VITAMIN E DEFICIENCY AND PHOTOSENSITIZATION OF ELECTRON-TRANSPORT CARRIERS IN MICROSOMES

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1. Introduction

Cellular and membrane damage mediated by endogenous chromophores, in the presence of visible light and oxygen has been reported in [1–6]. In particular, the aerobic illumination of rat liver microsomes with heterochromatic light ($\lambda > 400$ nm) showed a preferential inactivation of NADPH-cytochrome P450-reductase and cytochrome P450 [5] with a strong correlation between cytochrome P450 destruction and lipid peroxidation. Here, we used microsomal membranes isolated from both control and vitamin E-deficient animals to determine how the lack of vitamin E in these membranes would affect the sensitivity of hemo- and flavoproteins and lipids to photodamage.

Vitamin E-deficient rat liver microsomes, aerobically treated with visible light ($\lambda > 400$ nm) showed higher values for the destruction of cytochrome P450 and protoheme, which correlated well with increased levels of lipid peroxidation, when compared to control microsomes. These effects were abolished under anaerobiosis. The pronounced photoinactivation of NADPH-cytochrome P450-reductase was similar in both vitamin E-deficient and control samples, but could be considerably recovered by addition of the cofactor FMN and was partially reversed under nitrogen. Our results suggest that the photoinactivation of cytochrome P450 is mediated by lipid peroxides, whereas the photoinactivation of NADPH-cytochrome P450-reductase is not. Under the present conditions of illumination cytochrome b_5 and NADH-cytochrome b_5-reductase were not photosensitive.

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2. Materials and methods

Male Long-Evans rats (~100 g body wt) were purchased from Simonsen Labs. (Gilroy CA). Two different diets were from BioServ Inc. (Frenchtown NJ): Bio-Mix 1331 contained <1 IU vitamin E/100 g; Bio-Mix 1332 had 21 IU vitamin E/100 g. Over 3 months a group of 8 rats (controls) were fed Bio-Mix 1332 and another group of 8 rats (vitamin E-deficient animals) were fed Bio-Mix 1331. The vitamin E deficiency was estimated by red blood cell hemolysis [7]: vitamin E-deficient animals exhibited 93 ± 3% hemolysis, compared to 10 ± 3% hemolysis for controls.

Microsomes were prepared from perfused livers according to [8] and washed twice in K_2PO_4 (50 mM, pH 7.5). The pellets were kept in liquid N_2 until used.

2.1. *Illumination conditions*

Aerobic incubation of dark and light samples was as in [1,5]. Microsomal suspensions (10 ml at 2 mg protein/ml in a 50 mM K_2PO_4 buffer, pH 7.5) were placed in 50 ml Erlemeyer flasks and slowly shaken in a waterbath at 26 ± 1°C. Dark samples were incubated under the same conditions, but inside flasks covered with aluminium foil. All data are averages of results from ≥4 different microsomal preparations.

2.2. *Electron carriers*

The activity of the microsomal reductases was assayed as follows: NADPH-cytochrome P450-reductase was measured using cytochrome *c* as an electron acceptor [9]; NADH-cytochrome b_5-reductase was measured using ferricyanide as an electron acceptor [10]. The concentrations of cytochrome b_5 [11] and cytochrome P450 [12] were measured using difference spectra in an Aminco DW-2 spectrophotometer.

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The detection of malondialdehyde produced during lipid peroxidation was assayed as in [13]. Protoheme content was estimated using the differential extinction coefficient $\Delta\epsilon_{555-575}$ of 32.4 $mM^{-1} . cm^{-1}$ for the oxidized *vs* dithionite reduced spectra of the heme [14].

3. Results

The effect of visible light on microsomal electron-transport components shows a faster rate of inactivation of cytochrome P450 in the vitamin E-deficient microsomes compared to controls (fig.1). The NADPH-cytochrome P450-reductase was inactivated very rapidly during illumination but at approximately the same rate in control and vitamin E-deficient microsomes. Cytochrome b_5 levels were unaffected during illumination (not shown). Dark samples showed only a small inactivation of the NADPH-cytochrome P450-reductase and cytochrome P450 but no change in the levels of cytochrome b_5. A slight activation of the NADH-cytochrome b_5-reductase was observed in the illuminated samples (20–30%) in both control and vitamin E-deficient samples.

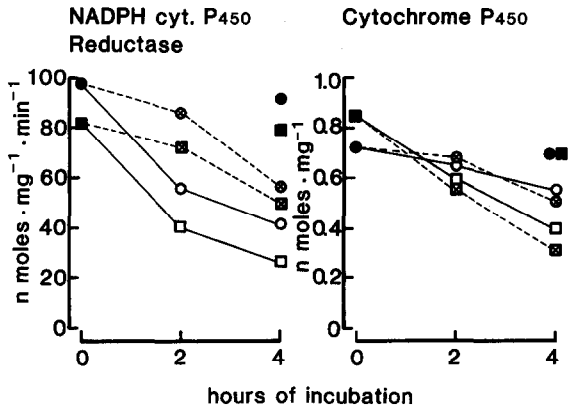

Fig.1. Effect of incubation of microsomes on the activity of NADPH-cytochrome P450-reductase and the content of cytochrome P450. Circles refer to normal samples and squares to vitamin E-deficient samples. Open symbols refer to illuminated samples while crossed symbols refer to samples kept in the dark in the presence of 0.5 mM NADPH. Dark controls (in the absence of NADPH) are indicated by filled symbols at the beginning and end of the 4 h incubation. Variability for each value of NADPH-cytochrome P450-reductase is ± 5 nmol . mg $protein^{-1}$. min^{-1}. Variability for each value of cytochrome P450 is 0.05 nmol/mg protein.

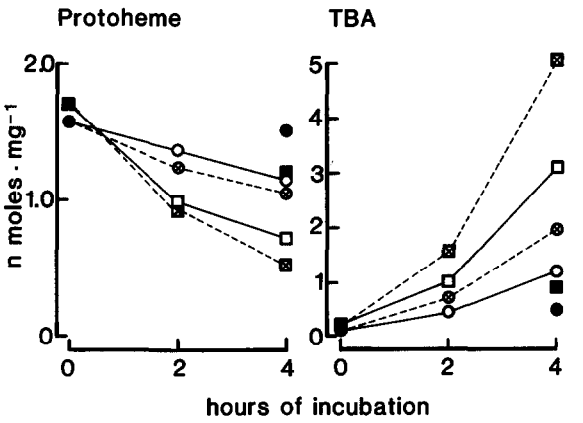

Fig.2. Effect of incubation of microsomes on the protoheme content and the levels of thiobarbituric (TBA) acid reactants. Symbols as in fig.1. Variability for each value of protoheme is ± 0.08 nmol/mg protein. Variability for each value of TBA at 2 and 4 h incubation is ± 0.2 nmol/mg protein, and at zero time ± 0.05 nmol/mg protein.

The protoheme content of the microsomal suspensions decreased while thiobarbituric acid-reactant materials increased during illumination (fig.2); the effects were always more pronounced in the vitamin E-deficient samples.

Under anaerobic conditions ($+N_2$) but in the presence of light there was no inactivation of cytochrome P450, no destruction of the protoheme and no measurable lipid peroxidation, but the NADPH-cytochrome P450-reductase was still inactivated up to 50% of the amount observed in the presence of air (in both control and vitamin E-deficient samples).

The dark incubation in the presence of NADPH increased the levels of thiobarbituric acid reactants, inactivated the NADPH-cytochrome P450-reductase, destroyed cytochrome P450 and reduced the protoheme content of the sample; the effects on lipids, cytochrome P450 and protoheme were considerably larger for the vitamin E-deficient microsomes (fig.1,2).

The destruction of cytochrome P450 was never accompanied by a stoichiometric rise in cytochrome P420. In most cases the shoulder in the difference spectra at 420 nm was hardly detectable (not shown). Also, post-incubation treatment of the preparations with reduced 1 mM glutathione did not recover cytochrome P450.

Post-incubation addition to the illuminated preparations (4 h) of 10 nmol FMN/mg protein made it possible to recover 70 ± 10% of the activity of

NADPH-cytochrome P450-reductase of the preparation kept in the dark.

The zero-time amount of cytochrome P450/mg protein was always somewhat larger in the vitamin E-deficient preparations, while the activity of the NADPH-cytochrome P450-reductase/mg protein was somewhat smaller in these samples as compared to controls. The zero-time levels of thiobarbituric acid reactants was always larger in vitamin E-deficient microsomes.

Preliminary data on the photoinactivation of aminopyrine demethylation show that this activity follows very closely the activity of the NADPH-cytochrome P450-reductase (not shown).

4. Discussion

During aerobic illumination with visible light, microsomes isolated from both control and vitamin E-deficient rats showed a strong correlation between rates of formation of thiobarbituric acid reactants and rates of destruction of cytochrome P450 and protoheme. Similarly, during the dark incubation in the presence of NADPH, the final amounts of thiobarbituric acid reactants and the destruction of cytochrome P450 and protoheme were highest in the vitamin E-deficient microsomes.

A strong correlation between cytochrome P450 destruction and lipid peroxidation during photooxidative damage was already demonstrated in the case of microsomes isolated from control animals; both were shown to be oxygen-dependent and to be protected by antioxidants and radical scavengers [5]. We have further shown that the disappearance of cytochrome P450 is due to a destruction of the protoheme, which also explains why P420 is never observed and P450 cannot be recovered with reduced glutathione [15].

Since cytochrome b_5 is much less sensitive to hydroperoxides than cytochrome P450 [16], this may explain why the former was virtually unaffected by the illumination. The fact that the photoinactivation of NADPH-cytochrome P450-reductase can be significantly reversed, and to the same extent, in both control and vitamin E-deficient microsomes by the subsequent addition of FMN suggests that it was to a large extent due to a loss of the cofactor. The greater photoreactivity of FMN as compared to FAD [17] may be the reason why the FMN + FAD-containing NADPH-cytochrome P450-reductase is much more photosensitive than the FAD-containing NADH-cytochrome b_5-reductase. The loss of FMN also does not correlate with lipid peroxidation.

Our results would therefore indicate that cytochrome P450 photoinactivation may be mediated by lipid peroxidation,which is larger in vitamin E-deficient microsomes, whereas the photoinactivation of NADPH-cytochrome P450-reductase may simply be due to the photosensitivity of the FMN cofactor of that enzyme.

The NADPH-dependent inactivation of the NADPH-cytochrome P450-reductase could be due to the high levels of thiobarbituric acid reactants (fig.2) formed in both the control and the E-deficient samples under these conditions. When high levels of thiobarbituric acid reactants are formed, the activity of the NADPH-cytochrome P450-reductase is first released from the microsomes and subsequently lost [18–20]. The larger values of thiobarbituric acid reactants found in the vitamin E-deficient sample did not correlate with a greater inactivation of the NADPH-cytochrome P450-reductase, suggesting that the effect is not mediated in a simple fashion by lipid peroxidation.

The lower values of NADPH-cytochrome P450-reductase activity and the higher values of cytochrome P450 in the vitamin E-deficient microsomes are interesting. Since the levels of thiobarbituric acid reactants are always higher in the vitamin E-deficient microsomes (even at zero-time) it is surprising that the levels of cytochrome P450 are increased unless we consider a mechanism whereby increased peroxidative damage in vivo may induce the synthesis of cytochrome P450. Well-known effects of phenobarbital, for example, include both increased levels of lipid peroxidation (in isolated hepatocytes) [21] and large induction of cytochrome P450. The decreased level of NADPH-cytochrome P450-reductase activity could be due to the fact that increased lipid peroxidation in vivo may release the enzyme from the microsomal membrane [18–20]. Further studies are being undertaken in microsomes to elucidate the effects of vitamin E deficiency on the activity and amount of electron-transport carriers, on their drug metabolizing rates and on their photosensitivity. These studies may help to clarify the structural *vs* antioxidant role of vitamin E in biological membranes and may also help to unravel the reports [22–24] on activities of drug metabolizing enzymes in liver microsomes isolated from vitamin E-deficient rats.

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