, ascorbate) were added to the control sample. Incubation was carried out at 37°C with constant stirring. Samples for assay of LPO products (from the quantity of malonyl dialdehyde (MDA) formed) [7], were taken at definite time intervals. The MDA content was determined from the absorption spectrum at 535 nm (coefficient of molar extinction 1.56×10^5 $M^{-1} \cdot cm^{-1}$) on a Specord spectrophotometer (East Germany). If necessary LPO was stopped by the addition of the antioxidant 4-methyl-2,6-di-tert-butylphenol in a concentration of 5×10^{-4} M. The protein content in the microsomes was determined by the biuret method, using bovine serum albumin as the standard.

EXPERIMENTAL RESULTS

Curves showing degradation of cytochrome P-450 as a result of the action of trypsin on native microsomes (curves 4 and 5), and also on membranes previously subjected to LPO, are given in Fig. 1. It will be clear from Fig. 1 that the process of proteolytic degradation takes place in two phases: during the first 1-3 min cytochrome P-450 was destroyed rapidly (v = 0.03 nmoles/mg protein/min), but later the velocity of degradation fell practically to 0 under the influence of trypsin in a concentration of 1×10^{-6} M, and to 0.08 and 0.0036 nmoles/mg protein/min under the influence of trypsin in a concentration of 2×10^{-5} M. Under the conditions of proteolysis chosen about 81% of cytochrome P-450 was virtually inaccessible for trypsin in a concentration of 1×10^{-6} M and 60% in a concentration of 2×10^{-5} M, even after 10 min of proteolysis.

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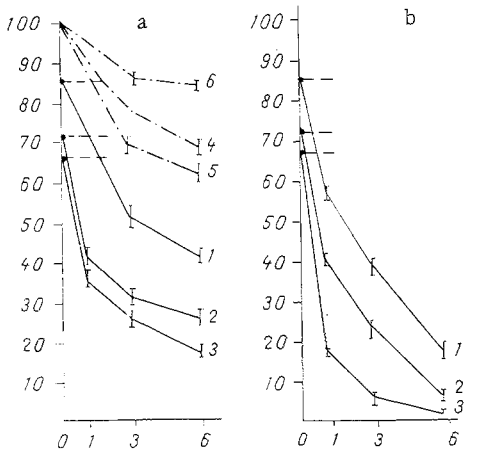

Fig. 1. Degradation of cytochrome P-450 during proteolysis by trypsin in concentrations of 1×10^{-6} M (a) and 2×10^{-5} M (b). 1, 2, 3) After preliminary induction of NADPH-dependent LPO: 1) 2.8 nmoles MDA/mg protein, 2) 4.1 nmoles MDA/mg protein, 3) 10 nmoles MDA/mg protein; 4, 5) with the same concentrations of trypsin and without induction of LPO; 6) during induction of LPO in the absence of trypsin. Abscissa, incubation time (in min); ordinate, changes in degradation of cytochrome P-450 (in %).

TABLE 1. Degradation of Cytochrome P-450 during Accumulation of 1 mM MDA in Rat Liver Microsomes during First 5 min of Incubation (M ± m)

System of LPO induction	Velocity of degradation of cytochrome P-450, nmole/mg protein/min	
	LPO + trypsin (2×10^{-5} M)	trypsin (2×10^{-5} M) + LPO
NADPH (0.5 mM) + Fe^{++} (10 μM)	0,158±0,034	0,183±0,037
NADH (0.5 mM) + Fe^{++} (10 μM)	0,152±0,023	0,171±0,027
Ascorbate (0.5 mM) + Fe^{++} (10 μM)	0,076±0,020	0,101±0,022

As a result of the combined action of trypsin and LPO two effects were observed: acceleration of the rapid reaction of cytochrome P-450 degradation and increase in the accessibility of cytochrome P-450 for protease.

The quantity of cytochrome P-450 destroyed as a result of the combined action of LPO and trypsin was greater than the resultant of these factors taken separately, i.e., it was nonadditive. These effects were intensified during accumulation of LPO products and an increase in the protease concentration (Fig. 1, 1-3). The effectiveness of degradation of cytochrome P-450 as a result of LPO, it will be noted, depended on the method of induction of free-radical oxidation of lipids, and diminshed in the order NADPH > NADH > ascorbate-dependent LPO (Table 1). The results are evidence of an increase in the velocity of proteolysis and in the accessibility for proteases of cytochrome P-450 in noninduced native membranes of liver microsomes. In a similar series of experiments with the microsomal fraction from the liver of rats induced beforehand with phenobarbital or 20-methylcholanthrene, it was shown that NADPH-dependent LPO also accelerates degradation and increases the accessibility of cytochrome P-450 for proteases; in this case, moreover, the effectiveness of absence of LPO products was found to be higher than in microsomes obtained from noninduced animals (Table 2). Practically total disassembly of cytochrome P-450 took place as soon as 3.1 nmoles MDA/mg protein had accumulated in the membrane (in the case of noninduced microsomes 10 nmoles MDA/mg protein).

TABLE 2. Changes (in %) in Accessibility of Cytochrome P-450 for Proteolytic Degradation Induced by NADPH-Dependent LPO in Liver Microsomes during Incubation for 5 min (M ± m)

Method of induction of microsomes	Trypsin concentration	
	$1 \cdot 10^{-6}$ M	$2 \cdot 10^{-5}$ M
Noninduced	54±2	71±7
Induced by phenobarbital	61±2	94±3,5
Induced by 20-methylcholanthrene	52±5	100±6,2

Consequently, both in induced and in native microsomal membranes LPO may behave as a factor which sharply increases the effectiveness of proteolytic degradation of cytochrome P-450. The essential point is that the converse also proved to be true, namely: as a result of minimal preliminary proteolysis (2 min) the velocity of subsequent degradation of cytochrome P-450 was sharply increased during accumulation of endogenous LPO products (Table 1). Reactions of LPO and the action of proteases are thus two mutually potentiating processes which, together, sharply increase the velocity of degradation of cytochrome P-450 and its accessibility for disassembly, and the LPO process itself can be regarded as a trigger mechanism making particular forms of cytochrome P-450 accessible for endogenous proteases.

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