Vitamin E deficiency has different effects on brain and liver phospholipid hydroperoxide glutathione peroxidase activities in the rat

Jean-Marie Bourre,*, Odile Dumont, Michel Clément, Lan Dinh, Marie-Thérèse Droy-Lefaix, Yves Christen

aINSERM U 26, Hôpital Fernand Widal, 200 rue du Faubourg Saint-Denis, 75475 Paris Cedex 10, Paris, France
bIPSEN, 24, rue Erlanger, 75781 Paris Cedex 16, France

Abstract

The effect of vitamin E deficiency on glutathione peroxidase activity (GPX) and on the activity of a selenoenzyme (phospholipid hydroperoxide glutathione peroxidase (PHGPX)) was measured in rat brain and liver. In brain, the activity of both enzymes was in the same range in homogenate and in microsomes. In contrast, in liver homogenate, PHGPX activity was approximately 20 times lower than that of GPX. Very interestingly, PHGPX activity was significantly decreased in brain microsomes by vitamin E deficiency, but slightly significantly increased in liver microsomes. In contrast, GPX activity was not affected in brain by vitamin E deficiency, but was significantly lower in liver homogenate and microsomes. Thus, PHGPX activity is partially controlled by vitamin E in membranes, and PHGPX is probably an enzyme different from GPX.

Keywords: Glutathione peroxidase; Phospholipid hydroperoxide glutathione peroxidase; Vitamin E; Desaturase; Polyunsaturated fatty acids; Brain; Liver

It is well known that the brain is especially susceptible to free radical damage since the membrane lipids are very rich in polyunsaturated fatty acids (PUFA). Free radicals contribute to neuronal loss in cerebral ischemia and hemorrhage, and may be involved in the degeneration of neurons during normal aging and also in various diseases such as Alzheimer’s disease, epilepsy, schizophrenia, tardive dyskinesia, and Parkinson’s disease. It has long been known that lipid peroxidation, which greatly influences membrane fluidity, lipid composition, and enzymatic activities is correlated with brain damage. Free radicals are also implicated in the initiation of lipid peroxidation. Glutathione peroxidase (GPX) play a key role against peroxidation [2].

Therefore, antioxidant defences in the brain have justifiably received considerable attention. The brain contains both enzymatic and non-enzymatic antioxidants that protect against free radical damage. The enzymatic antioxidants include catalase, superoxide dismutase, GPX, glutathione reductase and glucose-6-phosphate dehydrogenase. Known non-enzymatic antioxidants include vitamin E, beta-carotene, estrogen, ascorbic acid, and glutathione. Another form of GPX isolated from pig liver by Ursini et al. [22] acts directly on peroxidized phospholipids integrated into biomembranes and is therefore called phospholipid hydroperoxide glutathione peroxidase (PHGPX). Apart from this important functional difference and the apparently monomeric nature of PHGPX, the two enzymes appear similar. The molecular mass of PHGPX is close to the subunit molecular mass of GPX, and both enzymes contain selenium in almost identical stoichiometric amounts [22]. The enzyme is present in brain [24], and in various organs such as testis, intestinal epithelium, heart, retina, and kidney. Individual glutathione peroxidases could have tissue-specific functions [2]. PHGPX has been cloned recently in mouse fibroblasts [1]. Expression of PHGPX is responsible for the protection of host cells from lipid hydroperoxide-mediated injury [17]. Moreover, PHGPX is more active than GPX in preventing the elevation of liver lipid peroxide levels in selenium-deficient rats [9].

Interestingly, when lipid peroxidation was induced in liver microsomes by ascorbate and iron adenosine-diphosphate...
phate, inhibition by PHGPX and glutathione occurred only if vitamin E was also present [12]. These results suggest cooperation between the peroxidase and the free radical scavenger, which raises the question of what is the mechanism of cooperation and the role of the vitamin E. Speculations have been made on the effect of vitamin E on GPX activity, both in vivo [4,16,18,20] and in cell culture [7].

The aim of the present work was to study the relationship between PHGPX activity and dietary vitamin E content. Moreover, due to the similarity between GPX and PHGPX it still remains uncertain whether PHGPX should be considered an enzyme in its own right or rather a variant or derivative of GPX.

Female Sprague–Dawley rats from Ifa Credo (l’Arbresle, France) were fed a synthetic laboratory diet. Animals were maintained under standardized conditions of light (07:00–19:00), temperature (22 ± 1°C, humidity (70%) and received water ad libitum. From the 14th day of gestation, breeding female rats were fed a synthetic vitamin-E-deficient diet or the same diet enriched with All-rac-tocopherol (0.050 g/kg diet) (APAE-INRA, Jouy-en Josas, France).

Rats were decapitated and the liver and brain rapidly removed, weighed, and rinsed with homogenization medium. The liver was homogenized for 1 min in three volumes of 0.25 M sucrose, 20 mM Tris–HCl (pH 7.4). Following centrifugation at 10 000 × g for 15 min and at 105 000 × g for 1 h, the pellet was resuspended in 0.1 M Tris–HCl (pH 7.4) and 0.3 M KCl buffer solution and centrifuged again at 105 000 × g for 1 h to obtain the microsomal fraction. Brain microsomes were prepared using the same method with homogenization medium for 1 min in five volumes of 0.32 M sucrose, 0.1 M phosphate, NaCl 9/1000 buffer. The final pellet containing brain microsomes was resuspended in 0.1 M Tris–HCl (pH 7.4) and 0.3 M KCl buffer solution. Liver and brain microsomes were stored at −80°C for no longer than 2 weeks before use.

The substrate was synthesized according to Maiorino et al. [13]. Briefly, phosphatidylcholine polynsaturated fatty acids were oxygenated with soybean lipoxidase in the presence of bile salts. Phosphatidylcholine contained mainly linoleic acid, as originating from soybean (Sigma, Ref. P7443). At the end of the reaction, phospholipids were separated from bile salts and extracted with methanol. The substrate was stored at −20°C for no longer than 2 weeks before analysis. The number of hydroperoxide groups was then evaluated by colorimetric chemical assay based on reduction of hydroperoxides with potassium iodide according to van Kuijk et al. [23]. Starch formed a coloured complex with the triiodide ion produced by reduction of the hydroperoxides in the presence of an acid catalyst, aluminium chloride. Calibration was performed with solutions of known concentrations of hydrogen peroxide.

PHGPX was assayed as follows. One milligram of biological sample was added to a total volume of 2.5 ml containing 0.125 M Tris–HCl (pH 7.4), 6.25 mM EDTA, 0.125 mM NADPH (Sigma), 1.25 mM NaN₃ (Sigma), 3.75 mM reduced glutathione (Sigma), 5 µl of glutathione reductase (Sigma, specific activity 200 units/mg protein) and 15 µl of 20% (v/v) peroxide-free Triton X-100 (Sigma). After 5 min at 37°C for temperature equilibration, complete reduction of glutathione and PHGPX activation, the reaction was started by addition of 40 nmol of substrate and the change in absorption at 340 nm was recorded (Kontron Uvikon 930 spectrophotometer). GPX was assayed by the method of Tappel [19].

Rats were maintained on control and vitamin-E deficient diets during gestation and suckling and pups were fed the same diet as their dams for 4 weeks. At the end of the depletion period, four pups of each group were decapitated under light ether anesthesia. Livers and forebrains were rapidly removed and frozen. All the tissues were lyophilized and stored at −30°C until analysis of tocopherols. Tocopherols were determined by HPLC in serum and lyophilized tissues according to Ueda and Igarashi [21] with minor modifications [5,6]. The concentrations of alpha-tocopherol were calculated using calibration curves obtained with a 2500 Chromato-Integrator (Merck–Hitachi). Results are expressed as µg/g fresh weight. Protein was determined according to Lowry et al. [11]. Results are presented as means ± SD for four experiments using four rats from each dietary group. Data were analyzed using Student’s t-test for comparison between the two groups. Experimental protocols were approved and met French government guidelines (Ministry of Agriculture, authorization no. 03007, June 4, 1991).

Dietary vitamin E deprivation reduced vitamin E content in both forebrain and liver, (69.7 and 96.8%, respectively) (Table 1). Fig. 1 shows GPX and PHGPX activities in brain and liver measured in homogenate and microsomal preparations in deficient and control animal. In vitamin-E-deficient animals, GPX was not affected in brain homogenate or in brain microsomes, but was significantly decreased in liver homogenate and liver microsomes compared with controls. In vitamin-E-deficient animals, PHGPX activity was significantly decreased in brain microsomes, but not in brain homogenate. In contrast, the activity was significantly increased in liver microsomes, but not in liver homogenate. Thus, in brain, vitamin E deficiency significantly decreased microsomal PHGPX but not microsomal GPX. PHGPX and GPX were significantly and inversely altered in liver from deficient animals: PHGPX was significantly increased in liver microsomes, whereas GPX was significantly decreased in liver homogenate and microsomes.

<table>
<thead>
<tr>
<th>Vitamin E content of brain and liver of control and vitamin-E-deficient animals</th>
<th>Vit E⁺</th>
<th>Vit E⁻</th>
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<tr>
<td>Forebrain (vit E µg/g)</td>
<td>24.81 ± 1.59</td>
<td>7.51 ± 1.89</td>
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<tr>
<td>Liver (vit E µg/g)</td>
<td>35.73 ± 2.75</td>
<td>1.13 ± 0.34</td>
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When GPX and PHGPX activities were compared, activities were similar in brain homogenate and microsomes for both control and deficient animals, as well as in liver microsomes for control animals. In liver microsomes the PHGPX activity was similar to GPX activity for deficient animals. In contrast, in liver homogenate, PHGPX values were, respectively, about 20- and 17-fold lower than GPX values for control and deficient animals.

Unlike GPX, PHGPX can reduce membrane phospholipid hydroperoxides in situ without the necessity of prior hydrolysis by phospholipase A2. Thus, PHGPX has a more direct protective role and due to its interaction with vitamin E (also present in membrane) is probably more active. The cooperation between vitamin E and PHGPX in the protection against oxidative damage of membranes remains to be clarified. Both selenium and vitamin E are recognized as essential nutrients for prevention of oxidative damage. The obligatory presence of selenium for the enzymatic activity of the two seleno-dependent glutathione peroxidases is well known. In contrast, the role of vitamin E is still obscure. Moreover, it is believed that the major difference between GPX and PHGPX is that the reduction of phospholipid hydroperoxides in the membrane matrix is catalyzed uniquely by PHGPX. Others properties such as kinetic mechanisms of the peroxidase reactions, selenium content and the similarity of molecular weight between PHGPX and the monomer of GPX suggest a structural relationship between these two enzymes. Therefore, we considered it to be important to study the role vitamin E plays in the activity of both glutathione peroxidase enzymes.

GPX activity measured in brain microsomes could in fact correspond to the PHGPX activity. Moreover, GPX and PHGPX were not similarly distributed in different tissues: in contrast to liver, brain GPX activity in homogenate was the same as that of PHGPX in microsomes. This similar tissue distribution has already been reported [24].

Thus, very interestingly, PHGPX activity in liver and brain was not regulated in the same manner by vitamin E deficiency. Microsomal PHGPX activity was reduced in brain but increased in liver. We have previously found that vitamin E deficiency decreases GPX in liver [3], and we have attributed this to the impairment of selenium absorption during vitamin E deficiency. Compared with GPX, PHGPX activity was lower in liver microsomes, but PHGPX activity was increased in vitamin E-deficient microsomes.

These results show that, in addition to the difference in their substrate specificity and localization, these glutathione peroxidases have distinct functions. It seems likely that when vitamin E is withdrawn from the diet, PHGPX is required to prevent free radical generation by lipid hydroperoxides. Maiorino et al. [12] studied the interaction between vitamin E and PHGPX using the iron-dependent lipid peroxidation system. In this case, vitamin E has an effective antioxidant effect only if both PHGPX and glutathione are present, perhaps because PHGPX prevents formation of alkoxyl radicals against which vitamin E is a relatively weak antioxidant.

This result could be linked to the in vitro alteration of delta-6-desaturase by vitamin E in rat brain and liver [8]. In that study we showed that delta-6-desaturase activity in brain microsomes was increased by vitamin E; in contrast, this activity was reduced in the liver. In our present study, vitamin E deficiency could alter polyunsaturated fatty acid synthesis by delta-6-desaturation in the liver membrane.

The effect of vitamin E depletion on PHGPX activity in liver microsomes was different from that of selenium, showing that selenium acts by a different mechanism from that of vitamin E, although they are both required for prevention of oxidative damage. Interestingly, GPX and PHGPX are regulated differently in rat by dietary selenium [10]. Selenium depletion has been observed in brain in a patient with epilepsy [15], and PHGPX is speculated to play a key role in the defense of neuronal cells against oxygen radical formation and peroxidation processes.

The differences observed in this work between brain and liver microsomes could be a consequence of the distribution of GPX and PHGPX in this organelle, as it has been shown that PHGPX is predominant in the luminal phase of the endoplasmic reticulum, in contrast with GPX which predo-