Hypothesis

Do specific linoleate 13-lipoxygenases initiate β-oxidation?

Ivo Feussner[a,*], Hartmut Kühn[b], Claus Wasternack[a]

[a]*Institut für Pflanzenbiochemie, Weinberg 3, D-06120 Halle, Germany*
[b]*Institut für Biochemie, Universitätsklinikum Charité, Humboldt Universität Berlin, Hessische Str. 3-4, D-10115 Berlin, Germany*

Received 14 January 1997

Abstract The germination process of oilseed plants is characterized by a mobilization of the storage lipids which constitute the major carbon source for the growing seedling. Despite the physiological importance of the lipid mobilization, the mechanism of this process is not well understood. Recently, it was found that a specific linoleate 13-lipoxygenase is induced during the stage of lipid mobilization in various oilseed plants and that this enzyme is translocated to the membranes of the lipid storage organelles, the so called lipid bodies. Lipoxygenase expression was paralleled by the occurrence of enantiospecific hydro(pero)xy polyenoic fatty acid derivatives in the storage lipids suggesting the in vivo action of the enzyme. Furthermore, it was reported that oxygenated polyenoic fatty acids, in particular as 13(S)-hydro(pero)xy-9(Z),11(E)-octadecanoic acid [13(S)-H(P)ODE], are cleaved preferentially from the storage lipids when compared with their non-oxygenated linoleate residues. These findings may suggest that 13(S)-H(P)ODE may constitute the endogenous substrate for β-oxidation during lipid mobilization of oilseed plants.

Key words: *Cucumis sativus*; Lipid body; Lipoxygenase; β-Oxidation; Oxygenated polyunsaturated fatty acid

1. Introduction

Several developmental processes during the life cycle of a plant are characterized by changes in the composition and turnover of cellular lipids. The life cycle of seed plants starts with germination. For oilseed plants this process is characterized by a mobilization of the storage lipids which serve as a major carbon source for the growth of the seedlings. In the seeds the storage lipids are deposited in special organelles, the so-called lipid bodies, during seed maturation. These storage organelles which constitute intracellular droplets of triacylglycerols surrounded by a phospholipid monolayer are degraded during early stages of germination, and a new set of proteins which includes a specific linoleate 13-lipoxygenase (LOX) becomes detectable at their membranes [1,2].

LOXs (linoleate:oxygen oxidoreductase, EC 1.13.11.12) constitutes a family of dioxygenases and are widely distributed in plants and animals [3,4]. They catalyze the regio- and stereo-specific dioxygenation of polyunsaturated fatty acids containing a 1(Z),4(Z)-pentadiene system [4]. Although this family of enzymes has been studied intensively there is no general concept for their biological importance [5]. In the past, lipoxygenases have mainly been implicated in the biosynthesis of hormones such as jasmonic acid [6] or leukotrienes [7], but there is evidence for a biological role of mammalian lipoxygenases outside the arachidonic acid cascade [5]. In plants lipoxygenase-derived hydroperoxy fatty acids are further metabolized via several pathways (Fig. 1) [8,9]. Some of these degrading pathways are well characterized: (i) the hydroperoxide lyase pathway: oxidative cleavage of hydroperoxy fatty acids forming short chain aldehydes (C_6- or C_9-) and the corresponding C_{12}- or C_9-ω-keto fatty acids [10]; (ii) the allene oxide synthase pathway: conversion of hydroperoxy fatty acids to unstable allene oxides, which either undergoes non-enzymatic hydrolysis forming α- and γ-ketols, or is further metabolized to 12-oxo-phytoenoic acid by an allene oxide cyclase [8]; (iii) the peroxygenase pathway: [9,11]. Oxidation of unsaturated fatty acids into epoxy- or dihydrodiol derivatives via an intramolecular oxygen transfer using fatty acid hydroperoxides as co-substrates. These pathways were shown to convert fatty acid hydroperoxides to compounds of physiological importance, such as odors, jasmonates, and other oxylipins [6,8]. In addition to these well-characterized pathways, there are other routes for hydroperoxide metabolization which are not as well characterized. (i) LOX catalyzed hydroperoxidase reactions: Under certain conditions (such as low oxygen tension, LOXs are capable of catalyzing the homolytic cleavage of the O–O bond forming a hydroxy radical and an alkoxy radical which may rearrange to ketodienes [12]. (ii) A recently described divinyl ether synthase [13]. (iii) The reductase pathway: In mammals hydroperoxy lipids are rapidly reduced to their corresponding hydroxy compounds most probably via glutathione-dependent peroxidase reactions [14,15]. There are several types of glutathione peroxidases but only one of them the phospholipid hydroperoxide glutathione peroxidase is capable of reducing esterified hydroperoxy fatty acids [16]. However, in plants such peroxidases have not been characterized so far.

2. Hypothesis

The lipid bodies of cucumber seedlings contain a linoleate 13-LOX [17,18] which is capable of dioxygenating esterified linoleic acid residues at carbon atom 13 without the preceding action of a lipid hydrolyzing enzyme [19]. During early stages of germination this reaction leads to a strong increase in the degree of oxidation of the storage lipids and a preferential release of 13(S)-hydroxy-9(Z),11(E)-octadecadienoic acid (13(S)-HODE) from the lipid bodies. These data may suggest that mobilization of the fatty acids for β-oxidation is not initiated by a lipase but by a specific LOX isoform. In addi-

*Corresponding author. Fax: (49) 345-5582162.
E-mail: ifeussne@ipb.uni-halle.de

Dedicated to Prof. Dr. P. Matile on the occasion of his 65th birthday.

PII S0014-5793(97)00218-4

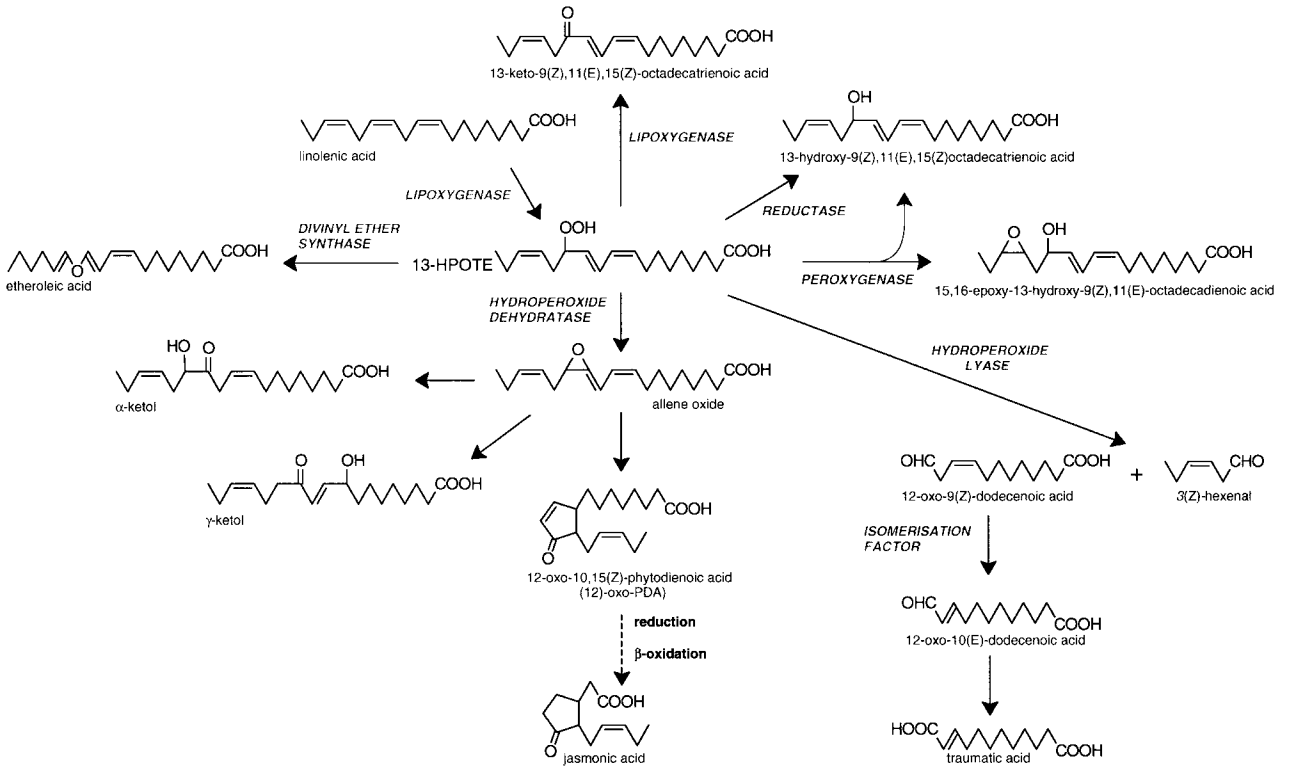

Fig. 1. Survey over lipid hydroperoxide metabolizing reactions derived from the LOX reaction, modified from [9].

tion to this specific LOX isoform two additional enzymes, a *hydroperoxylipid reductase* and a *hydro(pero)xy fatty acid-specific lipase* may be involved. Free 13(S)-HODE which is formed from the storage triacylglycerols via the sequential action of these enzymes may serve as endogenous substrate for glyoxysomal β-oxidation during germination. In this concept the stereo-specific dioxygenation of the storage lipids by a specific linoleate 13-LOX may play a crucial role as the first step of the lipid mobilization cascade.

3. Discussion

3.1. Properties of lipid body LOX

The lipid body lipoxygenase is highly expressed during the germination in various oilseeds [1,2,20]. After synthesis the enzyme appears to translocate to the lipid body membrane but the mechanism of the translocation process has not been studied so far. From analysis of the primary structure of the cDNA it seems that this enzyme is a soluble protein which translocates by a post-translational mechanism to the lipid body membrane [21]. Although the function of the N-terminal region of plant LOXs is not well understood [4] one might argue that binding of LOXs at specific membranes may be similar to that of 13-LOXs from mammalians. For these enzymes which do not have an N-terminal domain like plants it is suggested that the mechanism of the translocation occurs in a calcium-dependent way [5]. The lipoxygenase has a molecular weight of about 100 kDa, exhibits an alkaline pH-optimum (8.5) and an unusual positional specificity [18]. Linoleic acid and linolenic acid are converted to their 13(S)-hydroperoxy derivatives [13(S)-HPODE and 13(S)-HPOTE]. Arachidonic acid is oxygenated to a mixture of 15(S)-, and 12(S)- hydroperoxy-eicosatetraenoic acid [12(S)- and 15(S)-HPETE], but in addition substantial amounts of 8(S)-HPETE are also formed [18]. This positional specificity with arachidonic acid might be indicative of a different active site compared to other LOX, although the molecular mechanism could not explained so far. Binding of the enzyme to the lipid body membrane augmented its enzymatic activity 4-fold and alters its positional specificity in favor of 8(S)-HPETE formation [18]. The purified enzyme in vitro was capable of oxygenating trilinolein to mono-, di- and tri-HPODE derivatives (I. Feussner et al., unpublished data). Since these compounds have also been detected in germinating cucumber seedlings an involvement of this LOX in the germination process may be concluded [19]. In cucumber seedlings at day four of germination period about 50% of the phospholipids surrounding the storage lipids as a monolayer and about 20% of the triacylglycerols are oxygenated by the enzyme. Immunocytochemical analysis of cucumber cotyledons at the first day of germination revealed a specific localization of this enzyme at the membrane of lipid bodies [20]. The protein-chemical characteristics, the subcellular localization, the enzymatic properties and the biological dynamics of the lipid body lipoxygenase indicate that this particular isoform of linoleate 13-LOX is distinct from other lipoxygenase isoforms present in cucumber.

3.2. Physiological role of the lipid body LOX

Fractionation studies of the storage lipids during the germination process suggested a stepwise oxygenation process. At the beginning of the germination LOX products are preferentially detected in the lipid body phospholipids whereas at later stages the storage triacylglycerols become the major LOX substrates [19]. One may assume that the phospholipid monolayer which surrounds the storage lipids is the primary

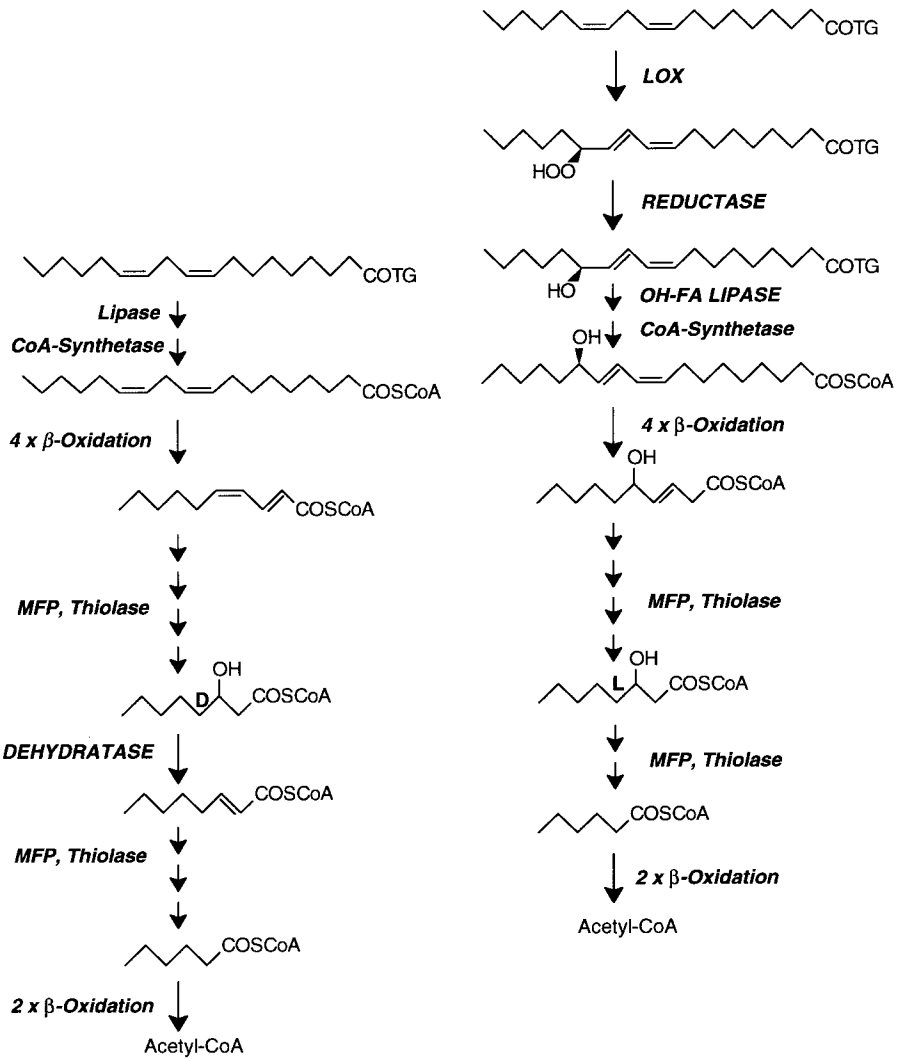

Fig. 2. Proposed pathways for the degradation of polyunsaturated fatty acids in plants. The left-hand side displays the peroxisomal degradation of linoleic acid according to [23]. The right-hand side shows the proposed LOX-dependent pathway. TG, triacylglycerol; MFP, multifunctional protein; OH-FA, hydroxy fatty acid.

target of LOX attack. During in vitro studies with artificial model membranes it has been shown that oxidation of a phospholipid layer greater than 5% leads to severe disturbances of its structure [22]. The high oxidation degree of the lipid body membrane may alter its structural integrity, allowing the linoleate 13-LOX and other cytosolic enzymes to come in contact with the storage lipids. In fact, as indicated above large amounts of specific LOX products were detected in the triacylglycerol fraction at later stages of the germination process. HPLC analysis combined with measurements of chemiluminescence, as well as ^{1}H-NMR data, indicated that large amounts of esterified 13(S)-hydroperoxy-9(Z),11(E)-octadecadienoic acids and small amounts of esterified 13(S)-HODE were present in the storage lipids (Feussner et al., unpublished data). Interestingly, we found that more 13(S)-HODE was released from the lipid bodies than linoleic acid suggesting that the lipase activity responsible for the fatty acid liberation appears to prefer oxygenated fatty acid derivatives. These in vivo findings are supported by recent in vitro data indicating a preferential release of 13(S)-HODE from isolated lipid bodies of cucumber seedlings [19]. In order to test whether this sequence of reactions is restricted to cucumber we analyzed lipid bodies from other oilseeds (soybean, tobacco, sunflower and rape [19]). Here again, large amounts of oxygenated fatty acids which may be due to the in vivo activity of a linoleate 13-LOX were detected in germinating seedlings.

Taken together these data prompted us to propose a new model for the breakdown of the storage lipids during germination of oil seed plants. This model which is depicted in Fig. 2 (right-hand side) differs from the scheme of the peroxisomal degradation of linoleic acid (Fig. 2, left-hand side) [23,24]. According to this model the initial step of lipid mobilization during germination is the LOX-catalyzed oxygenation of the lipid body membrane leading to a disruption of the integrity of the lipid storage organelles which makes the storage lipids accessible to cytosolic enzymes. At this stage the linoleate 13-lipoxygenase may come in physical contact with the storage triacylglycerols and oxygenate their linoleic acid residues forming 13(S)-HPODE-containing compounds. After being formed the hydroperoxy storage lipids are reduced to their corresponding hydroxy derivatives which are subsequently cleaved by a lipase which exhibits a specificity for oxygenated triacylglycerols. Alternatively, the hydroperoxy triacylglycerols are cleaved by the lipase and the resulting free 13-HPODE

is subsequently reduced to 13-HODE. At this stage of research the reducing enzyme as well as the 13-H(P)ODE specific lipase involved in cucumber germination have not been characterized. However, a lipase activity which prefers oxygenated ester lipids over their non-oxygenated counterparts has already been described in several plants [25]. For the reduction of the hydroperoxy lipids two enzymatic reactions may be envisaged: (i) a fatty acid-dependent peroxygenase using a polyenoic fatty acids as reducing equivalent [11] or (ii) a glutathione-dependent peroxygenase for which glutathione constitutes the reductant. Such a glutathione-dependent peroxygenase has recently been described in stressed citrus plants [26].

The final catabolic pathway of the storage lipids is the peroxisomal β-oxidation. This pathway provides reducing equivalents and acetyl-CoA which may be utilized by the seedling for synthetic processes. When 13(S)-HODE is used as substrate for β-oxidation instead of linoleic acid the first three cycles of β-oxidation are identical. The 4th step of β-oxidation is different with respect to the order of the enzymatic steps for both fatty acids leading either to 2(L)-hydroxy-octanyl-CoA or to 2(D)-hydroxy-octanyl-CoA. For a complete β-oxidation of 13(S)-HODE it is an absolute requirement that the chiral center at C_1 is in the L-configuration according to the Fischer-convention. Chiral phase HPLC of the 13-H(P)ODE formed by the lipid body LOX indicated that the enzyme in vitro and in vivo produces almost exclusively 13(S)-H(P)ODE (Cahn-Prelog nomenclature) which is $13L_S$-H(P)ODE according to the Fischer-convention. The lipid body LOX introduces molecular oxygen at the optimal steric configuration for a complete β-oxidation of the oxygenated fatty acid. In order to be further metabolized by β-oxidation, the derivative with the D-configuration, resulting from linoleic acid, has to be converted by an additional enzyme, the dehydratase. In contrast, 9-LOXs which are widely distributed in plants as well would be less effective for lipid mobilization. These enzymes convert linoleic acid to 9(S)-H(P)ODE which contains a chiral center of the D_S-configuration. This fatty acid derivative can only be partly β-oxidized because the 'wrong' stereochemistry at C_9 may prevent complete metabolization.

The metabolic scheme presented for the lipid mobilization during the germination process suggests a novel biological role for linoleate 13-LOXs. These enzymes appear to 'label' the storage lipids and thus tagging them for degradation by specific lipid hydrolyzing activities. By this way the plants may discriminate the normal lipid turnover during seed development and maturation from the massive breakdown of the storage lipids which occurs as the final catabolic event of the storage lipids during germination.

3.3. Do linoleate 13-LOXs in plants and animals have a common biological function?

In plants, lipoxygenase-initiated breakdown of cellular lipids or organelles may not be restricted to lipid mobilization during the germination process but may also be involved in other developmental processes, such as leaf senescence. In green leaves and during senescence specific linoleate 13-LOXs are induced [27–29]. Furthermore, senescence is accompanied by a massive breakdown of chloroplastidic glycolipids, which contain large amounts of linolenic acid and is a good substrate for linoleate 13-LOXs. It is suggested that the degradation of galactolipids proceed via a diacylglycerol intermediate [30] formed by cleaving off the carbohydrate moiety. It might be possible that a senescence-induced linoleate 13-LOX may be involved in the degradation of the diacylglycerol derivative. Interestingly, in barley induction of a senescence-induced glyoxysomal β-oxidation [31] is accompanied by the induction of a 13-LOX [32]. Here again 13(S)-H(P)OTE, the LOX-derived product of linolenic acid, might be the preferred endogenous substrate.

Because linoleate 13-LOXs are widely distributed in plants and animals one may ask the question whether these enzymes are of similar importance for animal physiology. Unfortunately, here again no comprehensive concept for the biological role of these enzymes has been presented. However, there are several hypothesis most of which may be categorized into two groups [5]: (i) formation of bioactive fatty acid metabolites such as 15(S)-HETE and 13(S)-HODE, (ii) oxygenation of complex substrates such as membrane constituents or lipoproteins altering the physico-chemical properties of these lipid-protein assemblies. In the later case, the metabolizing of biomembranes precede the degradation of organelles.

Searching for proteins involved in red blood cell maturation a linoleate 13-LOX was discovered more than 20 years ago [33]. This enzyme shows interesting biological dynamics; it is not present in reticulocyte precursor cells nor in immature reticulocytes [34]. During red blood cell maturation the appearance of this LOX coincides with the onset of the maturational breakdown of mitochondria [35]. These biological dynamics and the fact that non-specific LOX inhibitors slow down the degradation of mitochondria during in vitro maturation of rabbit reticulocytes suggested that the enzyme may be involved in the programmed degradation of these organelles [34]. In fact, specific 13-LOX products were detected in the membranes of rabbit reticulocytes [36] indicating the in vivo action of the enzyme. The degree of lipid peroxidation in the mitochondrial membranes was 4-fold higher than that of the plasma membrane [37] suggesting a preferential attack of the 13-LOX on mitochondria. A similar preferential oxygenation of mitochondrial membranes was reported for the in vitro interaction of the enzyme with different types of biomembranes [38]. Another example for the hypothesis that a linoleate 13-LOX may be involved in processes of organelle biogenesis is its potential role in macrophage development [39]. Although the immediate function of the enzyme and the mechanism of its up-regulation are not clear, one may hypothesize that the enzyme is implicated in the remodeling of the intracellular membranes during macrophage maturation. However, more work is needed to substantiate this hypothesis.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft.

References

[1] I. Feussner, H. Kindl, FEBS Lett. 298 (1992) 223–225.
[2] R. Radetzky, I. Feussner, R.R. Theimer, H. Kindl, Planta 191 (1993) 166–172.
[3] S. Rosahl, Z. Naturforsch. 51c (1996) 123–138.
[4] J.N. Siedow, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 145–188.
[5] Kühn, H. (1997) Prog. Lip. Res. (1997) in press.
[6] G. Sembdner, B. Parthier, Annu. Rev. Plant Physiol. Plant Mol. Biol. 44 (1993) 569–589.

[7] R.A. Lewis, A.F. Austen, R.J. Soberman, N. Engl. J. Med. 323 (1990) 645–655.
[8] M. Hamberg, J. Lipid Med. 6 (1993) 375–384.
[9] E. Blée, J. Joyard, Plant Physiol. 110 (1996) 445–454.
[10] K. Matsui, M. Shibutani, T. Hase, T. Kajiwara, FEBS Lett. 394 (1996) 21–24.
[11] E. Blée, A.L. Wilcox, L.J. Marnett, F. Schuber, J. Biol. Chem. 268 (1993) 1708–1715.
[12] G.A. Veldink, J.F.G. Vliegenthart, J. Boldingh, Prog. Chem. Fats Other Lipds 15 (1977) 131–166.
[13] A.N. Grechkin, M. Hamberg, FEBS Lett. 388 (1996) 112–114.
[14] Flohé, L. (1989) in: Glutathione: Chemical, Biochemical and Medical Aspects, Part A, pp. 643-731 (Dolphin, D., Poulson, R. and Avramovic, O. eds.), John Wiley, New York.
[15] R.F. Burk, K.E. Hill, Annu. Rev. Nutr. 13 (1993) 65–81.
[16] F. Ursini, M. Maiorino, R. Brigelius-Flohé, K.D. Aumann, A. Rovery, D. Schomburg, L. Flohé, Meth. Enzymol. 252 (1995) 38–53.
[17] I. Feussner, H. Kindl, Planta 194 (1994) 22–28.
[18] I. Feussner, H. Kühn, FEBS Lett. 367 (1995) 12–14.
[19] I. Feussner, C. Wasternack, H. Kindl, H. Kühn, Proc. Natl. Acad. Sci. USA 92 (1995) 11849–11853.
[20] I. Feussner, B. Hause, A. Nellen, C. Wasternack, H. Kindl, Planta 198 288 (1996) 11849–11853.
[21] M. Höhne, A. Nellen, K. Schwennesen, H. Kindl, Eur. J. Biochem. 241 (1996) 6–11.
[22] Y. Isaacson, C.D. Sherbourne, R.W. Gross, W.F. Stenson, Chem. Phys. Lipids 52 (1990) 217–226.
[23] Gerhardt, B. and Kleiter, A. (1995) in: Plant Lipid Metabolism, pp. 265–267 (Kader, J.-C. and Mazliak, P. eds.), Kluwer Academic Publ., Dordrecht.
[24] Kindl, H. (1987) in: The Biochemistry of Plants, Vol. 9, pp. 31–52 (Stumpf, P.K. and Conn, E.E. eds.) Academic Press, London.
[25] U. Stahl, A. Banas, S. Stymne, Plant Physiol. 107 (1995) 953–962.
[26] T. Beeort-Zahar, G. Benhayyim, D. Holland, Z. Faltin, Y. Eshdat, FEBS Lett. 366 (1996) 151–155.
[27] J. Royo, G. Vancanneyt, A.G. Perez, C. Sanz, K. Störmann, S. Rosahl, J.J. Sanchez-Serrano, J. Biol. Chem. 271 (1996) 21012–21019.
[28] L.Y. Zhang, M. Hamberg, Lipids 31 (1996) 803–809.
[29] T.R. Prakash, P.M. Swamy, P. Suguna, P. Reddanna, Biochem. Biophys. Res. Commun. 172 (1990) 462–470.
[30] H. Gut, P. Matile, Bot. Acta 102 (1989) 31–36.
[31] F. Vicentini, P. Matile, J. Plant Physiol. 142 (1993) 50–56.
[32] I. Feussner, B. Hause, K. Vörös, B. Parthier, C. Wasternack, Plant J. 7 (1995) 949–957.
[33] T. Schewe, W. Halangk, C. Hiebsch, S.M. Rapoport, FEBS Lett. 60 (1975) 149–152.
[34] S.M. Rapoport, T. Schewe, Biochim. Biophys. Acta 864 (1986) 471–495.
[35] Rapoport, S.M., Schewe, T. and Thiele, B.J. (1990) in: Blood, Cell Biochemistry, Vol. 1, pp. 151-194, Plenum, New York.
[36] H. Kühn, A.R. Brash, J. Biol. Chem. 265 (1990) 1454–1458.
[37] H. Kühn, J. Belkner, R. Wiesner, Eur. J. Biochem. 191 (1990) 221–227.
[38] H. Kühn, J. Belkner, R. Wiesner, A.R. Brash, J. Biol. Chem. 265 (1990) 18351–18361.
[39] B.D. Levy, M. Romano, H.A. Chapman, J.J. Reilly, J. Drazen, C.N. Serhan, J. Clin. Invest. 92 (1993) 1572–1579.