Structural Elucidation of Oxygenated Storage Lipids in Cucumber Cotyledons

IMPLICATION OF LIPID BODY LIPOXYGENASE IN LIPID MOBILIZATION DURING GERMINATION*

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Vol. 272, No. 34, Issue of August 22, pp. 21635–21641, 1997

At early stages of germination, a special lipoxygenase is expressed in cotyledons of cucumber and several other plants. This enzyme is localized at the lipid storage organelles and oxygenates their storage triacylglycerols. We have isolated this lipid body lipoxygenase from cucumber seedlings and found that it is capable of oxygenating in vitro di- and trilinolein to the corresponding mono-, di-, and trihydroperoxy derivatives. To investigate the in vivo activity of this enzyme during germination, lipid bodies were isolated from cucumber seedlings at different stages of germination, and the triacylglycerols were analyzed for oxygenated derivatives by a combination of high pressure liquid chromatography, gas chromatography/mass spectrometry, and nuclear magnetic resonance spectroscopy. We identified as major oxygenation products triacylglycerols that contained one, two, or three 13S-hydroperoxy-9(Z),11(E)-octadecadienoic acid residues. During germination, the amount of oxygenated lipids increased strongly, reaching a maximum after 72 h and declining afterward. The highly specific pattern of hydroperoxy lipids formed suggests the involvement of the lipid body lipoxygenase in their biosynthesis.

These data suggest that this lipoxygenase may play an important role during the germination process of cucumber and other plants and support our previous hypothesis that the specific oxygenation of the storage lipids may initiate their mobilization as a carbon and energy source for the growing seedling.

Lipoxygenases (LOXs) are non-heme iron-containing dioxygenases that catalyze the regio- and stereoselective oxygenation of polyenoic fatty acids to their corresponding hydroperoxy derivatives (1). They are widely distributed in plants and animals (2, 3). In mammals, LOXs are classified into 9- and 13-LOXs with respect to their positional specificity of arachidonic acid oxygenation (4). Since arachidonic acid is either not present in higher plants or is a minor constituent of cellular lipids, plant LOXs may be classified into 9- and 13-LOXs with respect to their positional specificity in linoleic acid oxygenation. Recently, a more comprehensive classification of plant LOXs has been proposed based on the comparison of their primary structures (5). Although plant and mammalian lipoxygenases have been extensively characterized with respect to their protein chemical and enzymatic properties, there is no general idea of their biological importance (1).

In plants, 13-LOXs have been implicated in the biosynthesis of jasmonic acid (6). This phytohormone has been shown to be an important mediator in the wound response of plants to herbivore attack (7, 8). For many years, plant and animal LOXs have been considered to oxygenate mainly free polyenoic fatty acids, forming oxygenated derivatives that may exhibit biological activities (2, 4, 9). On the other hand, more recent studies have suggested that certain plant (10, 11) and mammalian lipoxygenases (12–14) are able to oxygenate not only free polyenoic fatty acids but also ester lipid substrates, such as phospholipids (15) and cholesterol esters (16) and even more complex lipid-protein assemblies such as biomembranes (17) and lipoproteins (18, 19). Although these experiments indicate the ability of certain LOXs to accept complex ester lipids as substrates, it is unclear whether such reactions actually occur in vivo. In mammals, the in vivo activity of 15-LOXs on membrane lipids was measured in rabbit reticulocytes (13, 20) as well as in atherosclerotic lesions of rabbits (21) and humans (22). We recently extended these findings to plants (23). Investigating the metabolism of storage lipids in germinating cucumber seedlings, we found that a special isoform of linoleate 13-LOX is synthesized at very early stages of germination, which translocates to the lipid storage organelles (lipid bodies) and oxygenates the storage triacylglycerols rather than free linoleic acid released by the action of a lipid-hydrolyzing enzyme. Moreover, we found large amounts of oxygenated free linoleic acid derivatives in the cytosol of seedlings, suggesting that these derivatives are preferentially cleaved from the lipid stores. Although the enzyme responsible for this release has not been characterized, these data suggest a mobilization of the storage lipids for β-oxidation by a specific isoform of linoleate 13-LOX (24) without initiation by a lipase.

For direct evidence on the in vivo activity of the lipid body LOX in cucumber seedlings, we analyzed the chemical structure of the oxygenated storage lipids occurring during germination and found that hydroperoxy derivatives of linoleate-containing triacylglycerols constitute the major oxygenated storage lipids. Since these compounds exhibit a high enantiomeric specificity, they are likely to be formed by the in vivo action of the lipid body LOX.

* Supported by Deutsche Forschungsgemeinschaft Grant SFB 363/C5 (to I. F. and C. W.) and by Deutsche Forschungsgemeinschaft Grant Ku 961/2–2 (to H. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This paper is dedicated to Prof. Dr. B. Parthier on the occasion of his 65th birthday.

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(Received for publication, April 29, 1997)
EXPERIMENTAL PROCEDURES

Materials—The chemicals used were from the following sources: standards of chiral and racemic hydroxy fatty acids from Chayman Chemicals; methanol, hexane, and 2-propanol (all HPLC grade) from Baker; 1,3-dilinolein and trilinolein from Sigma (Germany). Seeds of cucumber (Cucumis sativus L. cv. Chinesishe Schlange) and of sunflower (Helianthus annuus L. cv. Albena) were used for the experiments.

Plant Growth and Isolation of Lipid Bodies and LOX—Cotyledons of 10 dry seeds or of seedlings germinated for various time periods in the dark at 27 °C and 100% humidity were used. Lipid bodies were prepared by ultracentrifugation (25), and its LOX was purified to apparent homogeneity by selective extraction with Brij 99 (26).

Oxygenation of Linoleic Acid-containing Neutral Lipids by the Lipid Body LOX—In vitro oxygenation of 1,3-dilinolein and trilinolein was carried out by adding 100 μg of the purified cucumber lipid body LOX with these substrates (800 μg final concentration) in 0.2 ml sodium borate buffer (pH 8.5) for 45 min at room temperature. The substrates were added as aqueous stock suspensions (20 μl), which were prepared by emulsifying dilinolein and trilinolein in 2.5 ml of Tris- HCl buffer (pH 7.5) containing 15% (w/v) arabic gum with a tip sonifier (27). The reaction products were extracted with three volumes of a chloroform/methanol mixture (2:1, by volume). After recovery of the organic phase, solvents were evaporated under vacuum, and the lipids were reconstituted in 0.1 ml of HPLC solvent A (see below).

Lipid Extraction from Cucumber Seedlings and Sample Workup—The isolated lipid bodies were resuspended in 0.1 M phosphate buffer, pH 7.4, and the suspension was sonicated to achieve a homogeneous dispersion. After acidification to pH 3, the lipids were extracted. After removal of the acidic phase, the neutral phase was evaporated, and the lipids were reconstituted in chloroform. Aliquots were treated with triphenylphosphine to reduce hydroperoxide compounds to their corresponding hydroxy derivatives. These aliquots were diluted with methanol to a final volume of 0.4 ml. Then 0.08 ml of 40% (w/v) KOH was added, and the samples were hydrolyzed under argon atmosphere for 20 min at 60 °C. After cooling down to room temperature, the samples were extracted with chloroform/methanol mixture (2:1, by volume). After recovery of the organic phase, solvents were evaporated under vacuum, and the lipids were reconstituted in 0.1 ml of HPLC solvent A (see below).

Analysis—HPLC analysis was carried out on a Shimadzu HPLC system or on a Beckman HPLC system, both of them coupled to a diode array detector. RP-HPLC of the free fatty acid derivatives was carried out on a Nucleosil C-18 column (Macherey-Nagel, KS system, 250 × 4 mm, 5-μm particle size) with a solvent system of methanol/water/acetic acid (85/15/0.1 by volume) and a flow rate of 1 ml/min. For calculation of the hydroxylinoleic acid:linoleic acid ratio, the absorbances at 234 nm were recorded. For detection of hydroperoxy derivatives, these aliquots were diluted with methanol to a final volume of 0.4 ml. Then 0.08 ml of 40% (w/v) KOH was added, and the samples were hydrolyzed under argon atmosphere for 20 min at 60 °C. After cooling down to room temperature, the samples were extracted with chloroform/methanol mixture (2:1, by volume). After recovery of the organic phase, solvents were evaporated under vacuum, and the lipids were reconstituted in chloroform. Aliquots were treated with triphenylphosphine to reduce hydroperoxide compounds to their corresponding hydroxy derivatives. These aliquots were diluted with methanol to a final volume of 0.4 ml. Then 0.08 ml of 40% (w/v) KOH was added, and the samples were hydrolyzed under argon atmosphere for 20 min at 60 °C. After cooling down to room temperature, the samples were extracted with chloroform/methanol mixture (2:1, by volume). After recovery of the organic phase, solvents were evaporated under vacuum, and the lipids were reconstituted in 0.1 ml of HPLC solvent A (see below).

Acidic Lipid Extraction from Cucumber Seedlings and Sample Workup—The isolated lipid bodies were resuspended in 0.1 M phosphate buffer, pH 7.4, and the suspension was sonicated to achieve a homogeneous dispersion. After acidification to pH 3, the lipids were extracted. After removal of the acidic phase, the neutral phase was evaporated, and the lipids were reconstituted in chloroform. Aliquots were treated with triphenylphosphine to reduce hydroperoxide compounds to their corresponding hydroxy derivatives. These aliquots were diluted with methanol to a final volume of 0.4 ml. Then 0.08 ml of 40% (w/v) KOH was added, and the samples were hydrolyzed under argon atmosphere for 20 min at 60 °C. After cooling down to room temperature, the samples were extracted with chloroform/methanol mixture (2:1, by volume). After recovery of the organic phase, solvents were evaporated under vacuum, and the lipids were reconstituted in 0.1 ml of HPLC solvent A (see below).

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Structural Elucidation of the Oxygenated Storage Lipids—In vivo formation of oxygenated storage lipids during germination of cucumber seedlings described recently (23).

In Vitro Oxygenation of Linoleic Acid-containing Neutral Lipids by the Cucumber Lipid Body LOX—The lipid bodies of cucumber seedlings contain specifically oxygenated ester lipids, and preliminary structural analysis of these compounds suggested that they may be formed in vivo by the lipid body LOX (23). To find out whether this enzyme is capable of oxygenating neutral ester lipids, it was purified to apparent electrophoretic homogeneity from the lipid bodies of cucumber seedlings and was incubated with 1,3-dilinolein and with trilinolein for 25 min at room temperature, and the lipids were analyzed by RP-HPLC for the occurrence of oxygenated derivatives. From Fig. 1 (upper trace), it can be seen that two major products (I and II), both of which contain the characteristic conjugated diene chromophore (insets), were formed during the oxygenation of 1,3-dilinolein. Both compounds were prepared, reduced with triphenylphosphine, and hydrolyzed under alkaline conditions. RP-HPLC analysis of the hydrolysis mixture indicated that product II contained equimolar amounts of linoleic acid and 13-HODE. The chemical structure of both linoleic acid and 13-HODE was confirmed by co-chromatography with authentic standards in RP- and SP-HPLC as well as by GC/MS (data not shown). These data suggested that product II may constitute a mono-oxygenated dilinolein derivative. Analyzing the hydrolysis mixture of product I by HPLC, we mainly detected 13-HODE; only trace amounts of linoleic acid were detected, suggesting that product I was a dilinolein derivative containing two 13-HODE residues. The lower trace in Fig. 1 shows an HPLC analysis of the oxygenation products formed during the reaction of the lipid body LOX with trilinolein. Three major products (A, B, and C), each of them containing a conjugated diene chromophore, were observed. HPLC analysis of their hydrolysis products (data not shown) suggested that product C was a mono-oxygenated trilinolein, product B a double-oxygenated trilinolein, and product A a triply oxygenated trilinolein.

These in vivo data indicate that the lipid body LOX is capable of oxygenating di- and triacylglycerols and thus may be responsible for the in vivo formation of oxygenated storage lipids during germination of cucumber seedlings described recently (23).

Structural Elucidation of the Oxygenated Storage Lipids—In a lipid extract of 4-day-old cucumber seedlings, a large amount of 13S-HODE was found when the lipids were extracted under reducing conditions and when the extracts were hydrolyzed prior to RP-HPLC analysis (23). For more detailed structural analysis of the oxygenation products, we separated components of lipid extracts obtained from 4-day-old cucumber cotyledons under nonreducing conditions and without previous hydrolysis. To detect the mono lipids, the absorbance at 234 nm and the peroxide-induced luminescent chemiluminescence were recorded simultaneously. In Fig. 2, a representative chromatogram of such an analysis is shown. Five major products, some of which appear to be heterogeneous, can be separated. Product 1 co-migrated with triple-oxygenated trilinolein (see above). Products 2 and 4 co-eluted with double-oxygenated and mono-oxy-
generated trilinolein, respectively. Interestingly, all major compounds detected at 234 nm appeared in the chemiluminescence assay, indicating that all fractions contain hydroperoxy lipids. However, exact quantification of the hydroperoxide content by chemiluminescence was problematic under our assay conditions. Therefore, we determined the hydroperoxide:hydroxide ratio by ¹H NMR measurements (see below).

For further structural information, the oxygenation products 1–5 were isolated by RP-HPLC, reduced with triphenylphosphine, and hydrolyzed under alkaline conditions. The resulting free oxygenated fatty acid derivatives containing the conjugated diene chromophore were isolated by RP-HPLC and were further analyzed by SP- and CP-HPLC as well as by GC/MS. For product 1, which co-migrated with the triple-oxygenated trilinolein in RP-HPLC, 13-HODE was detected as the major product in the hydrolysis mixture; only trace amounts of linoleic acid and other fatty acids were found (Table I). The lack of linoleic acid and oleic acid was also indicated by the absence of olefinic protons in ¹H NMR. These data suggest that product 1 may constitute a triacylglycerol containing three HODE residues. In HPLC analysis of the hydrolysis mixture of product 2,
substantial amounts of linoleic acid were detected (linoleic acid:HODE ratio of 1:1.7). This value and the fact that product 2 co-chromatographed with a double-oxygenated trilinolein suggested that product 2 constitutes a triacylglycerol containing two HODE residues and one nonoxygenated linoleic acid. For product 3, which migrated close to the double-oxygenated trilinolein in RP-HPLC, a strong preponderance of HODE over linoleic acid was found in the hydrolysis mixture (Table I). However, in GC/MS large amounts of oleic acid, stearic acid, and myristic acid were detected. These analytical data suggest that product 3 may constitute a triacylglycerol containing two HODE residues and one other fatty acid. For product 4, which co-chromatographed with a standard of mono-oxygenated trilinolein, a linoleic acid:HODE ratio of about 1:0.4 was calculated. In GC/MS, no significant amounts of other fatty acids were detected. These data are consistent with a chemical structure of product 4 as triacylglycerol containing one HODE and two nonoxygenated linoleic acid residues. For product 5, a linoleic acid:HODE ratio of about 1:0.6 was analyzed. In addition, substantial amounts of other fatty acids such as oleic acid, stearic acid, and myristic acid were detected in the hydrolysis mixture. These data suggest that product 5 may constitute a mixture of triacylglycerols that contain one HODE residue and two other fatty acids. It should be mentioned that product 5 showed considerable heterogeneity when the lipid extracts of various preparations were compared.

To determine the mechanism of biosynthesis of the oxygenated storage lipids, more detailed structural information is necessary. For separation of the positional and geometric HODE isomers, the hydrolysis mixtures of triphenylphosphine-treated compounds 1–5 were analyzed by SP-HPLC. In Fig. 3, a representative chromatogram of such an analysis is shown for product 4. 13-HODE(Z,E) was the major oxygenated linoleic acid isomer. In addition, only small amounts of 9-HODE(E,E) and of the corresponding all-E isomers were detected. Recording the absorbance at 270 nm (data not shown), keto linoleic acid:linoleic acid ratio of about 1:0.6 was analyzed. In addition, substantial amounts of other fatty acids such as oleic acid, stearic acid, and myristic acid were detected in the hydrolysis mixture. These data suggest that product 5 may constitute a mixture of triacylglycerols that contain one HODE residue and two other fatty acids. It should be mentioned that product 5 showed considerable heterogeneity when the lipid extracts of various preparations were compared.

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**Fig. 3.** SP-HPLC analysis of product 4 after reduction and alkaline hydrolysis. Product 4 was prepared from the lipid extracts of 4-day-old etiolated cucumber cotyledons by RP-HPLC (see Fig. 2). The hydroperoxy lipids were reduced and hydrolyzed under alkaline conditions (see “Experimental Procedures”). The hydroxy fatty acids were prepared by RP-HPLC and further analyzed by SP-HPLC on a Zorbax SIL column (DuPont, 250 × 4.6 mm, 5-μm particle size) with a solvent system of hexane/2-propanol/acetic acid (100/20/0.1 by volume) and a flow rate of 1 ml/min. The absorbance at 234 nm was recorded. Inset, CP-HPLC analysis of the enantiomer composition of 13-HPODE carried out on a Chiralcel OD column (Daicel Chemical Industries, 250 × 4.6 mm, 5-μm particle size), with a mobile phase of hexane/2-propanol/acetic acid (95/5/0.1 by volume) and a flow rate of 1 ml/min. 13-KODE, 13-keto-9(Z),(11)E-octadecadienoic acid.

**Fig. 4.** Low field region of 1H NMR spectra of oxygenated storage lipids and of authentic 13S-HPODE and 13S-HODE. Products 1–4 were prepared by RP-HPLC as described in the legend to Fig. 1.
acid isomers (13-KODE) were also detected; they accounted for about 7% of the oxygenated linoleic acid derivatives. The structure of all products was confirmed by UV spectroscopy and GC/MS of the native compounds and of their hydrogenated derivatives (data not shown). Similar results were obtained for all oxygenated storage lipids (products 1–5). Analysis of the enantiomer composition of 13-HODE/Z,E by chiral phase HPLC (inset) indicated a strong preponderance of the S-isomer over the corresponding R-isomer (Table I). Taken together, the analytic data indicate a remarkably high specificity of the biosynthesis of the oxygenated storage lipids, suggesting that they are formed by the in vivo action of a LOX.

Table II

| Position | 1H Chemical shift | 1H Multiplicity | 1H Coupling constant | 13C Chemical shift | 13C Coupling constant |
|---------|------------------|-----------------|----------------------|------------------|----------------------|
| 9       | 5.443            | dt              | 10.8/7.8             | 132.8            |
| 10      | 5.977            | dd              | 11.1/10.8            | 127.9            |
| 11      | 6.504            | dd              | 15.2/11.1            | 125.8            |
| 12      | 5.670            | dd              | 15.2/6.6             | 135.8            |
| 13      | 4.193            | dt              | 6.6/6.6              | 72.8             |

Quantification of the Peroxide Content in the Storage Lipids—The high product specificity detected implicated an involvement of a LOX in the biosynthesis of the oxygenated storage lipids. Although the chemiluminescence measurements suggested the in vivo formation of hydroperoxy lipids (Fig. 2, lower trace), there was no direct proof for the assumption that the majority of the oxygenated storage lipids constituted specific hydroperoxy derivatives and thus may originate from a LOX pathway. To test this hypothesis, we calculated the hydroperoxy:hydroxy lipid ratio by 1H NMR measurements. This method takes advantage of the different chemical shifts of the dienoic protons. In Fig. 4 (lower traces), the low field regions of the 1H spectra of 13-HODE and 13-HPODE are shown, and in Table II selected 1H and 13C NMR data are summarized. All low field signals were unambiguously assigned, based on the connectivities of the 1H,1H COSY two-dimensional spectra (data not shown). The assignment of the corresponding 13C signals followed from the proton-detected one-bond 1H,13C chemical shift-correlated two-dimensional NMR experiments (HMOC). Earlier published assignments of C-11 and C-12 of 13-HODE (29) have to be interchanged.

The olefinic protons of 13-HODE and 13-HPODE show distinct differences in chemical shifts (H-12, δ 0.10; H-11, δ 0.06). The integrals of the H-11 signals were chosen for calculation of the hydroperoxide:hydroxide ratio because these signals were not superimposed with other 1H signals, as H-9 and H-10 were. From the 1H NMR spectra of the four major oxygenated triacylglycerols (Fig. 4), it can be concluded that in all fractions of the storage lipids was also proved by the HMOC spectra. In Fig. 5, a comparison of the low field region of the one-bond 1H,13C shift correlation two-dimensional NMR spectrum (HMOC) of product 2 and the relevant parts of the HMOC spectra of 13-HPODE and 13-HODE (insets) are shown. The 13C chemical shift of C-13 (δ 86.8) indicates 13-HPODE to be the main lipid oxygenation product.

Time Course of the in Vivo Formation of Oxygenated Ester Lipids during Germination—The steady state concentration of LOX products in the storage lipids did increase in a time-dependent manner during germination (Fig. 6). During onset of germination (12 h), small amounts of mono-oxygenated triacylglycerols (products 4 and 5) were detected. At later stages (48 h), the oxygenated lipids were augmented, and the share of double and triple oxygenation products was increased. After 72 h of germination, we observed a decline of the oxygenation products, suggesting an exhaustion of the storage lipid (not shown).

Oxygenated Triacylglycerols in Other Plants—To determine whether the oxygenation of storage lipids during early stages of germination is restricted to cucumber, we analyzed lipid extracts of lipid bodies of 4-day-old sunflower seedlings for the occurrence of oxygenated fatty acids by NMR (Fig. 7). Large amounts of hydroperoxy and hydroxy fatty acids could be detected. Comparison of the amounts of oxygenated lipids present in the storage lipids of cotyledons of cucumber and sunflowers at day 4 of germination indicated a 3-fold higher concentration in cucumber seedlings. Moreover, in sunflowers we observed an

FIG. 5. Low field region of the one-bond 1H,13C shift correlation two-dimensional NMR spectrum (HMOC) of fraction 2. Comparison with the relevant parts of the HMOC spectra of 13-HPODE and 13-HODE (insets) as well as the 1H chemical shift of C-13 (δ 86.8) indicate 13-HPODE to be the main lipid oxygenation product.

TABLE II

Selected date of 1H and 13C NMR of authentic standards of 13-HODE and 13-HPODE

The solvent was CDCl3 in 13C measurements the chemical shifts (ppm) of cross peaks in HMOC spectra are given.
almost equal distribution (1:1.26) of hydroperoxy and hydroxy lipids, suggesting that the peroxide-reducing capacity is much higher in sunflowers. These data indicate similarities in the mechanism of lipid mobilization of cucumber and sunflowers.

**DISCUSSION**

Mobilization of storage lipids that are utilized as carbon and energy source in growing seedlings is an essential process in germination of cucumber and of various oil seed plants. Unfortunately, the mechanism of this process is far from clear. For a long time, it has been assumed that lipid mobilization is initiated by the liberation of free fatty acids from the storage lipids. The free fatty acids are subsequently transported to peroxisomes and there may undergo β-oxidation. However, a lipase activity responsible for fatty acid liberation from the lipid storage organelles has not been detected in cucumber. A specific lipid body LOX induced during early stages of germination was recently reported (23). It appears to translocate to the lipid storage organelles to oxygenate the storage lipids. This oxygenation was recently suggested to be the initiation of the mobilizing cascade of the storage lipids (24). In addition to the lipid body LOX, this metabolizing cascade should also involve a lipid hydrolyzing enzyme, most probably a triacylglycerol lipase. This enzyme may cleave preferentially oxygenated triacylglycerols. Although this enzyme has not been characterized with respect to its protein chemical and enzymatic properties, there is experimental evidence for its existence. (i) Analysis of the amount and composition of free fatty acids in the seedlings’ cytosol indicated large amounts of 13S-HODE but almost no linoleic acid. (ii) In vitro experiments indicated that, from isolated lipid bodies, only 13S-HODE is liberated into the surrounding medium. Although these preliminary data suggest the existence of a lipase that preferentially cleaves oxidized storage lipids, there is no direct proof for the in vivo activity of such an enzyme. Recently, lipases that appear to prefer oxygenated ester lipids over nonoxygenated lipids have been described for microsomal membranes of various plants (30).

Our NMR data indicate that the majority of the oxygenated storage lipids are present in cucumber seedlings as 13-HPODE derivatives. Together with the enantiomer composition, these data strongly suggest a storage lipid oxygenation by a LOX, whereas other enzymatic and/or nonenzymatic oxygenating pathways are highly improbable. However, a specific product pattern alone does not prove the in vivo action of a LOX. Recently, it was observed that myoglobin in the presence of hydrogen peroxide is capable of oxygenating linoleic acid preferentially to 9S-HPODE (31), and it may be possible that still unknown enzymes or nonenzymatic catalysts are also capable of producing a specific product pattern. However, since the formation of specifically oxygenated storage lipids in cucumber seedlings is paralleled by the expression of the lipid body LOX, which is capable of oxygenating triacylglycerols in vitro, it appears reasonable to assume a causal relation between LOX expression and storage lipid oxygenation.

The accumulation of hydroperoxy lipids within the lipid bodies of germinating cucumber seedlings is quite remarkable. Rough estimates showed that about 15% of polyunsaturated fatty acids in the storage lipids at day 4 of germination are present as hydroperoxy derivatives, and thus the lipid storage organelles are characterized by a high oxidizing potential. In contrast, in intact mammalian cells hydroperoxy lipids can hardly be detected, even in cells in which LOXs are expressed at high levels (20, 32). This leads to the conclusion that the reducing capacity of animal cells, which is mainly due to the catalytic activity of glutathione peroxidases (33, 34), is much lower.
higher than that of the seedlings. Nevertheless, it is quite surprising that there is no obvious damage in seedlings by such high peroxide concentrations. It may be possible that the peroxides are kept inside the lipid bodies and thus may not interfere with other metabolic pathways for which peroxides may be toxic.

The sequence of metabolic events involved in the breakdown cascade of storage lipids following LOX-catalyzed oxygenation remains to be investigated. Looking at the metabolic flux of peroxisomal β-oxidation, it appears to be necessary that lipid peroxides are reduced before entering the β-oxidation cascade. In principle, there are two different ways for oxygenated lipids to enter β-oxidation. (i) The hydroperoxy triacylglycerols are reduced to the corresponding hydroxy derivatives from which hydroxy fatty acids may be subsequently liberated. These hydroxy fatty acids are then transported to the peroxisomes and may undergo β-oxidation. This order of events accords with the fact that mainly hydroxy fatty acids were detected in the cytosol and in our in vitro liberation assays (23, 24). (ii) The hydroperoxy triacylglycerols formed by the LOX are directly cleaved, and the free hydroperoxy fatty acids are reduced in the cytosol to the corresponding hydroxy compounds. Several metabolic routes including the peroxidase activity of LOX (35) and the peroxynitrite pathway (36) have been shown to reduce peroxo fatty acids in plants. Most of these pathways require a reductant and lead to the formation of other secondary products like epoxy hydroxy compounds, keto dienes, α-ketols, etc. In addition, one should consider the possibility that “glutathione peroxidase-like pathways” that reduce peroxo fatty acids directly to the hydroxy compounds may be involved (37).

Although most of the experiments reported here were carried out with cucumber seedlings, there are reports on the occurrence of a special lipid body LOX in other plants (25, 38, 39). We have detected large amounts of oxygenated storage lipids in sunflower and soybean, suggesting that the mechanisms involved in lipid mobilization during cucumber germination may also occur in other plants. Thus, the concept of implicating a special LOX in the mobilization cascade of storage lipids may be of a general pathway for plants utilizing lipids as a major carbon and energy source for seedling growth. Inhibitor studies and/or experiments with transgenic plants in which the lipid body LOX gene is specifically knocked out remain to be carried out in the future to verify this concept.

Acknowledgment—We thank Dr. S. Rosahl for critical reading of the manuscript.

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