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MODIFICATION OF RAT LIVER CYTOCHROME P-450 BY MALONDIALDEHYDE AND 4-HYDROXYNONENAL

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INTRODUCTION

The content of the active form of the microsomal hemoprotein, cytochrome P-450, directly depends on its interrelationships with other components of the microsomal membrane (Lyakhovich & Tsyrlow, 1978). These interrelationships are significantly changed after alterations in the structure of lipid and protein membrane components (Polyakova, Gromova & Tsyrlov, 1987). Cytochrome P-450 is a hydrophobic protein (Lu & Levin, 1974), therefore many laboratories have studied the hydrophobic interactions betwen cytochrome P-450 and its lipid surrounding. It has been demonstrated that the conversion of cytochrome P-450 to its inactive form, cytochrome P-420, is related to heme hydrophobic environment, which state is maintained by lipid compounds of the microsomal membrane and the hemoprotein conformation (Imai & Sato, 1967). A methodological approach to these investigations is a modification of membrane compounds and SH-groups of cytochrome P-450 by different agents.

Aldehydic products of lipid peroxidation, namely malondialdehyde (MDA) and 4-hydroxynonenal (HNE), are very reactive compounds (Esterbauer, Schaur & Zollner, 1991). Toxic effects of these aldehydes are linked up with the modification of different cell structures by their reactions with either SH-groups or ε-amino groups to form semimercaptals or Schiff bases, respectively. Recently we have demonstrated that HNE actively interacts with the hydrophobic area of membrane proteins and membrane lipids (Buko, Artsukevich, Maltsev & Shareyko, 1994).

The aim of this study was to compare the changes in lipid and protein components of rat liver microsomal membranes modified by MDA or HNE with the alterations in cytochrome P-450 content and activities of cytochrome P-450 dependent reactions. We also attempted to clarify the possible mechanisms of the MDA and HNE interactions with the rat liver microsomal membrane.

MATERIALS AND METHODS

All the spin and fluorescent probes were obtained from Sigma (USA). MDA was prepared from 1,1,3,3-tetramethoxypropane (Aldrich, USA) by acid hydrolysis. HNE was a generous gift of Prof. H. Esterbauer (University of Graz, Austria). Other chemicals were of reagent class purity.

Male rats weighing approximately 250 g were used for liver microsomal membrane preparation. The animals were decapitated after the liver perfusion *in situ* with 1 mM sodium bicarbonate. Microsomes were isolated from whole liver homogenates by centrifugation at 105 000 × *g* as described elsewhere (Buko & Sushko, 1988). Protein concentration was measured in membranes by the method of Lowry, Rosebrough, Farr & Randall (1951). Cytochrome P-450 and P-420 content was determined from the carbon monooxide difference spectrum upon dithionite reduction according to Omuro and Sato (1964). NADPH-cytochrome P-450 reductase activity was measured as described by Dallner (1963).

Preincubation of liver microsomal membranes with MDA of HNE as well as with aliphatic aldehydes or *p*-chloromercuribenzoate (PCMB) was

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performed in 50 mM Tris-HCl buffer, pH 8.0 at 22°C during 5 min. Cytochrome P-450 and P-420 content as well as NADPH-cytochrome P-450 reductase activity were measured after preincubation of microsomes with MDA or HNE during 5 min.

All the ESR spectra were taken at room temperature (22°C) on an ERS-220 spectrometer (Germany). The usual instrumental parameters were: the microwave power, 5 mW; the center of the field, 3300 G; the scan range, 100 G. Quartz tubes of 1 mm internal diameter were used. 5-, 12- and 16-doxyl derivatives of stearic acid were used for a transversal scanning of liver plasma membranes. The spin label, 1-oxyl-2,2,6,6-tetramethylpiperidyl-PCMB (OTMP-PCMB) covalently modifying hemoprotein SH-group was also used in the experiment to evaluate microviscosity of the heme hydrophobic environment. The rotational correlation time and the lipid bilayer structural order were calculated using a routine method (Griffith & Jost, 1979).

The fluorescence spectra of pyrene bound to lipid components of the microsomal membrane or the hydrophobic area of membrane proteins were recorded on an LOMO-SDL-2 spectrofluorimeter (St. Petersburg, Russia). Potassium ferricyanide was used as a quencher of the fluorescent probe.

RESULTS AND DISCUSSION

The preincubation of liver microsomes with MDA resulted in a significant decrease of cytochrome P-450 content (Figure 1). A similar effect was obtained after the preincubation of microsomes with PCMB, a specific modifier of SH-groups. A decrease of cytochrome P-450 content was observed at MDA or PCMB concentrations higher than 10 μM. The similarity of the both curves can indicate a similar mechanism of the decrease of cytochrome P-450 content under the influence of either MDA or PCMB. The data obtained by Lyakhovich & Tsyrlov (1978) suggest that PCMB inactivates cytochrome P-450 via its conversion to the inactive form, cytochrome P-420, bound to SH-group at the hemoprotein active center. MDA also induced a formation of cytochrome P-420 in liver microsomes (Figure 1) but this effect was not so significant as the effect of PCMB.

HNE dramatically decreased the cytochrome P-450 content at concentrations lower than 10 μM similarly to the effects of aliphatic aldehydes (Figure 2). Cytochrome P-450 was not detected at HNE concentrations higher than 10 μM. HNE induced the production of cytochrome P-420 at concentrations of 1 μM and higher. We did not observe a stoichiometry between the recorded values of cytochrome P-450 decrease and cytochrome P-420 increase under the influence of MDA or HNE. As suggested by Lyakhovich, Tsyrlov, Mishin and Gromova (1973), only the degree of lowering cytochrome P-450 content really reflects the rate of hemoprotein inactivation by lipid peroxidative reactions.

The structural profile of the microsomal membrane was investigated by ESR spectroscopy using

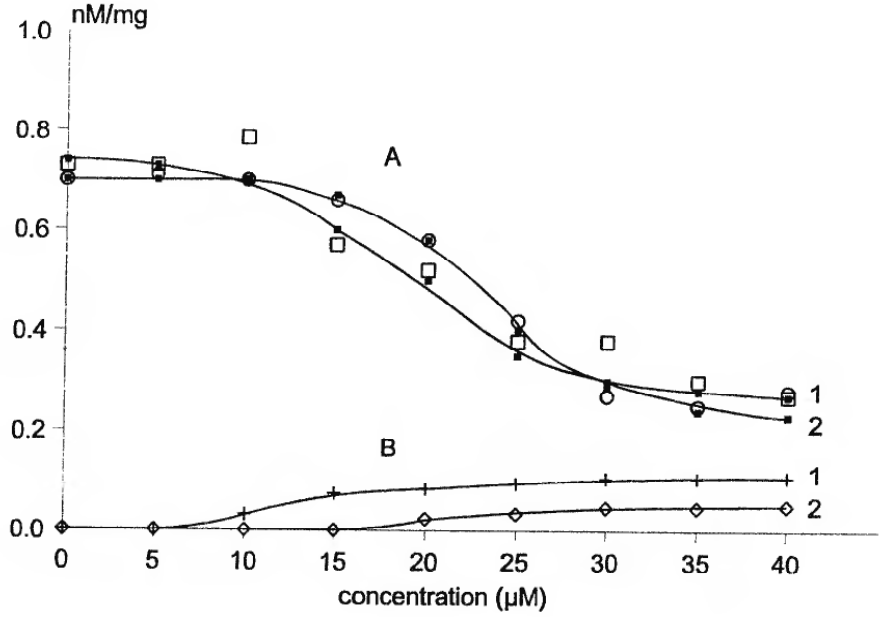

Fig. 1. The decrease of cytochrome P-450 content (A) and the production of cytochrome P-420 (B) by rat liver microsomes in the presence of MDA (1) or PCMB (2)

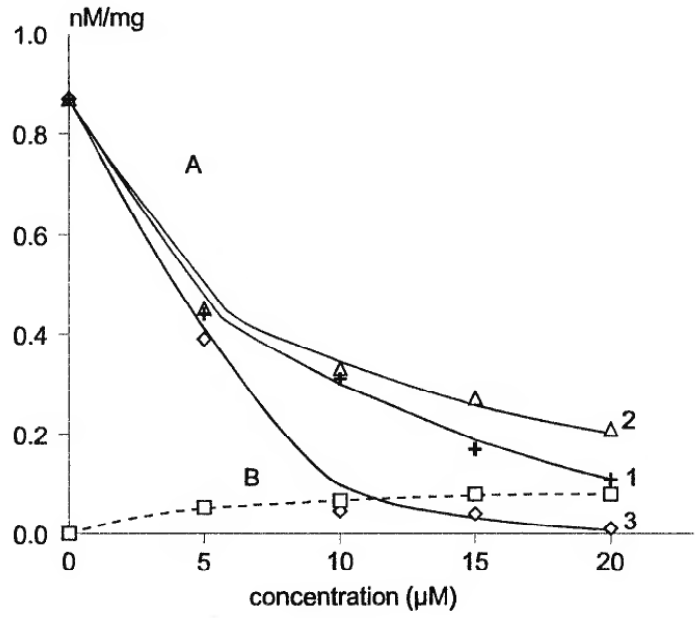

Fig. 2. Effects of aldehydes (1 – HNE; 2 – caprylic aldehyde; 3 – valeric aldehyde) on cytochrome P-450 content (A) and the effect of HNE on cytochrome P-420 formation (B) by rat liver microsomes

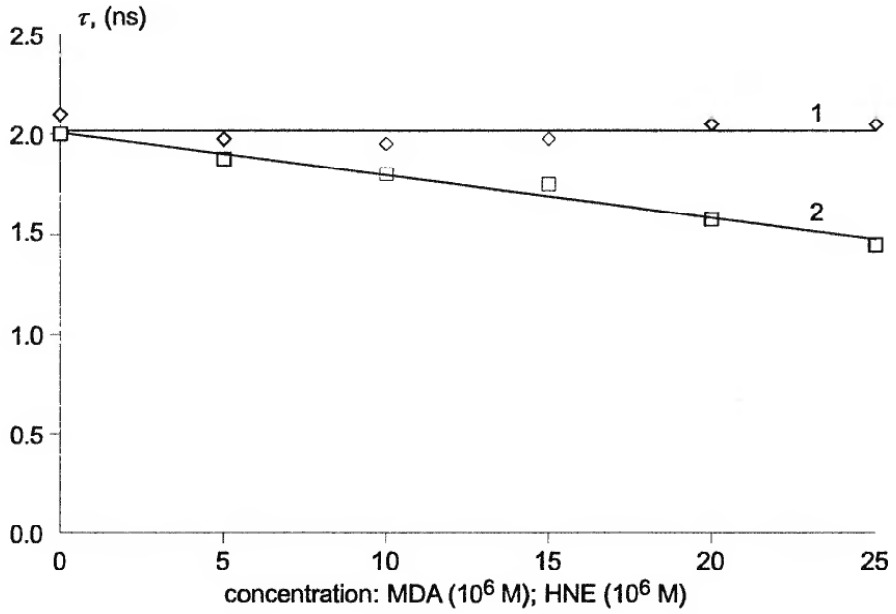

Fig. 3. The effect of HNE (1) or MDA (2) on the rotational correlation time (τ) of the carbodiimide spin label bound to active groups of surface located membrane proteins of rat liver microsomes

doxyl derivatives of stearic acid (DSA) having different locations of the nitroxyl residue. MDA increased the microviscosity of polar and non-polar zones of membrane phospholipids gathered around the nitroxyl radicals located at C_5, C_{12} and C_{16}, increasing the rotational correlation time of the corresponding spin probes (Figure 3, 4). HNE also increased the microviscosity of the microsomal membrane in the hydrophobic areas of the lipid bilayer at C_{12} and C_{16} but decreased the rotational correlation time of 5-DSA. The latter data suggest that HNE fluidized the surface leaflet of the microsomal membrane.

The intrinsic protein fluorescence is very sensitive to the changes in hydrophobicity of the whole molecule and of its separate areas. MDA decreased the intensity of fluorescence of microsomal protein tryptophane residues which was especially evident at MDA concentration of 10 μM (Figure 5). These data suggest that MDA significantly increased the

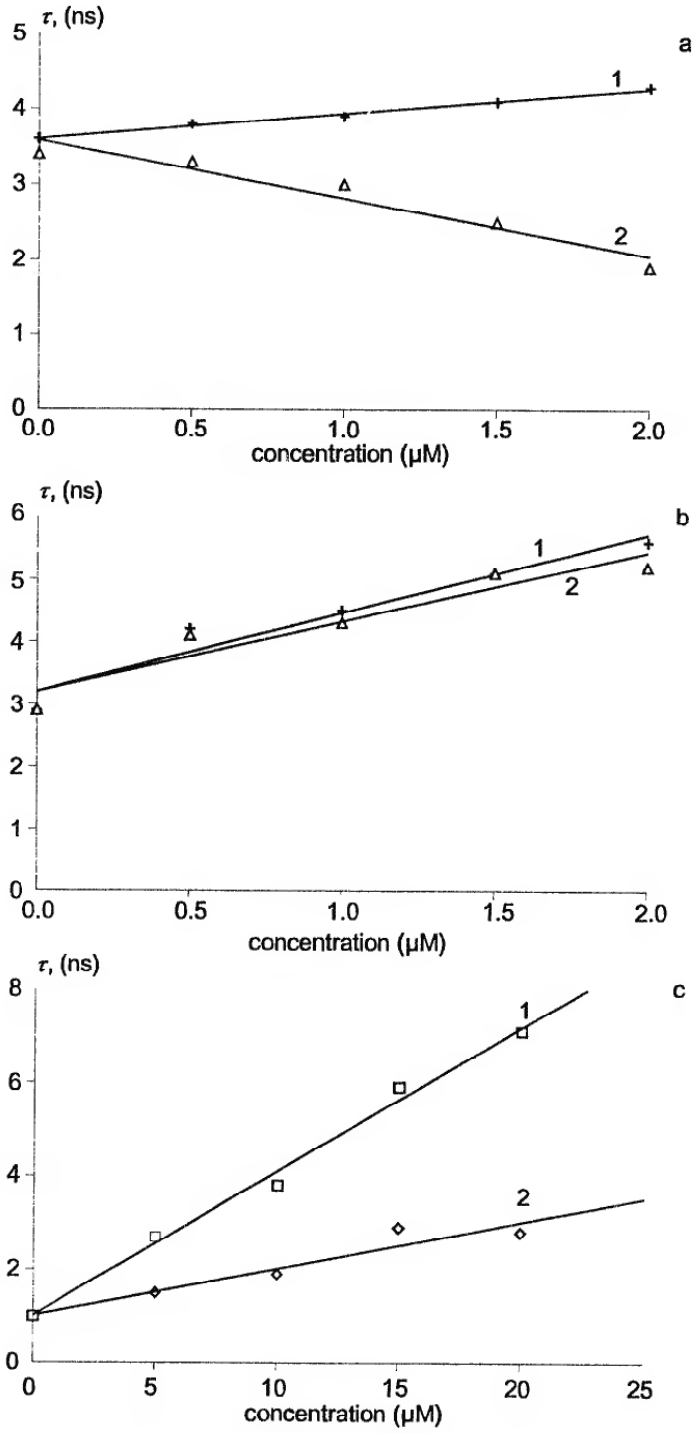

Fig. 4. The rotational correlation time (τ) of hydrophobic spin probes, doxyl derivatives of doxylstearic acid with different localizations of the nitroxyl radicals (a) 16-DSA; b) 12-DSA; c) 5-DSA) bound to lipid bilayer of rat liver microsomal membrane after pretreatment with MDA (1) or HNE (2)

number of hydrophobic areas in the membrane, mostly those surrounding tryptophane residues.

The fluorescence spectra of the fluorescent probe, pyrene, were changed under the influence of MDA (Figure 6). MDA decreased the ratio of spectra intensity at 372 nm and 398 nm and increased the formation of pyrene eximers bound to the lipid bilayer (λ_f = 470 nm) after stimulation of tryptophane residues (λ_{ex}= 280 nm). This indicates a decrease of polarity of the surrounding of pyrene molecules and, i.e. an increase in hydrophobicity.

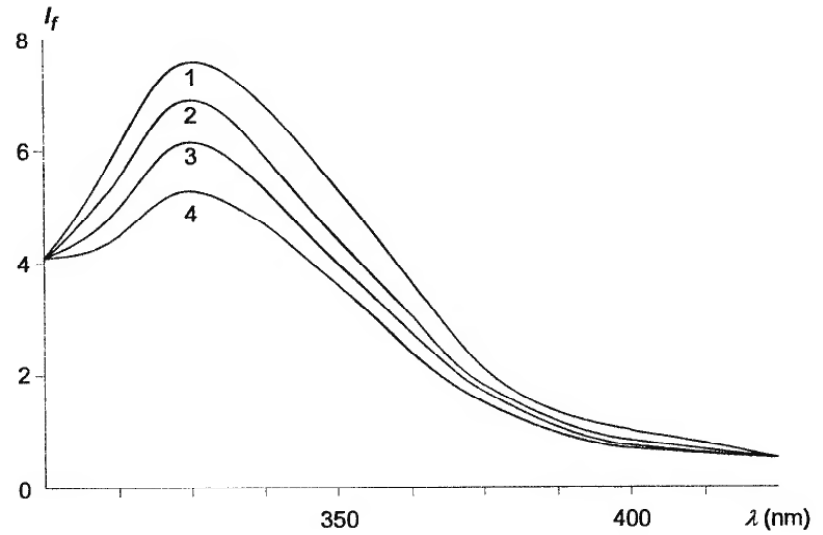

Fig. 5. Tryptophane fluorescence of rat liver microsomal membrane (λ_{ex} = 280 nm) under influence of different MDA concentrations (1 – control; 2 – MDA 10^6 M; 3 – MDA 2×10^6 M; 4 – MDA, 5×10^6 M)

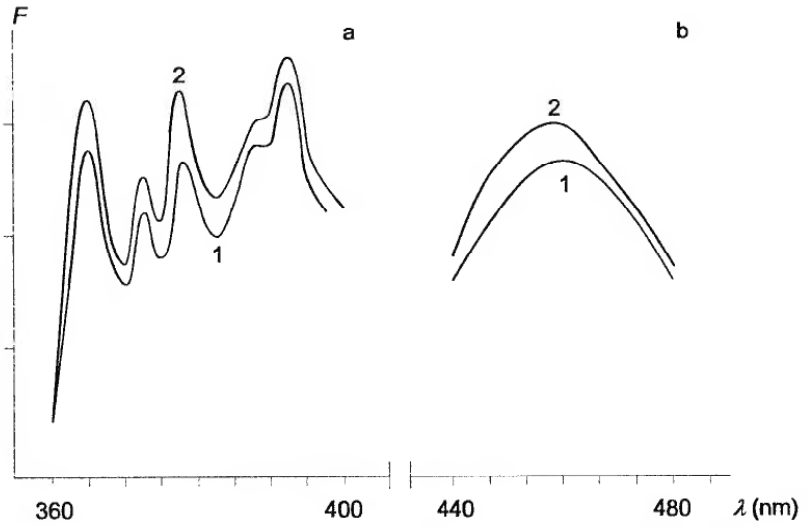

Fig. 6. The effect of MDA (30 μM) on the fluorescence excitation spectra of pyrene, localized in lipid bilayer of rat liver microsomal membrane in regions of (a) monomer (λ_{f1} = 372 nm; λ_{f2} = 398 nm) and (b) eximer (λ_f = 470 nm) fluorescence; 1 – control; 2 – MDA – treated membranes

Earlier we demonstrated that HNE enhanced the microviscosity of the lipid surrounding of the spin label OTMP-PCMB bound to SH-groups of membrane proteins whereas MDA lowered this parameter (Buko *et al.*, 1994). OTMP-PCMB was effectively quenched by potassium ferricyanide in microsomes preincubated with MDA, but not in the native microsomal membrane (Figure 7). This action developed most effectively at MDA concentrations higher than 20 μM.

The interactions of MDA with the microsomal membrane led to structural changes in hydrophobic areas of membrane proteins and non-polar zones of phospholipids. Simultaneously, the microviscosity of cytochrome P-450 heme surrounding as well as the tryptophane residues and non-polar zones of the lipid bilayer were increased, whereas the hydrophobicity of SH-groups environment was decreased. As some authors suggested (Imai & Sato, 1967), the lability of the cytochrome P-450 spectrum after its interactions with different reagents is maintained by changes in the hydrophobicity of heme surrounding. The SH-groups which are responsible for the native hemoprotein spectrum are located in the hydrophobic area formed by membrane phospholipids. We propose that the conversion of cytochrome P-450 to cyto-

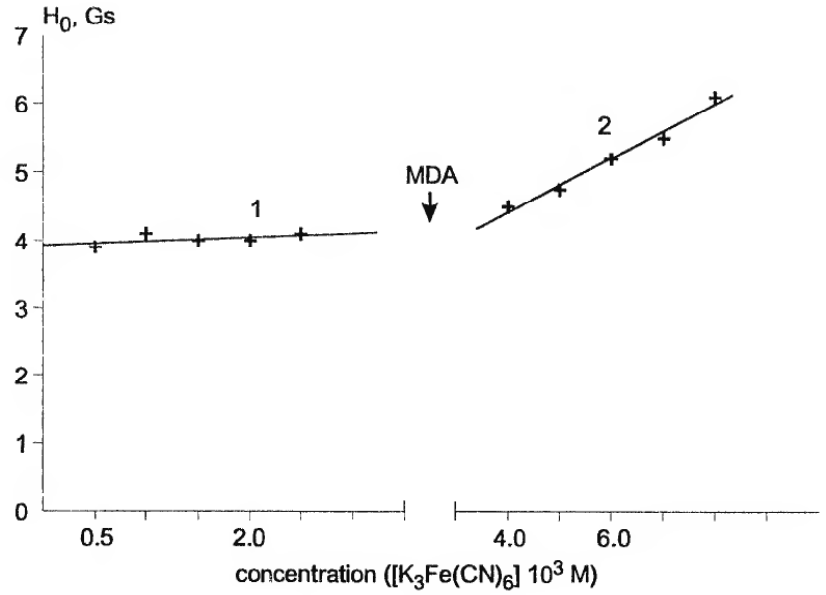

Fig. 7. ESR spectrum changes of the spin label OTMP-PCMB bound to SH-groups of microsomal membrane protein at different concentrations of potassium ferricyanide. 1 – in the absence of MDA; 2 – in the presence of MDA (30 μM)

chrome P-420 in our experiments is a result of "direct attacks" of the SH-ligand by aldehydes.

The inactivation of cytochrome P-450 occurred at an MDA concentration of 10 μM, wheras the changes in microviscosity were observed at lower concentrations. At the same time potassium ferricyanide quenched the spin label OTMP-PCMB bound to membrane protein SH-groups only at MDA concentrations higher than 10 μM. On the basis of these data we can propose the following mechanism of the cytochrome P-450 inactivation by MDA. MDA modified the surface layer of the microsomal membrane, interacting with surface SH- and amino groups and forming protein cross-linkages. This led to neutralisation of the charges located on the membrane surface and to changes in the protein conformation, probably cytochrome P-450 conformation, which promoted formation of channels or pockets in the hemoprotein facilitating the MDA penetration to more hydrophobic areas. Simultaneously, the hydrophobicity of cytochrome P-450 lipid environment was decreased. All the above preceeded the binding of MDA to an SH-group of the cytochrome P-450 active center and created conditions for these interactions.

HNE as a hydrophobic compound penetrated into the hydrophobic areas of the heme, causing their fluidization. As a result, the coordination of SH-group to the heme takes place in a more hydrophobic envinronment. The disturbances in the hydrophobic interactions of heme surrounding are suggested to be a factor leading to the inactivation of cytochrome P-450 by this aldehyde.

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