and in the supernatant. A similar pattern also was observed by Ide [6] after dissociation of deoxyribonucleoproteins by x rays in experiments in vivo. The results now obtained are in agreement with the view that one of the functions of histones is to determine the nuclear localization of DNA [3].

LITERATURE CITED

1. S. Yu. Abakumova, N. G. Kutsenko, and M. I. Lerman, Dokl. Akad. Nauk SSSR, 227, 742 (1976).
2. O. E. Demidenko, Izv. Akad. Nauk Latv. SSR, No. 11, 69 (1975).
3. Ya. G. Érenpreis, Usp. Sovrem. Biol, 81, 37 (1975).
4. K. Burton, Biochem. J., 62, 315 (1956).
5. A. Girolamo, E. Henshaw, and H. H. Hiatt, J. Mol. Biol., 8, 479 (1964).
6. H. Ide, Annot. Zool. Jpn., 42, 142 (1969).
7. M. T. Kuo, W. Meinke, and G. F. Saunders, Proc. Nat. Acad. Sci. USA, 72, 5004 (1975).
8. O. H. Lowry et al., J. Biol. Chem., 193, 265 (1951).

RESISTANCE OF DIFFERENT FORMS OF CYTOCHROME P-450 OF RAT LIVER TO FACTORS DESTABILIZING THE MICROSOMAL MEMBRANE

O. A. Gromova, N. E. Zakharova, V. V. Lyakhovich, and I. B. Tsyrlov

UDC 612.351.11.014.46

The effect of factors destabilizing the membrane of the liver microsomes on the spectral properties of cytochrome P-450 (P-448) was investigated in intact rats and rats receiving phenobarbital (PB) or 3-methylcholanthrene (MC). Considerable resistance of microsomes induced by PB and MC to enzymic and nonenzymic peroxidation of polyunsaturated fatty acids of membrane phospholipids was discovered. A clear difference was shown in the sensitivity of cytochrome P-448 and cytochrome P-450 of intact rats and rats receiving PB to in vitro treatment with sodium deoxycholate. The results indicate structural changes in the microsomal membrane during induction by PB and MC, which are two different types of inducers of the monooxygenases of the liver.

KEY WORDS: cytochrome P-450; induction; peroxidation of lipids; deoxycholate.

The system of monooxygenases, responsible for the metabolism of drugs, toxins, and hormones in the liver, is a membrane-bound polyenzymic complex with the property of reversibly increasing its activity in the response to administration of various xenobiotics to animals [4, 11]. Phenobarbital (PB) and the polycyclic hydrocarbon 3-methylcholanthrene (MC) are representatives of two large chemically heterogeneous groups of inducers. The basic differences between the properties of the components of the above-mentioned enzyme system, when induced by PB and MC, have now been sufficiently well described [2, 4, 11], but little information is yet available on the organization of the membrane structures of the microsomes and their mutual relations with the enzymic complexes.

Cytochrome P-450 is the terminal region of the electron transport chain in the microsomes and lies in the hydrophobic zone of the membrane [1, 12]; its action depends on the character of the hydrophobic environment, in the creation of which a leading role is played by phospholipids.

Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 553-555, May, 1977. Original article submitted August 13, 1976.

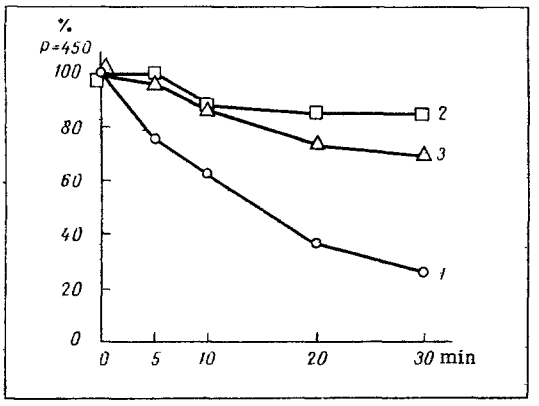

Fig. 1

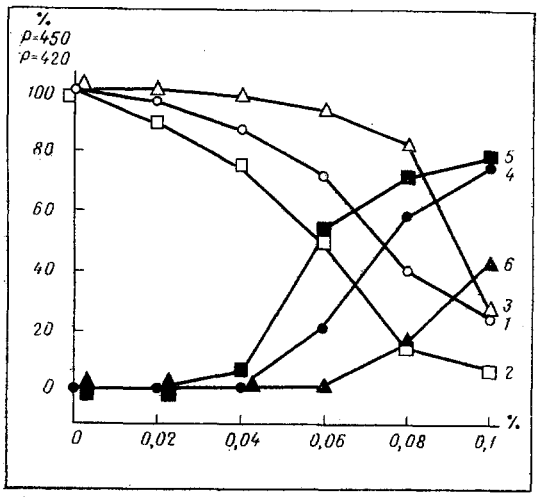

Fig. 2

Fig. 1. Comparative changes in content of CO-binding hemoproteins in liver microsomes of intact rats (1) and rats receiving PB (2) or MC (3) during enzymic POL. Initial concentration of C-P-450 0.83 nmole/mg protein, PB-P-450 2.03 nmole/mg protein, and P-448 1.56 nmole/mg protein. Concentration of microsomal protein 1 mg/ml. For other conditions, see Table 1. Abscissa, incubation time (in min); ordinate, concentration of cytochrome P-450 (in % of initial).

Fig. 2. Effect of DOC on conversion of control cytochrome P-450 and its induced forms: 1) C-P-450; 2) PB-P-450; 3) P-448; 4) C-P-420; 5) PB-P-420; 6) MC-P-420. Protein concentration 1 mg/ml. Abscissa, concentration of DOC in incubation medium (in %); ordinate, concentration of cytochrome P-450 and of its converted form P-420 (in % of initial).

The object of this investigation was to study the resistance of different forms of cytochrome P-450 to activation of peroxidation of polyunsaturated fatty acids of phospholipids and also after treatment of the microsomes with deoxycholate (DOC), an anionic detergent rupturing hydrophobic bonds.

EXPERIMENTAL METHOD

Male Wistar rats weighing 240-280 g were used. For 3 days the rats were given PB-Na in a dose of 100 mg/kg body weight or MC in a dose of 20 mg/kg intraperitoneally. The liver microsomes were isolated by differential centrifugation [14]. A suspension of microsomes containing 30-50 mg protein/ml was kept at 0-4°C during the experiment. Protein was determined by Lowry's method [10]. The content of cytochromes P-450, P-448, and P-420 was determined spectrophotometrically by the method of Omura and Sato [12] on a Model 356 (Hitachi) dual-beam differential spectrophotometer. Ascorbate- and NADPH-dependent peroxidation of lipids (POL) were activated by the method of Hochstein and Ernster [7]. The rate of oxygen uptake at 26°C was determined on the LP-60 polarograph with a stationary platinum electrode; the accumulation of malonic dialdehyde (MDA) also was recorded [13]. To determine the resistance of cytochrome P-450 during activation of POL in the microsomes, samples were incubated in a Warburg apparatus at 26°C. After incubation for 5, 10, 20, and 30 min the quantity of P-450 and MDA was measured. The POL reaction was stopped by the addition of 0.3 mM EDTA. Conversion of P-450 into P-420 was induced by incubating preparations of microsomes in 0.1 M phosphate buffer, pH 7.4, in the presence of DOC-Na (0.02-0.1%) for 5 min followed by recording the differential spectra of the cytochromes. To analyze the results standard extinction coefficients were used: for the control (C-P-450), PB-induced (PB-P-450), or MC-induced (P-448) CO-binding hemoproteins 91 $mM^{-1} \cdot cm^{-1}$, and for cytochrome P-420 111 $mM^{-1} \cdot cm^{-1}$.

EXPERIMENTAL RESULTS

Activation of enzymic POL led to degradation of cytochrome P-450 and the loss of its characteristic spectral properties. Incubation of the microsomes of the intact animals for 30 min in the presence of excess NADPH and ADP-Fe^{3+} complex led to a decrease in the content of cytochrome P-450 to 25% of its initial level (Fig. 1). Under similar conditions of incubation, the content of CO-binding hemoproteins in preparations of microsomes induced by PB or MC fell by a much smaller degree – by 15 and 50%, respectively.

TABLE 1. Rate of Uptake of Oxygen and MDA Accumulation during POL in Liver Microsomes of Intact Rats and Rats Receiving PB and MC (M ± m)

Object	Rate of oxygen uptake, nanoatoms O_2/min/mg protein		MDA, nmoles/mg protein/min
	NADPH - dependent POL	ascorbate-dependent POL	
Control microsomes	194±11	145±10	2,5±0,27
PB microsomes	69±4	39±4	0,1±0,03
MC microsomes	120±10	53±5	0,36±0,05

Legend. Incubation medium (in mmoles: KCl 125, Tris-HCl (pH 7.4) 20, NADPH 0.8 (for ascorbate 1), ADP 4, $FeCl_3$ 15; protein 2 mg/ml.

The results of investigation of POL showed that the observed "resistance" was evidently not due to the properties of the induced cytochrome. The rate of uptake of oxygen and the accumulation of MDA, the final product of peroxidation of unsaturated fatty acids (Table 1), indicate that the liver microsomes of rats receiving PB, and also MC, were much more resistant to NADPH- and ascorbate-dependent POL. This effect was particularly marked in microsomes induced by PB.

The resistance of the PB and MC microsomes thus revealed was due, it can tentatively be suggested, to an increase in the hydrophobic character of the microsomal membrane as a result of induction and, consequently, these microsomes would be more resistant to the action of any factor directed against hydrophobic bonds. It will be clear from Fig. 2 that weakening and destruction of hydrophobic interaction during treatment of preparations of microsomes with increasing concentrations of DOC caused conversion of cytochrome P-450 into the functionally inactive form cytochrome P-420. A clear difference was observed in resistance to conversion of control cytochrome P-450 and of its forms induced by PB and MC. For instance, conversion into the inactive form of cytochrome P-448 was observed with higher concentrations of DOC and was less marked than in the control cytochrome P-450. Cytochrome PB-P-450 was distinguished by its much greater sensitivity to the action of the detergent than the control hemoprotein, and by even greater sensitivity than cytochrome P-448.

Resistance of microsomal membranes induced by MC to POL thus correlated with resistance to conversion of cytochrome P-448 built into these membranes. Resistance to conversion of P-448 after treatment of microsomes by "chaotropic" substances, organic solvents destabilizing hydrophobic interaction [8], has been described in the literature.

It can be postulated that the increasing importance of hydrophobic interactions in the maintenance of the structure of the microsomal membranes during induction by MC is responsible for their relative stability.

No correlation was observed in these experiments between the resistance of PB microsomes to POL and the sensitivity of the PB-P-450 to detergent treatment. It must be remembered that the factors used to modify the membrane structure differed with respect to their target object. The reason for the observed resistance of the microsomes to POL during PB induction was evidently a decrease in the concentration of polyunsaturated acids which are the substrate for POL [3].

On account of differences in the response to PB stimulation in the proliferating membranes of the endoplasmic reticulum, the relative proportions of the individual components are evidently changed [4, 6, 9] and, consequently, so also is the interaction between them. A more hydrophilic, "loose" packing of the microsomal membrane can be postulated in the zone of cytochrome P-450, and this possibly makes the hemoprotein more accessible for the detergent.

So far as MC induction is concerned, it is not accompanied by appreciable proliferation of the endoplasmic reticulum, by an increase in the microsomal protein and phospholipids [5], or by any change in the composition of the fatty acids [3]. The ability of MC and other polycyclic hydrocarbons to interact with the phospholipids of artificial membranes [15] suggests that the unique stabilization of the microsomal membranes by

induction with MC can be due to a nonspecific membrane effect. With their powerful donor–acceptor properties, compounds of this type may have an inhibitory action on POL processes.

During induction of microsomal monooxygenases, besides an increase in enzyme synthesis de novo, there are also definite changes in the properties of and interaction between the structural components of the membrane which, in turn, may be responsible for the observed differences in the manifestations of functional activity of membrane-bound enzymes and, in particular, of cytochrome P-450.

LITERATURE CITED

1. A. I. Archakov, L. F. Panchenko, G. I. Bachmanova, et al., Dokl. Akad. Nauk SSSR, 196, 223 (1971).
2. A. H. Conney, Pharmacol. Rev., 19, 317 (1967).
3. S. C. Davison and E. D. Wills, Biochem. Soc. Trans., 1, 362 (1973).
4. L. Ernster and S. Orrenius, Fed. Proc., 24, 1190 (1965).
5. H. Glaumann, Chem. Biol. Interact., 2, 369 (1970).
6. J. R. Hayes, U. K. Marcel, and T. C. Campbell, Biochem. Pharmacol., 22, 1120 (1973).
7. P. Hochstein and L. Ernster, Biochem. Biophys. Res. Commun., 12, 388 (1963).
8. Y. Imai and P. Siekevitz, Arch. Biochem., 144, 143 (1971).
9. Y. Kuriyama and T. Omura, J. Biochem. (Tokyo), 69, 695 (1971).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. N. J. Mannering, Metabolism, 20, 228 (1971).
12. T. Omura and R. Sato, J. Biol. Chem., 239, 2379 (1964).
13. R. C. Stancliff, M. A. Williams, K. Utsumi, et al., Arch. Biochem., 131, 629 (1969).
14. J. R. Tata, in: Subcellular Compounds, London (1969), p. 83.
15. N. D. Weiner, J. Chawdry, and A. Felmeister, J. Pharm. Sci., 60, 425 (1971).