Original Contribution

Vitamin E differentially regulates the expression of peroxiredoxin-1 and -6 in alveolar type II cells

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Abstract

Vitamin E is the primary lipophilic antioxidant in mammals. Lack of vitamin E may lead to an increase of cytotoxic phospholipid-peroxidation products (PL-Ox). However, we could previously show that alimentary vitamin E-depletion in rats did not change the concentrations of dienes, hydroperoxides, and platelet-activating factor-related oxidation products in alveolar type II cells (TII cells). We hypothesized that vitamin E deficiency increases the activity of enzymes involved in the degradation of PL-Ox. Degradation of PL-Ox may be catalyzed by phospholipase A2, PAF-acetylhydrolase, or peroxiredoxins (Prx’s). Alimentary vitamin E deficiency in rats increased the expression of Prx-1 at the mRNA and protein levels and the formation of Prx-SO3, but it did not change the expression of Prx-6 or the activity of phospholipase A2 and PAF-acetylhydrolase in TII cells. H2O2-induced oxidative stress in isolated TII cells activated protein kinase Ca (PKCa) and increased the expression of Prx-1 and Prx-6. Inhibition of PKCa in isolated TII cells by long-time incubation with PMA inhibited PKCa and Prx-1 but not Prx-6. We concluded that the expression of Prx-1 and -6 is selectively regulated in TII cells; PKCa regulates the expression of Prx-1 but not Prx-6. Prx-6 expression may be closely linked to lipid peroxidation.

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Vitamin E belongs to the endogenous cellular defense system against oxidative stress [1]. Vitamin E deficiency frequently induces oxidative stress and lipid peroxidation in different cells and tissues, indicating a disruption of the dynamic balance between oxidants and the endogenous defense system [2–5]. Oxidative stress is a very important factor in the development of acute lung injury. Acute lung injury may occur in response to oxidative air pollutants or during hyperoxic ventilation and may also occur in response to the abrupt change in oxygen tension during the transition from prenatal to postnatal life. Premature neonates tolerate this change poorly and are at high risk of developing acute lung injury [6,7]. In addition to having immature lungs, these infants also exhibit a relative vitamin E deficiency, compared to mature newborns and adults [8,9]. It has been suggested that this vitamin E deficiency contributes to acute and chronic lung injury and other neonatal disorders [10]. To date, our knowledge about the role of vitamin E in oxidative lung injury is still fragmentary.

Previously, we described the effects of alimentary vitamin E deficiency in rats on different aspects of the metabolism in alveolar type II cells (TII cells), an important epithelial cell of the lung. We showed that in vivo depletion of vitamin E caused an increased expression of heat shock proteins HSP32 and HSP70 [11], immunological dysregulation [12], apoptotic sensitization [13], and a reduction of surfactant lipid biosynthesis [14]. Lipid peroxidation in response to vitamin E depletion has so far not been investigated in TII cells.

Phospholipid (PL) peroxidation damages cell membranes and is involved in oxidative cell injury [15,16]. The
concentration of PL-peroxidation products (PL-Ox) in cellular membranes is balanced by sufficient amounts of membrane-bound lipophilic antioxidants like vitamin E or by the two-step repair mechanism of oxidized PL. This classic concept involves the action of two enzymes: phospholipase A2 (PLA2) catalyzes the excision of oxidized fatty acids, followed by reacylation forming a new intact PL species.

Peroxiredoxins (Prx’s) represent a new family of antioxidant enzymes which are able to reduce toxic PL-hydroperoxides to nontoxic hydroxy derivatives [17–19]. Therefore, it would be plausible to expect Prx’s to be increased in response to the depletion of lipophilic antioxidants, as in alimentary vitamin E deficiency. The family of Prx’s consists of six isoforms [18] and comparison of the frequency of the individual isoforms in 22 bovine tissues showed that all six Prx’s are expressed in all tissues but to very different extents. Prx-1 and -6 are the most abundant isoforms in the lung [20]. The increase of the Prx-1 expression at the mRNA and protein levels in response to oxygen in newborns suggests that this enzyme may help to protect the lung from excessive oxidative stress during the transition from prenatal to postnatal life [21,22]. Prx-6 is also up-regulated after birth [23,24]. An overexpression of this Prx-6 in a lung-derived cell line protects against membrane damage mediated by PL peroxidation [25].

The active-site cysteine of Prx’s is selectively oxidized during catalysis, whereby oxidation of at least Prx-1, -2, and -3 seems to be reversible [26–29]. Overoxidation yielding Prx-SO3 in vivo is a sensitive indicator of oxidative exposure of cells [28,29].

The aim of the present work is to investigate whether lipid peroxidation is increased in TII cells of rats in response to alimentary vitamin E deficiency and whether phospholipase A2 and platelet-activating factor (PAF)-acetyl hydrolase activity or the expression of Prx-1 and -6 is increased under these conditions.

Materials and methods

Materials

The diets used were obtained from Altromin (Lage, Germany). The vitamin E-depleted diet contained less than 2 mg α-tocopherol per kilogram; the vitamin E-enriched diet contained 400 mg RRR-α-tocopherol (Sigma, Taufkirchen, Germany) per kilogram. Phorbol-12-myristate-13 acetate (PMA) was from Calbiochem–Novabiochem (Bad Soden, Germany). L-3-Phosphatidylcholine-1-palmitoyl-2-[1-14C]oleoyl (sp act 52.2 mCi/mmoll) was purchased from Amersham Biosciences Europe (Freiburg, Germany) and hexadecyl-2-acetyl-[3H]-sn-glycero-3-phosphatidylcholine (sp act 7.10 Ci/mmoll) was from DuPont NEN (Boston, MA, USA). All other chemicals of the highest purity grade were obtained from Merck (Darmstadt, Germany).

Feeding regimen

Wistar rats (body mass 80 to 90 g) received the control diet or vitamin E-depleted diet ad libitum for 5 weeks. Thereafter, some vitamin E-depleted rats received the vitamin E-enriched diet ad libitum for 3 days.

Preparation of alveolar type II cells

TII cells were isolated using the elastase method as described by Dobbs et al. [30]. Viability was controlled by trypan blue staining and purity by Harris type hematoxylin staining of the isolated TII cells. Viability and purity ranged from 90 to 95% and 87 to 93%, respectively. Freshly isolated TII cells were suspended in Dulbecco’s minimal essential medium (DMEM; Gibco BRL, Life Technologies, Paisley, Scotland) and aliquots were shock-frozen in liquid N2 and stored at −80°C.

Primary culture of TII cells

Freshly isolated TII cells were suspended in DMEM containing 10% fetal calf serum (Gibco BRL), and H2O2 (Aldrich, Germany; final concentration 500 μM) was added. The cells were cultured in the presence of H2O2 for 8 h at 37°C on cell culture dishes (Gibco BRL). Cells were scraped off and washed twice with DMEM and once with PBS and were thereafter used for membrane preparation and lysis. Lysis of TII cells and membrane preparation were performed as outlined in previous communications [31,32].

Determination of conjugated dienes and lipid hydroperoxides (LOOH)

Conjugated dienes were determined in lipid extracts according to the procedure described by Fuhrman et al. [33]. TII cell homogenates were extracted with hexane: isopropanol (3:2; v/v). The upper hexane phase was evaporated to dryness in a stream of N2 and redissolved in 1 ml of hexane, and the content of conjugated dienes was detected spectrophotometrically at 234 nm against a hexane blank. The extinction E234 was calculated per milligram of cell protein.

Lipid hydroperoxides were determined using a commercial kit (Lipid Peroxidation Assay Kit II; Calbiochem, Merck) according to the instructions of the manufacturer. The assay is based on the oxidation of ferrous ions to ferric ions by hydroperoxides under acidic conditions. The ferric ions bind with xylene orange to form a stable complex which can be measured at 560 nm. The concentration of hydroperoxides is calculated from the absorbance and the hydroperoxide apparent molar extinction coefficient 4.31 × 104 M−1 cm−1.
**Determination of PAF-related compounds**

Oxidative degradation of polyunsaturated fatty acyl residues in phosphatidylcholine (PC) results in the formation of PCs containing short-chain aldehydes, or carbonic acids. The determination of these PAF-related compounds was carried out as described previously [34,35]. The method involves lipid extraction, chromatographic enrichment of PCs with short acyl chains, derivatization with 9-(chloromethyl)anthracene, solid-phase extraction of the derivatives, and reversed-phase HPLC with fluorescence detection. The fragmented PCs were quantified by their fluorescence yield relative to the internal standard 1-palmitoyl-2-suberoyl-PC [35].

**Phospholipase A2 assay**

1-Palmitoyl-2-[1-14C]oleoyl-PC (2.5 µCi) in dimethyl sulfoxide (DMSO) was added to TII cell homogenate and incubated in buffer (75 mM Tris base, 5 mM CaCl2, pH 8.5, or 40 mM acetate buffer, 5 mM EDTA, pH 4.0) for 60 min. The radioactive fatty acid that split off was extracted by the addition of Dole reagent [36]. The extracted radioactivity was measured in a scintillation counter (Wallac 1410; Pharmacia, Uppsala, Sweden). The specific radioactivity of the substrate was the basis for the calculation of the phospholipase A2 activity in picomoles of fatty acid split off per milligram of cell protein in 60 min.

**PAF-acetylhydrolase assay**

PAF-acetylhydrolase degrades PAF-related compounds; the activity was determined as previously described [37]. [3H]PAF in 1% Tween 20 was added as substrate to TII cell homogenate in buffer (0.5 M Hepes, 5 mM EGTA, pH 7.2) and incubated at 37°C for 30 min. Thereafter, chloroform: methanol (4:1; v/v) and 10 mM Tris–HCl, 5 mM EDTA, pH 7.5, were added to the incubation mixture and carefully mixed. The radioactivity of the upper phase and the specific radioactivity of the substrate were used for the calculation of the PAF-acetylhydrolase activity in nanomoles of acetate split off per milligram of cell protein in 60 min.

**Western blot analysis**

The protein concentrations of protein kinase Cα (PKCα) in the cellular membrane fractions, Prx-1 and -6, and oxidized Prx’s (Prx-SO3) in lysates of TII cells were estimated by SDS–PAGE with subsequent Western blot analysis using specific antibodies for the determination of the individual proteins as previously described [38,11,12,39]. PKCα was identified using rabbit anti PKCα antiserum (Calbiochem, La Jolla, CA, USA); for the identification of Prx-1 and -6 and for Prx-SO3 we used specific polyclonal antibodies from rabbit (LabFrontier, Seoul, South Korea). The determination of actin using mouse monoclonal anti-β-actin clone AC-15 (Sigma) on the blots was carried out as loading control. Peroxidase-conjugated goat anti-rabbit IgG and goat anti mouse IgG were from Pierce (Bonn, Germany) and were used as secondary antibodies.

**Reverse transcriptase and real-time quantitative PCR**

Total RNA was isolated using the RNasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Samples were further treated with DNase I (DNA-free; Ambion, Austin, TX, USA) to avoid any DNA contamination. Quality of RNA was assessed by form-aldehyde agarose gel electrophoresis.

Reverse transcription was performed with 0.5 µg RNA added to the reaction mix containing Amplitaq Gold DNA polymerase, RNase inhibitor, Amplitaq DNA polymerase, and random hexamers (Applied Biosystems, Foster City, CA, USA). Negative reverse transcriptase (RT) (no enzyme) and no-template (no RNA) controls were also included. The RT thermal cycle was 10 min at 25°C, 60 min at 37°C, and 5 min at 95°C.

For real-time PCR, Prx-1 and -6 were detected using SYBR Green. Primers are shown in Table 1. Primers were purchased from Metabion (Martinsried, Germany). Primers were designed with Primerdesigner (Applied Biosystems). The correct product size was then confirmed by electrophoresis. The PCR mixture (25 µl total volume) consisted of 0.3 µM each primer, 2× reaction buffer (dNTP, HotGoldstar DNA polymerase, uracil-N-glycosylase, 5 nM MgCl2), SYBR Green, and 1 µl of template. Amplification and detection were performed using Taqman (Applied Biosystems) with the following cycle profile: 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C + 1 min at 60°C.

**Analysis of real-time PCR results**

The mRNA of three animals was pooled. All results were normalized to β-actin, which is expressed constitutively in all tissues. Quantification was performed by determining the threshold cycle (C_T). C_T is proportional to the amplified starting copy number of cDNA (or RNA). All reactions were performed in triplicate and controlled by a no-template control.

<table>
<thead>
<tr>
<th>Gene of interest</th>
<th>Forward and reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prx 1</td>
<td>TGTGGATTTCTCACAGGCATCTG</td>
</tr>
<tr>
<td>Prx 6</td>
<td>AGCGTCAGTCGGTGAAGAGT</td>
</tr>
<tr>
<td>β-Actin</td>
<td>GGAAATCTTGTGCGTACGAT</td>
</tr>
</tbody>
</table>

All primers are written 5’ to 3’ and were purchased from Metabion (Martinsried, Germany).
reaction. The quantity of mRNA was calculated by normal-
ing the $C_T$ of genes of interest to the $C_T$ of the
housekeeping protein β-actin of the same RNA probe.
The difference between $C_T$ of control and that of vitamin E
deficiency or vitamin E refeeding ($\Delta \Delta C_T$) was used to
calculate the increase of mRNA as $2^{\Delta \Delta C_T}$.

**Inhibition of PKCα**

TII cells were incubated in the presence of PMA (final
concentration 10 μM) solubilized in DMSO (final concen-
tration 0.01%, v/v) for 18 h at 37°C [39]. Thereafter PKCα
was determined by Western blot analysis in the membrane
protein fraction isolated as previously described in detail
[40]. Prx-1 and -6 expression was measured by Western blot
analysis of TII cell lysates.

**Other methods**

Vitamin E and its oxidation product vitamin E quinone
were determined by HPLC of hexane extracts from TII cells
according Catignani and Bieri [41]. Total protein was
determined with Bradford’s reagent (Sigma).

**Statistic analysis**

Significance of differences between means of control and
means of vitamin E depletion or between means of control
and means of vitamin E refeeding were assessed by
Student’s $t$ test.

**Results**

**Vitamin E content in TII cells**

Alimentary vitamin E deficiency in rats caused a
decrease of vitamin E and an increase of vitamin E quinone
in isolated TII cells (Table 2). These results replicate the
results of previous investigations [11–14,39]; the data were
shown to document that the feeding regime caused differ-
ences in the vitamin E contents in TII cells also in the
present experiments. Freshly isolated TII cells were used for
all experiments.

*The cellular vitamin E level did not affect formation
of PL-Ox or the activity of phospholipase A2 and
PAF-acetylhydrolase*

We expected that the reduction of cellular vitamin E by a
factor of 10 would lead to an elevation in PL-Ox in TII cells.
The concentrations of various lipid oxidation products,
including conjugated dienes, LOOH, PAF-related comp-
ounds, and malondialdehyde did not change significantly
(Table 2). One possible reason for this unexpected finding
may be that PL-Ox are unstable and were lost during TII cell
isolation and purification. Another reason might be that
enzyme activities that participate in the degradation of PL-
Ox are increased concomitantly. However, we did not find
any significant alteration in different types of phospholipase
A2 activities in TII cells (Ca$^{2+}$-dependent alkaline PLA2,
Ca$^{2+}$-independent acidic PLA2, and PAF-acetylhydrolase;
see Table 2).

**Effects of cellular vitamin E content on the expression of
Prx’s in TII cells**

Because Prx’s are also able to detoxify PL-hydroper-
oxides, we hypothesized that members of this enzyme
family may compensate for vitamin E deficiency in our
model. In agreement with this assumption, the expression of
Prx-1 increased in response to vitamin E depletion in rats at
both the protein and the mRNA level in TII cells. Refeeding
of vitamin E to vitamin E-depleted rats reversed the
overexpression of Prx-1, suggesting a tight relationship
between vitamin E level and Prx-1 activity (Fig. 1). In
contrast, the expression of Prx-6 was not significantly
affected by the cellular vitamin E concentration (Fig. 2).
Fig. 3 shows that the formation of Prx-SO$_3^-$, a by-product of
Prx activity, increased in response to the decrease of the

![Table 2](image-url)

<table>
<thead>
<tr>
<th>Vitamin E content</th>
<th>Control rats</th>
<th>Vitamin E-depleted rats</th>
<th>Vitamin E-refed rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E (pg × 10$^8$ cells$^{-1}$)</td>
<td>74 ± 12</td>
<td>6 ± 4</td>
<td>296 ± 86</td>
</tr>
<tr>
<td>Vitamin E quinone (ng × 10$^6$ cells$^{-1}$)</td>
<td>12 ± 6</td>
<td>4 ± 2</td>
<td>20 ± 6</td>
</tr>
<tr>
<td>% of vitamin E + quinone</td>
<td>14</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Conjugated dienes (E$$_{234}$ × mg$^{-1}$ cell protein)</td>
<td>0.090 ± 0.001</td>
<td>0.078 ± 0.011</td>
<td>0.100 ± 0.013</td>
</tr>
<tr>
<td>Lipid hydroperoxides (ng × mg$^{-1}$ cell protein)</td>
<td>0.39 ± 0.03</td>
<td>0.40 ± 0.16</td>
<td>0.50 ± 0.08</td>
</tr>
<tr>
<td>PAF-related compounds (pmol × 10$^6$ cells$^{-1}$)</td>
<td>8.8 ± 0.7</td>
<td>8.5 ± 0.1</td>
<td>8.7 ± 1.0</td>
</tr>
<tr>
<td>Phospholipase A2 (Ca$^{2+}$; pH 8.5)</td>
<td>116 ± 9</td>
<td>110 ± 25</td>
<td>115 ± 3</td>
</tr>
<tr>
<td>(pmol × mg$^{-1}$ cell protein × 60 min$^{-1}$)</td>
<td></td>
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</tr>
<tr>
<td>Phospholipase A2 (EDTA; pH 4.0)</td>
<td>232 ± 18</td>
<td>228 ± 31</td>
<td>263 ± 46</td>
</tr>
<tr>
<td>(pmol × mg$^{-1}$ cell protein × 60 min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAF acetylhydrolase</td>
<td>1.02 ± 0.10</td>
<td>0.96 ± 0.11</td>
<td>1.06 ± 0.53</td>
</tr>
<tr>
<td>(nmol × 10$^6$ cells$^{-1}$ × 60 min$^{-1}$)</td>
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</tr>
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</table>
vitamin E content in TII cells. Again, refeeding of vitamin E reversed this effect on Prx-SO₃ concentration.

**PKCα regulates the expression of Prx-1 but not of Prx-6**

Vitamin E-dependent modulation of PKCα is so far the only known nonantioxidant property of vitamin E in TII cells [12]. We inhibited PKCα in isolated TII cells by long-time incubation (18 h) in the presence of 10 μM PMA as previously described [39]. Thereafter, the expression of Prx-1 and -6 was measured (Fig. 4). Inhibition of PKCα decreased the expression of Prx-1, whereas the expression of Prx-6 did not change (Fig. 4).

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Fig. 1. Effects of vitamin E on the expression of Prx-1 at the mRNA and protein levels in TII cells. (A) Western blot analysis of Prx-1 and β-actin. β-Actin was determined as a loading control. (B) Expression of Prx-1 protein was normalized to that of β-actin. The ratio of Prx-1 to β-actin of control was 1. mRNA was estimated from the cycles in the Taqman analysis as 2^ΔΔCT; control was 1 (see Materials and methods). Values of n = 3 experiments are given as means ± standard deviation. Asterisk indicates a significant difference compared to control (p < 0.05).

Fig. 2. Effects of vitamin E on the expression of Prx-6 at the mRNA and protein levels in TII cells. Details are as described for Fig. 1.

Fig. 3. Effects of vitamin E on the expression of Prx-SO₃ in TII cells. (A) Western blot analysis of Prx-SO₃ and actin. β-Actin was determined as loading control. (B) Expression of Prx-SO₃ was normalized to that of β-actin. The ratio of Prx-SO₃ to β-actin of control was 1. Values of n = 3 experiments are given as means ± standard deviation. Asterisk indicates a significant difference compared to control (p < 0.05).

Fig. 4. Effects of long-time incubation (18 h) of TII cells with 10 μM PMA on the expression of PKCo, Prx-1, and -6 in TII cells. (A) Western blot analysis of PKCo, Prx-1, and -6. (B) Expression of PKCo, Prx-1, and -6 protein was normalized to that of β-actin. The ratio of control was 1. Values of n = 3 experiments are given as means ± standard deviation. Asterisk indicates a significant difference compared to control (p < 0.05).
H$_2$O$_2$-induced stress activates PKC$_\alpha$ and increases the expression of Prx-1 and Prx-6 of TII cells

It is a moot point whether alimentary vitamin E deficiency in rats caused an increase of PL-Ox in TII cells, which was lost thereafter due to cell isolation and purification, or whether a compensatory increase in Prx-1 immediately degraded the PL-Ox formed in vivo. Culture of cells in the presence of H$_2$O$_2$ for 8 h exhibits oxidative stress in TII cells. We found a doubling of the LOOH concentration 5 min after H$_2$O$_2$ exposure in TII cells. After 8 h incubation, however, the LOOH concentration showed no difference compared to control. PKC$_\alpha$ showed, in response to H$_2$O$_2$ treatment of isolated TII cells for 8 h, a small (1.5 ± 0.3 in relation to control = 1) but significant activation, and the expression of Prx-1 and Prx-6 increased (13.3 ± 5.4 and 1.5 ± 0.2 in relation to control = 1; Fig. 5). The activation of PKC$_\alpha$ was rather small but it has to be taken into account that H$_2$O$_2$ added to, or generated in, the cell medium is completely degraded in the first 60 min of incubation. Also the stimulatory effect of paraquat-generated H$_2$O$_2$ on the PKC activity in human lung cells (WI-38) [43] occurs within minutes and might be subsequently decreased in the course of further incubation.

Discussion

Alimentary vitamin E deficiency in rats caused a decrease of vitamin E concentration in TII cells by 90% compared to control. However, the reduction of this important lipophilic antioxidant did not induce an increase of PL-Ox in freshly isolated TII cells. This result indicates that either vitamin E depletion in vivo caused no significant lipid peroxidation in TII cells or in vivo formed PL-Ox were lost during the time it took to isolate and purify the TII cells. The latter assumption has not been investigated so far but it has been shown that after oxidative stress in vivo, PL-Ox were stable enough to be found in blood plasma [44,45], epithelial lining fluid [46,47], and lung tissue [48,49]. In isolated TII cells, H$_2$O$_2$-generated PL-Ox were eliminated within 8 h. However, it seems to be also a plausible interpretation that a compensatory increase of Prx-1 balanced the enrichment of PL-Ox in TII cells by the immediate conversion of the highly reactive PL-hydroperoxides to less reactive hydroxy derivatives.

Interestingly, the Prx-6 expression did not change significantly in response to vitamin E content in TII cells. In contrast, Fisher and his group showed that Prx-6 expression increased in response to oxidative stress caused by hyperoxia, H$_2$O$_2$, or paraquat in both whole lung and a rat lung epithelial cell line (L2 cells) [50]. Although the definitive reason for this discrepancy is not clear, it might be assumed that alimentary vitamin E depletion in rats causes in TII cells—if anything—less of an oxidative stress than hyperoxia, H$_2$O$_2$, or paraquat. Treatment of isolated TII cells with H$_2$O$_2$ increased lipid peroxidation and expression of Prx-6, Prx-1, and activated PKC$_\alpha$. Together with the results of the vitamin E feeding experiments, these findings suggest that alimentary vitamin E deficiency in rats did not induce oxidative stress reflected by lipid peroxidation in TII cells. The higher level of Prx-SO$_3$, normally seen as a sensitive indicator of oxidative exposure, may merely be the result of the increase in total Prx protein concentration in our experiments.

In addition to its property as a radical scavenger, vitamin E exhibits nonantioxidative properties. Vitamin E modulates the activity of PKC$_\alpha$ in TII cells [12] and various other cell types [51,52]. Inhibition of PKC inhibits the oxygen-mediated increase of Prx-1 mRNA in A549 cells [21], indicating that the modulation of PKCs may be involved in the regulation of Prx’s in the lung. Furthermore, protein phosphorylation was shown to be one of the factors that affect Prx activity [24,12] and in parallel the expression of Prx-1 but not Prx-6. H$_2$O$_2$ induced lipid peroxidation and activated PKC$_\alpha$ of TII cells in primary culture [43]. Consequentially, we found an increased expression not only of Prx-6 but also of Prx-1.

In summary, we conclude that activation of PKC$_\alpha$ mediates a reversible increase of the Prx-1 expression in TII cells isolated from vitamin E-depleted rats, whereas lipid peroxidation did not increase under these conditions. Therefore, the vitamin E-induced increase of Prx-1 expression is not directly coupled to a higher cellular content of PL-Ox. In contrast to Prx-1, the elevation of Prx-6 expression seems to be closely linked to increased lipid peroxidation and might represent a powerful mechanism protecting TII cells against accumulation of cytotoxic...

Fig. 5. Effects of hydrogen peroxide on the expression of Prx-SO$_3$ in TII cells. Details are as described for Fig. 4.
amounts of PL-Ox. Therefore the missing increase of Prx-6 expression in response to vitamin E depletion is another argument corroborating the assumption that alimentary vitamin E depletion in rats does not increase lipid peroxidation in TII cells. The differentially regulated expression of Prx-1 and Prx-6 in TII cells may be seen as an indication that the individual members of the peroxiredoxin family have different metabolic function or show a graded activation in response to the extent of the oxidant load; Prx-1 expression already reacts very sensitively at marginal oxidative exposure without increased lipid peroxidation, but with activation of PKCo, whereas Prx-6 expression reacts to severe oxidative exposure accompanied by lipid peroxidation independent from the modulation of PKCo.

References


tively modify LDL, and affects their secretory properties. *Atherosclerosis* **111:**65–78; 1994.


