Lipoxygenase Forms Located at the Plant Plasma Membrane

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In cucumber cotyledons (*Cucumis sativus* L.) containing several soluble and particulate forms of lipoxygenase (LOX), the location of LOX forms in microsomes has been studied. We concentrated on the question whether the plasma membrane contains one or more forms of LOX. As methodology, we applied both the two-phase partition with polyethylene glycol/dextran and density gradient flotation of plasma membrane-enriched membrane fractions. Both methods show that a high percentage of the microsomal LOX can be attributed to the plasma membrane. Emphasis was put on the findings that the LOX located at the plasma membrane consisted of a species behaving as an integral membrane protein and another form characterized as a peripheral membrane protein by solubilization with carbonate. With long distance SDS-PAGE and immunodecoration using anti-lipid body LOX antiserum, it is possible to distinguish between microsomal LOX forms by small but significant differences in size. Treatment of seedlings with methyl jasmonate led to an enhanced level of LOX at the plasma membrane.

Introduction

Lipoxygenases (Gardner, 1991; Siedow, 1991; Vick and Zimmerman, 1987) represent a group of O_2-dependent enzymes catalyzing the regioselective dioxygenase reaction with 1,4-pentadiene cis-polyunsaturated fatty acids. Plant lipoxygenases (LOX) act on linoleate and catalyze the formation of either 9- or 13-hydroperoxide C_{18}-derivatives (Siedow, 1991). Subsequent enzymatic conversions by allene oxide synthase (Song *et al.,* 1993; Song *et al.,* 1993) and allene oxide cyclase (Gardner, 1991) lead to phytodienoic acid and, after chain shortening, to the hormone-like C_{12}-derivatives of jasmonic acid (Sembdner and Parthier,1993). An involvement of lipoxygenase in pathogen defense mechanisms has been postulated (Gardner, 1991; Hildebrand, 1989), and an elicitor-induced LOX has been found among the LOX genes in soybean and *Arabidopsis thaliana* (Bell and Mullet, 1991; Bell and Mullet, 1993). A soluble type-2 LOX was characterized as elicitor-induced enzyme in tobacco cell suspension culture (Fournier *et al.,* 1993).

While a considerable amount of data has been obtained by studying the soluble lipoxygenase forms little is known about the localization of LOX in organelles and particularly on membranes. Recently, new forms of lipoxygenase have been found in lipid bodies from cotyledons (Feussner and Kindl, 1992; Radetzky *et al.,* 1993; Feussner and Kindl, 1994) and in paraveinal mesophyll cell vacuoles of green leaves (Grayburn *et al.,* 1991; Tranbarger *et al.,* 1991). In the latter case, lipoxygenase is regarded as vegetative storage protein thought to be involved in the temporary storage of nitrogen. Membrane-associated forms of LOX have been studied in tomato fruits (Droillard *et al.,* 1993).

To investigate a potential function of LOX in a signal transduction chain originating from the plasma membrane and leading to the site of jasmonate synthesis we subjected the plasma membrane as potential site of LOX activity to a thorough investigation. The present paper deals with LOX and its intracellular location with emphasis on plasma membrane isoenzymes. The investigations revealed the presence of LOX forms in plasma membrane both as peripheral and integral membrane proteins.

Abbreviations: ER, endoplasmic reticulum; LOX, lipoxygenase.

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Materials and Methods

Plant material and isolation of crude organelle fractions

Cucumber (*Cucumis sativus*, Chinesische Schlangengurken) seedlings were grown in the dark for 4 days at 26 °C. Preparation and subfractionation of crude homogenates from cotyledons were carried out as described by Kirsch *et al.* (1989). The enrichment and separation of large organelles and microsomes were performed by differential centrifugation at 2000×*g*, 1000×*g* and 100,000×*g* according to Sturm and Kindl (1983). Separation into an ER-containing light microsomal fraction and a plasma membrane-containing heavy microsomal fraction was carried out by layering a suspension with crude microsomes onto a sucrose cushion (5 ml) consisting of 31% (w/w) sucrose and by centrifuging the 36-ml tube for 4 h at 27,000 rpm in a Kontron rotor TST-28.3. The pellet designated *heavy microsomes* was used for the further purification of plasma membrane. The membranes banding at the top of the 31%-cushion designated as *light microsomes* represented the main portion of the ER.

Treatment of seedlings during germination with methyl jasmonate

Cucumber seedlings were grown in the dark for 4 days in three different TLC chambers (volume: 3 l). In chamber 1, 1.0 mg (-)-jasmonic acid methylester dissolved in 100 µl ethanol and spread on a filter paper was administered. Chamber 2 without these compounds and chamber 3 with 100 µl ethanol contained the control plants.

Purification of plasma membrane by two-phase partition

A suspension of the heavy microsome fraction was subjected to two-phase partition according to Larsson (1985) . A 27 g two-phase partition system consisting of dextran T500 and polyethylene glycol 4000 was used (Kirsch *et al.*, 1989).

Enrichment of plasma membrane and the ER by sucrose density gradient centrifugation

A density gradient ranging from 20–50% (w/w) sucrose was prepared in a 36-ml centrifuge tube in the following way: Onto a 1.5 ml-cushion of 50% (w/w) sucrose, the microsomal pellet resuspended in 44% (w/w) sucrose (1.5 ml) and 7 ml of 40% (w/w) sucrose were layered. On top of these layers, a linear gradient formed from 13 ml 40% (w/w) sucrose and 13 ml 20% (w/w) sucrose was loaded. All sucrose solutions were prepared in a buffer consisting of 50 mM Tris-HCl (pH 7.5), 10 mM KCl, 1.5 mM EDTA, and 0.1 mM $MgCl_2$. The centrifugation was at 100 000 x *g* (av.) for 87 h.

Analysis of membrane proteins

Peripheral membrane proteins were solubilized by treating the membranes with 100 mM sodium carbonate in a Potter homogenizer. Following standing for 30 min, the membranes were separated from the aqueous phase by centrifugation at 45 000 rpm (Beckman rotor SW60) for 60 min. From the membranes obtained in the pellet, LOX was solubilized by intensive mixing with 1% (v/v) Brij 99 in phosphate buffer saline (Feussner and Kindl, 1994).

Phase partition between a Triton X-114-rich phase and an aqueous phase was carried out as described earlier (Feussner and Kindl, 1994). Only proteins solubilized previously in Triton X-114 were subjected to the partition procedure.

Enzymatic tests and other assays

LOX enzyme activity (EC 1.13.11.12) was measured according to Axelrod *et al.* (1981). In the case of plasma membrane or heavy microsomes, tetcyclacis, 5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-[5.4.$10^{2,6}$.$0^{8,11}$]-dodeca-3,9-diene was administered (Rademacher *et al.*, 1987) to control the extent to which consecutive reactions of the allene oxide synthase type interfere with the LOX test. Tetcyclacis was a gift of C. Wasternack (Halle).

Marker enzymes were determined by established methods: ATPase (EC 3.6.1.3), vanadate-sensitive ATPase, azide-sensitive ATPase, and nitrate-sensitive ATPase (LeBel *et al.*, 1978; Kirsch *et al.*, 1989), galactosyl transferase (EC 2.4.1.46) (Kirsch *et al.*, 1989; Larsson, 1985), glucan synthase II (EC 2.4.1.34) (Larsson, 1985); NADH: cytochrome reductase (EC 1.6.2.2) (Postius and Kindl, 1978; Sturm and Kindl, 1983). The protein content was estimated by the procedures described by Lowry *et al.* (1951). Chromatofocussing

was done as reported earlier (Feussner and Kindl, 1994). Electrophoresis (SDS-PAGE) was carried out on 30 cm long gels using a modified Laemmli system (Laemmli, 1970), with 4 M urea in the stacking and separating gels. Lipoxygenases on Western blots were detected as described in detail (Feussner and Kindl, 1994).

Results

Isolation of plasma membrane and localization of LOX within the fraction of heavy microsomes

The concept of confining a well known and intracellularly almost ubiquitous enzyme activity to a membrane depends mainly on the relyable purification of the particular membrane. By focusing on the potential contaminations and the specific enzyme activities of markers, we built the necessary platform to have the properties of this LOX form under scrutiny. The enrichment or isolation of plasma membrane can be achieved by two different procedures. Procedure A is based on the behaviour of plasma membrane to go into the upper phase of a two-phase partition developed by Larsson (1985). Procedure B, a sucrose density gradient flotation (Sturm and Kindl, 1983), depends on the equilibrium density of plasma membrane which is sufficiently distinct from the densities of other microsomal membranes. In both cases, it was found by preliminary studies that a preceding subfractionation of the microsomes is highly advantageous. To this end, a microsomal pellet was resuspended and loaded onto a cushion consisting of 31% sucrose. A subsequent centrifugation led (a) to a membrane fraction on top of the cushion and representing mainly the endoplasmic reticulum (light microsomes), and (b) to a pellet with membranes of densities higher than 32% sucrose (heavy microsomes).

Starting out with *heavy microsomes*, we subjected the membranes to the two-phase partition with dextran as lower phase and polyethylene glycol as upper phase. Table I summarizes the data on LOX activity and the controls using marker enzymes for membranes potentially contaminating the plasma membrane preparation. Crude microsomes are known to contain cytosolic contaminations and also LOX forms not attributable to plasma membrane. Therefore, the best proof of plasma membrane located LOX is the increase of specific enzyme activity during the step leading from heavy microsomes to plasma membrane, *i. e.* the increase from 29 to 37 nkat/mg protein. During the same operation, the specific activities of vanadate-sensitive ATPase increased from 821 to 5263 nkat/mg protein while the one of the endoplasmic reticulum (ER) marker decreased from 5.4 to 1 nkat/mg protein. Glucan synthase II increased in its specific activity during the preparation of plasma membrane. The activity of nitrate-sensitive ATPase was below the sensitivity of the assay already after the separation of light mirosomes from heavy microsomes. Nitrate-sensitive ATPase was fully recovered in the light microsomes and banded, upon densitiy gradient centrifugation, at a density corresponding to 21% (w/w) sucrose (data not shown).

Utilizing long distance SDS-PAGE for detecting small differences in molecular mass of LOX forms, we were able to compare rather exactly the sizes of LOX forms appearing during subfractionation of microsomes. Fig. 1 shows that both the preparation of heavy microsomes and the preparation of the upper phase in two-phase partition resulted in the removal of a 94 kDa LOX form. For this and later comparisons, the position of soluble soybean LOX was set as 95 kDa (Fig. 1, lane 7). The purified plasma membrane contained exclusively the 98 kDa form. The molecular mass of the smaller light microsomal LOX form corresponded

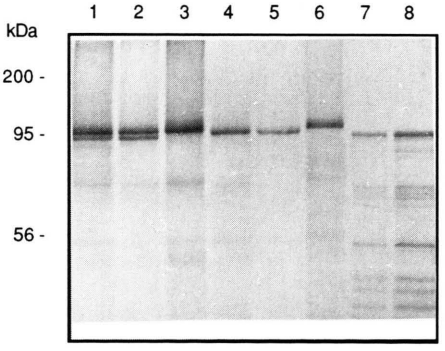

Fig. 1. Analysis of lipoxygenase (LOX) forms obtained during the preparation of plasma membrane by two-phase partition. Lane 1: crude microsomes; lane 2: light microsomes; lane 3: heavy microsomes; lane 4: two-phase parition, lower phase; lane 5: two-phase parition, lower phase (after repeating the partition of the first lower phase); lane 6: two-phase partition, upper phase (plasma membrane); lane 7: soybean LOX, 0.5 μg; lane 8: soybean LOX, 2.5 μg.

Table I. Survey of enrichments and contaminations determined by using marker enzymes of various organelles. The data were taken from a preparation using 45 g cotyledons.

Marker enzyme and proteins	Enzyme activities Crude microsomes	Heavy microsomes	Plasma membrane
Lipoxygenase	21,000 nkat	816 nkat	71 nkat
LOX (spec. act.)	68 nkat/mg	29 nkat/mg	37 nkat/mg
ATPase (total)	108,900 nkat	98,900 nkat	10,160 nkat
Vanadate-sensitive ATPase	28,000 nkat	23,000 nkat	10,000 nkat
Nitrate-sensitive ATPase	18,000 nkat	<50 nkat	<50 nkat
Azide-sensitive ATPase	10,000 nkat	2,600 nkat	100 nkat
NADH-cyt c reductase	3,600 nkat	150 nkat	2 nkat
Glucan synthase II	19,500 nkat	16,600 nkat	15,000 nkat
Galactosyl transferase	100 nkat	13 nkat	<1 nkat
Protein	307 mg	28 mg	1.9 mg

to the size of cytosolic LOX form from soybean seeds. It is evident that two-phase partition separates the 98 kDa form (upper phase) from the 94 kDa form (lower phase).

Applying the method of flotation in a density gradient, we prepared plasma membrane and the ER in highly enriched form. Unlike the two-phase partition, this procedure does not yield plasma membrane in the highest purified form, but it allows to prepare the organelles in a form suitable for direct comparison of yields. First, the *heavy microsomes* were separated. The plasma membrane was localized using vanadate-sensitive ATPase as marker (Fig. 2). Peaks of vanadate-sensitive ATPase are seen in fractions 47/49, fractions 41/43, and in the range around fractions 27–31. The latter fractions contain the plasma membrane as evidenced by analysis based on two-phase partition (data not shown). Fractions 47/49 correlate with the zone where the membrane suspension was applied to the gradient (see arrow). Second, the *light microsomes* were fractionated in the same form. NADH:cytochrome c reductase used as ER marker floted up to densities corresponding to

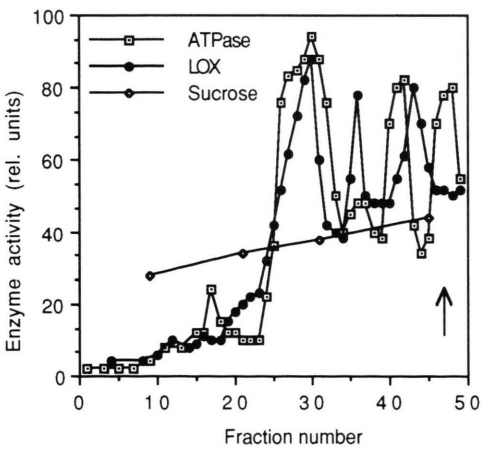

Fig. 2. Subfractionation of the heavy microsome fraction by sucrose density gradient centrifugation. 100 relative units correspond to 34 nkat (LOX) or 5 μkat (vanadate-sensitive ATPase). 40 relative units are equivalent to a sucrose density of 40% (w/w). The arrow indicates the position where the membrane suspension was applied to the gradient.

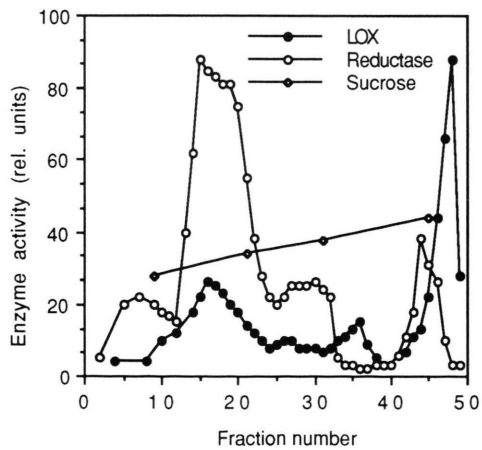

Fig. 3. Subfractionation of the light microsome fraction by sucrose density gradient centrifugation. 100 relative units correspond to 34 nkat (LOX) or 5 nkat (NADH:cytochrome reductase). 40 relative units are equivalent to a sucrose density of 40% (w/w).

29% sucrose (Fig. 3). We infer from the data that the ER houses the main portion of LOX attributed to the light microsomal fraction. However, the amount of LOX at the ER was not as high as the one at the plasma membrane.

When heavy microsomes used immediatly after preparation, or heavy microsomes used after stripping off the peripheral proteins, were assayed for LOX activity in the absence or presence of 10 – 100 μM tetcyclacis, an inhibitor of allene oxide synthase, very small but significant differences were observed. In the presence of the inhibitor, the amount of conjugated diene structure was approximately 5 – 8% higher than in the standard asssay. This indicates that the occurrence of a minor amount of allene oxide synthase at the membranes has to be implicated.

Demonstration of two distinct forms of plasma membrane LOX

To investigate the concept that one of the lipoxygenases in the plasma membrane may be an integral membrane protein responsible for signal transduction while another form may be destined for the extracellular compartment, we scrutinized by more than one method whether LOX forms occur distinct in the mode of membrane binding. Applying the partition within Triton X-114 phase and water for separating the integral proteins, we found that a high percentage of LOX behaved like an integral membrane protein (Fig. 4). An independent method for isolating peripheral membrane proteins is by removing them from the membrane by carbonate washings. Fig. 5 presents the analyses on Western blots of the respective subfractions. It provides additional evidence that light microsomes and heavy microsomes indeed contain different LOX proteins.

LOX forms found in the fraction of peripheral proteins obtained after carbonate washings showed, upon isoelectric focussing, a pI of 4.1 and 4.6. Both forms exhibited highest enzyme activities at pH values of 5.

The integral LOX form was tested for its substrate specificity. When identical amounts of membrane suspensions were assayed using as substrates linolate and arachidonate (50 μM each), respectively, the ratio of enzyme activities was 1.0 to 1.6.

Methyl jasmonate treatment results in an increase of plasma membrane LOX

As an increasing number of reports exists that methyl jasmonate activates the expression of LOX genes we investigated the level of LOX proteins under these environmental conditions. Such approaches are necessary as long as we are unable to attribute genomic or cDNA sequences to distinctly located isoforms. As a first contribution to this goal, the affect of methyl jasmonate to the station-

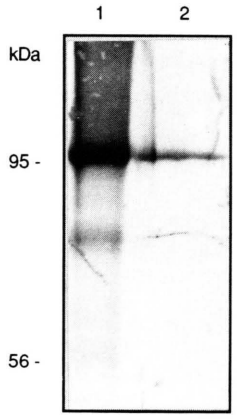

Fig. 4. Analysis of the LOX amount located in the plasma membrane fraction and then distributed between integral and peripheral proteins by two phase partion using Triton X-114. Lane 1: integral proteins located in the Triton X-114 phase; lane 2: peripheral proteins distributed in the aqueous phase.

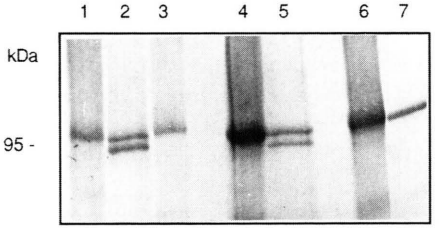

Fig. 5. Distribution of membrane-bound lipoxygenase (LOX) forms between the fractions of integral and peripheral proteins. As the fraction of peripheral membrane proteins, we defined the aqueous subfraction obtained by treating purified membranes intensively with 100 mM sodium carbonate. Lane 1: heavy microsomes; lane 2: light microsomes; lane 3: plasma membrane (prepared by two-phase partition); lane 4: light microsomes, integral proteins; lane 5: light microsomes, peripheral proteins; lane 6: plasma membrane, integral proteins; lane 7: plasma membrane, peripheral proteins.

ary concentrations of LOX at the plasma membrane was studied.

In three identical plasma membrane preparations containing virtually identical amounts of total protein, both the values of LOX amount assayed by Western blot (Fig. 6) and the values of LOX activity (data not shown) indicate a significant increase of LOX in the preparation from methyl jasmonate treated seedlings. Both the fraction of heavy microsomes consisting mainly of plasma membrane and the fraction of light microsomes contained enhanced levels of LOX in preparations after treatment with methyl jasmonate.

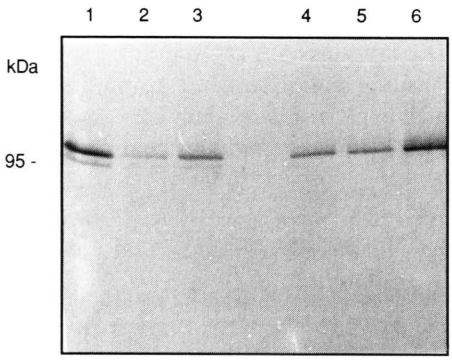

Fig. 6. Comparison of lipoxygenase (LOX) amounts in light microsomes and heavy microsomes upon treatment of seedlings with methyl jasmonate. Lane 1: light microsomes, after treatment with methyl jasmonate; lane 2: light microsomes, control; lane 3: light microsomes, untreated; lane 4: heavy microsomes, untreated; lane 5: heavy microsomes, control; lane 6: heavy microsomes, after treatment with methyl jasmonate.

Discussion

Various hypotheses implying a signal transduction chain originating from the border of the plant cell (Farmer and Ryan, 1992) and being based on hydrolytic activities or oxygen burst require plasma membrane located enzymes like LOX as a prerequisite (Ricker and Bostock, 1994). To contribute by clarifying the question whether there is indeed a plasma membrane located LOX we provide here multiple evidence for such a location. As the isolation of highly purified plasma membrane is much more crucial than the preparation of other organelles we need a number of investigations on this subject to get unequivocal acceptance of the presence and modulation of LOX at the plasma membrane.

Microsomal lipoxygenase was recently detected in senescing carnation flowers (Rouet-Mayer *et al.*, 1992) and characterized by a sharp pH-optimum of its activity at pH 6.1. In green tomato fruits, Droillard *et al.* (1993) identified a membrane-associated LOX. A polypeptide with an apparent molecular weight of 92 kDa was attributed to the tonoplast and to the plasma membrane. In contrast to the highly hydrophobic LOX in leukocytes and the plasma membrane LOX described here, the LOX from tomato fruits was characterized as membrane-associated protein, based on solubilization and phase separation in the Triton X-114 system. It was speculated that the more acidic pI of the microsomal LOX, compared to the cytosolic forms, is responsible for the binding of the enzyme to the membrane. Our data do not agree with such an interpretation. Furthermore, the clear distinction in molecular weight shown by improved SDS-PAGE suggests that in contrast to the smaller cytosolic forms (approximately 94 kDa) the plasma membrane LOX also differs in structure from the soluble counterparts. We put emphasis on the statement that plasma membrane LOX is structurally different from the main cytosolic LOX forms: both by molecular mass and by its behaviour upon two-phase partition with Triton X-114.

Further evidence for a LOX differing from other microsomal and cytosolic forms is provided by the pronounced selectivity towards arachidonic acid as substrate. In contrast to most other LOX forms, *e. g.* the one in tomato fruits (Regdel *et al.*, 1994), the plasma membrane LOX shows a clear preferrence for arachidonate as substrate. As arachidonate metabolism may be implicated in fungus-plant interaction, the capability of plasma membrane LOX to convert fungus-derived arachidonate is in agreement with a potential function of this LOX form in signal transduction.

The analysis of the microsomal fraction from cucumber cotyledons revealed that more than half of its LOX activity is confined to the heavy microsomal subfraction. This statement was corroborated when immunological assays were used to determine the amounts of LOX protein. Furthermore, improved purification of this subfraction by flotation of their membranes according to their equilibrium densities demonstrated that within the heavy microsomal fraction the plasma membrane comprised at least 60% of the total LOX amount.

Thus, proceeding from microsomes and following the LOX distribution step by step of the subfractionation, the plasma membrane represents the predominant site of LOX localization.

In contrast to the improved subfractionation by centrifugation, a method which does not lead to great losses of LOX, the procedure of two-phase partition may yield plasma membrane of even higher purification. However, the latter method results in lower yields and may therefore be very useful for unequivocal localization of an enzyme to the plasma membrane but is less useful for providing a clear balance sheet for purification steps. In these terms, we use the data with the plasma membrane purified by two-phase partition for clearly attributing LOX to the plasma membrane.

A great deal of evidence suggests that two forms of LOX are contained in the plasma membrane fraction, an integral form and a peripheral form. Aside from the stringent removal of peripheral proteins by carbonate washings, the two-phase partition based on Triton X-114 yielded hard evidence for the presence of an integral form of plasma membrane LOX. LOX enzyme activity and LOX protein amounts in the fraction of integral proteins likewise are predominant in the subfraction containing integral membrane proteins.

The profiles of marker enzymes clearly show that the contaminations of the plasma membrane prepared by two-phase partition were less than 5% concerning the ER and tonoplast. The specific enzyme activity of LOX and vanadate-sensitive ATPase are increasing during the last step, *i. e.* the treatment of heavy microsomes by two-phase partition, while the specific activity of NADH:cytochrome c reductase decreases significantly. Analyzing the LOX forms on Western blots during two-phase partition (Fig. 5) and comparing the lower phase to the plasma membrane-containing upper phase we obtained unequivocal evidence that a complete separation of the 94 kDa LOX form from the upper phase was achieved. Therefore, no light microsomal 94 kDa LOX nor any of the predominating 94 kDa cytosolic LOX forms (Feussner and Kindl, 1994) does contaminate the plasma membrane preparation. The clear evidence for a co-localization of plasma membrane marker and the 98 kDa LOX form behaving as integral membrane protein fully agrees with the data obtained with the centrifugation techniques.

Assaying LOX activity on membranes potentially containing an array of reactions, we had to consider consecutive steps causing a decrease in the amount of the hydroperoxide produced by the LOX reaction. As the spectroscopic test for LOX activity is based on the formation of a conjugated diene structure any reaction subsequently converting the diene product of LOX reaction to other products may cause that one observes values of diene products lower than actually corresponding to the LOX-dependent formation of the diene hydroperoxide. The consecutive step of LOX reaction at a microsomal membrane is most likely a reaction catalyzed by an allene oxide synthase (or allene oxide dehydrase). This enzyme, a cytochrome P450 protein, can selectively be inhibited by tetcyclacis. But as the presence of tetcyclacis in the enzyme test led only to a very small increase of the hydroperoxide concentration during the LOX test with membranes we infer that a consecutive step, most likely the conversion of diene hydroperoxide to allene oxide, contributed to the overall conversion to a rather low extent.

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Axelrod B., Cheesbrough T. M. and Laakso, S. (1981), Lipoxygenase from soybean. Meth. Enzymol. **71**, 441–451.

Bell E. and Mullet J. E. (1991), Lipoxygenase gene expression is modulated in plants by water deficit, wounding, and methyl jasmonate. Mol. Gen. Genet. **230**, 456–462.

Bell E. and Mullet J. E. (1993), Characterization of an *Arabidopsis* lipoxygenase gene responsive to methyl jasmonate and wounding. Plant Physiol. **103**, 1133–1137.

Droillard M. J., Rouet-Mayer M. A., Bureau J. M. and Lauriere C. (1993), Membrane-associated and soluble lipoxygenase isoforms in tomato pericarp – characterization and involvement in membrane alterations. Plant Physiol. **103**, 1211–1219.

Farmer E. E. and Ryan C. A. (1992), Octadecanoid-derived signals in plants. Trends Cell Biol. **2**, 236–241.

Feussner I. and Kindl H. (1992), A lipoxygenase is the main lipid body protein in cucumber and soybean cotyledons during the stage of triglyceride mobilization. FEBS Lett. **298**, 223–225.

Feussner I. and Kindl H. (1994), Particulate and soluble lipoxygenase isoenzymes. Comparison of molecular and enzymatic properties. Planta **194**, 22–28.

Fournier J., Pouenat M.-L., Rickauer M., Rabinovitch-Chable H., Rigaud M. and Esquerre-Tugaye M.-T. (1993), Purification and characterization of elicitor-induced lipoxygenase in tobacco cells. Plant J. **3**, 63–70.

Gardner H. W. (1991), Recent investigations into the lipoxygenase pathway of plants. Biochim. Biophys. Acta **1084**, 221–239.

Grayburn W. S., Schneider G. R., Hamiltonkemp T. R., Bookjans G., Ali K. and Hildebrand D. F. (1991), Soybean leaves contain multiple lipoxygenases. Plant Physiol. **95**, 1214–1218.

Hildebrand D. F. (1989), Lipoxygenases. Physiol. Plant. **76**, 249–253.

Kirsch T., Rojahn B. and Kindl H. (1989), Diphosphatase related to lipid metabolism and gluconeogenesis in cucumber cotyledons: Localization in plasma membrane and etioplasts but not in glyoxysomes. Z. Naturforsch. **44c**, 937–945.

Laemmli U.K. (1970), Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**, 680–685.

LeBel D., Poirier G. G. and Beaudoin A. R. (1978), A convenient method for the ATPase assay. Anal. Biochem. **85**, 86–89.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951), Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265–275.

Postius C. and Kindl H. (1978), The occurrence of phenylalanine ammonia-lyase and cinnamic acid p-hydroxylase on the endoplasmic reticulum of cell suspension cultures of *Glycine max*. Z. Naturforsch. **33c**, 65–69.

Rademacher W., Fritsch H., Graebe J. E., Sauter H. and Jung J. (1987), Tetcyclacis and triazoe-type plant growth retardants: their influence on the biosynthesis of gibberellins and other metabolic processes. Pestic. Sci. **21**, 241–252.

Radetzky R., Feussner I., Theimer R. R. and Kindl H. (1993), Transient occurrence of lipoxygenase and glycoprotein gp49 in lipid bodies during fat mobilization in anise seedlings. Planta **191**, 166–172.

Regdel D., Kühn H. and Schewe T. (1994), On the reaction specificity of the lipoxygenase from tomato fruits. Biochim. Biophys. Acta **1210**, 297–302.

Ricker K. E. and Bostock R. M. (1994), Eicosanoids in the *Phytophthora infestans*-potato interaction: lipoxygenase metabolism of arachidonic acid and biological activities of selected lipoxygenase products. Physiolog. Molec. Plant Pathol. **44**, 65–80.

Rouet-Mayer M. A., Bureau J. M. and Lauriere C. (1992), Identification and characterization of lipoxygenase isoforms in senescing carnation petals. Plant Physiol. **98**, 971–978.

Sembdner G. and Parthier B. (1993), The biochemistry and the physiological and molecular actions of jasmonates. Annu. Rev. Plant Physiol. **44**, 569–589.

Siedow J. N. (1991), Plant lipoxygenase – Structure and function. Annu. Rev. Plant Physiol. **42**, 145–188.

Song W. C., Baertschi S. W., Boeglin W. E., Harris T. M. and Brash A. R. (1993), Formation of epoxyalcohols by a purified allene oxide synthase – implications for the mechanism of allene oxide synthesis. J. Biol. Chem. **268**, 6293–6298.

Song W. C., Funk C. D. and Brash A. R. (1993), Molecular cloning of an allene oxide synthase – a Cytochrome-P450 specialized for the metabolism of fatty acid hydroperoxides. Proc. Natl. Acad. Sci. USA **90**, 8519–8523.

Sturm A. and Kindl H. (1983), Fucosyl transferase activity and fucose incorporation in vivo as markers for subfractionating cucumber microsomes. FEBS Lett. **160**, 165–168.

Tranbarger T. J., Franceschi V. R., Hildebrand D. F. and Grimes H. D. (1991), The soybean 94-kilodalton vegetative storage protein is a lipoxygenase that is localized in paraveinal mesophyll cell vacuoles. Plant Cell **3**, 973–987.