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Biochemical Characterization of the Dual Positional Specific Maize Lipoxygenase and the Dependence of Lagging and Initial Burst Phenomenon on pH, Substrate, and Detergent during Pre-steady State Kinetics

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The wound-inducible lipoxygenase obtained from maize is one of the nontraditional lipoxygenases that possess dual positional specificity. In this paper, we provide our results on the determination and comparison of the kinetic constants of the maize lipoxygenase, with or without detergents in the steady state, and characterization of the dependence of the kinetic lag phase or initial burst, on pH, substrate, and detergent in the pre-steady state of the lipoxygenase reaction. The oxidation of linoleic acid showed a typical lag phase in the pre-steady state of the lipoxygenase reaction at pH 7.5 in the presence of 0.25% Tween-20 detergent. The reciprocal correlation between the induction period and the enzyme level indicated that this lag phenomenon was attributable to the slow oxidative activation of Fe (II) to Fe (III) at the active site of the enzyme as observed in other lipoxygenase reactions. Contrary to the lagging phenomenon observed at pH 7.5 in the presence of Tween-20, a unique initial burst was observed at pH 6.2 in the absence of detergents. To our knowledge, the initial burst in the oxidation of linoleic acid at pH 6.2 is the first observation in the lipoxygenase reaction. Kinetic constants (K_m and k_{cat} values) were largely dependent on the presence of detergent. An inverse correlation of the initial burst period with enzyme levels and interpretations on kinetic constants suggested that the observed initial burst in the oxidation of linoleic acid could be due to the availability of free fatty acids as substrates for binding with the lipoxygenase enzyme.

Keywords: Detergent, Initial burst, Kinetics, Lag phase, Lipoxygenase, Mechanism

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Introduction

Most lipoxygenases (LOXs, EC 1.13.11.12) contain non-heme iron, and occur ubiquitously in both animals and plants (Siedow, 1991; Brash, 1999). LOX is a dioxygenase, which catalyzes the hydroperoxidation of polyunsaturated fatty acids containing one or more (1Z, 4Z)-pentadiene structures in the presence of molecular oxygen. LOX-catalyzed reactions are generally regiospecific and stereospecific (Feussner *et al*., 2002). In plants, linoleic acid (LA) or linolenic acid (LNA) is oxygenated at the C-9 or C-13 of the hydrocarbon backbone of the unsaturated fatty acid, by 9-LOX or 13-LOX, respectively. The corresponding (9S)-hydroperoxy or (13S)-hydroperoxy derivatives are further metabolized into a variety of biologically active compounds, *via* seven metabolic branches. The LOX pathway is responsible for the initiation of the biosynthesis of a number of physiologically important oxylipins which can function as elicitors of the expression of defense genes (Reymond *et al*., 1998; Agrawal *et al*., 2003; Lee *et al*., 2005), antimicrobial compounds against biotic stresses (Lee *et al*., 2004; Agrawal *et al*., 2004), and inducers of hypersensitive cell death (Göbel *et al*., 2003). For example, jasmonate biosynthesis is initiated by the 13-LOX pathway and the peroxidation of lipids in the hypersensitivity response is mediated primarily by 9-LOX (Jalloul *et al*., 2002; Montillet *et al*., 2005); leaf aldehydes are synthesized *via* either the 9-LOX or 13-LOX pathway. Previous experiments have shown that the orientation of the substrate molecule determines positional specificity (Egmond *et al*., 1972), whereas some recent studies have indicated that the frameshift re-alignment of the substrate may peform a crucial function in both positional and stereo specificities (Coffa *et al*., 2005). Interestingly, a few nontraditional LOXs (Feussner and Westernack, 2001) oxygenate at C-9 and C-13 positions and produce both 9-hydroperoxy and 13-hydroperoxy positional isomers. These dual positional specific LOXs are found in plants (Hughes *et al*., 1998; Hughes *et al*., 2001; Kim *et al*., 2001) and animals

(Bryant *et al*., 1982). The metabolic roles and a detailed understanding of the catalytic mechanisms exploited by the dual positional specific LOX remain unknown.

The LOX-catalyzed reaction in general has a characteristic kinetic lag phase in the early stage of the reaction (Smith *et al*., 1972). It has been assumed that the kinetic lag phase observed in the pre-steady state is attributable to the conversion of the LOX from Fe (II) to the catalytically active Fe (III) by the hydroperoxide product (Slappendel *et al*., 1982). The induction period in the kinetic lag phase could, in fact, be partially eliminated by the addition of the hydroperoxide product (Smith *et al*., 1972). However, the initiation of the LOX reaction with the oxidized LOX (Fe (III)) rather than Fe (II) LOX does not result in the complete disappearance of the induction period (Schilstra *et al*., 1993), which is in apparent contradiction with this assumption. Two distinct kinetic models regarding the origin of kinetic lag phase have been proposed (Schilstra *et al*., 1994) for explaining this apparent contradiction. A multiple-site model assumes that both the Fe (II) and Fe (III) forms of LOX are catalytically active, whereas in a two-step model only the Fe (III) form of the enzyme is active.

Polyunsaturated fatty acid (PUFA), a substrate of LOX, aggregates to form micelles in an aqueous phase and detergents are frequently used to solubilize aggregated micelles in the LOX reaction. The incorporation of substrate molecules into detergent micelles complicates the situation regarding the availability of substrate molecules for binding with the LOX enzyme. Substrate availability is one of the obstacles to the characterization of the kinetics of the LOX reaction unless the role of detergents in the steady and pre-steady state of the LOX reaction can be clarified. We have reported previously that the wound-inducible LOX obtained from maize is one of the nontraditional LOXs that possess dual positional specificity, and that the expression of LOX is modulated by both methyl jasmonate and wounding (Kim *et al*., 2002; Kim *et al*., 2003) *in vivo*. In this paper, we have first determined and then compared the kinetic constants of the maize LOX with or without detergents in the steady state, and more importantly, investigated the dependence of the kinetic lag phase or initial burst phenomenon, on pH, substrate, and detergent in the pre-steady state of the LOX reaction.

Materials and Methods

Reagents. LA and LNA with 99% purity, stored in sealed ampoules, were purchased from Sigma-Aldrich. All other chemicals were obtained either from Sigma-Aldrich or from Duchepa.

Heterologous expression and purification of LOX. The enzyme was heterologously expressed and purified as previously reported (Kim *et al*., 2003), with some slight modifications. The pZL1/LOX (6C02E12) was acquired from the University of Missouri-Columbia Clone Distribution Center, and digested with *Sal*I and *Xba*I. The resultant 1.3 kb fragment was then employed as a polymerase chain reaction (PCR) template. Two primers were used to introduce a *Bgl*II site (underlined) at the 5' side (5'-GGAAGATCTTCCATGCG CACCTCAATGGCAAC-3') and an *Eco*RI site (underlined) at the 3' side (5'-CCGGAATTCCGGTCAAATGGAGATGCTGTTTG-3') of the 6C02E12 sequence. The PCR product was then cloned into pGEM-T Easy vector for amplification, and subcloned into the *Bgl*II/*Eco*RI sites of pRSETB to generate the recombinant plasmid (pRSETB/LOX). *E. coli* BL21(DE3) pLysS cells were transformed with pRSETB/LOX vector and grown for 4 h at 25°C, and LOX expression was induced with the addition of isopropyl-β-D-thio-galactopyranoside (1 mM). The cells were grown for an additional 14 h at 25°C, harvested by centrifugation (3,000 × *g*, 15 min, 4°C), briefly washed with 50 mM Tris-HCl (pH 7.5), and resuspended in extraction buffer (50 mM Tris-HCl, pH 7.5) containing 10% glycerol, 0.1% Tween-20, 1 mM EDTA, 500 mM NaCl, 1% 1,10-phenanthroline, 0.2 mM PMSF, and 100 μg/ml egg white lysozyme. The resuspended cells were then disrupted by sonication and centrifuged, and the supernatant was loaded on the His-Tag column (ProBond Resin, Invitrogen, USA). The LOX was eluted with 50 mM Tris-HCl (pH 6.0), 350 mM imidazole, and the purity of the LOX was confirmed by SDS-PAGE.

Enzyme assay. The enzyme activity was determined by the modification of the previously reported procedure (Surrey, 1964) depending on the presence of detergents. In the presence of detergents, the assay mixture contained 50 mM Tris-HCl (pH 7.5), 2.5 mM substrate, 0.25% Tween-20, 0-20 mg of LOX in a 3 ml of final volume. In the absence of detergents, ethanol was used to solubilize substrate molecules and the assay mixture contained 50 mM Tris-HCl (pH 7.5), 1.0 mM substrate, 0.8% ethanol, 0-20 mg of LOX. The initial reaction velocity was determined at 25°C by following the formation of the conjugated diene of hydroperoxydienoic acid (HPODE) or hydroperoxytrienoic acid (HPOTE) at 234 nm by using a Perkin-Elmer spectrophotometer. The amount of HPODE or HPOTE was calculated using a molecular extinction coefficient of 25,000 cm^{-1} M^{-1}. The buffers used for pH profiling were 50 mM sodium phosphate for pH 6.0-6.8, Tris-HCl for pH 7.4-7.9, and sodium borate for pH 8.0-8.9.

Kinetic measurements in the steady state. The lag or initial burst period was excluded and data points in the steady state period were selected for measuring the initial velocity in the steady state. Kinetic constants (K_m and k_{cat}) were calculated by the Lineweaver-Burk plot. The ranges of substrate concentration employed for Lineweaver-Burk plot were 0.5-2.5 mM LA or LNA in 0.25% Tween-20, 0.05-0.5 mM LA in 0.8% ethanol, 0.05-1.0 mM LNA in 0.8% ethanol.

Determination of induction or initial burst period in the pre-steady state. For the pre-steady state kinetic experiments, the formation of HPODE or HPOTE was monitored for 0-4 min depending on the amount of enzyme using the conditions used for the kinetic measurements. The initial linear line in the pre-steady state was separated from the second straight line in the steady state. The induction or initial burst period was calculated from the initial linear time period in the pre-steady state of the LOX reaction.

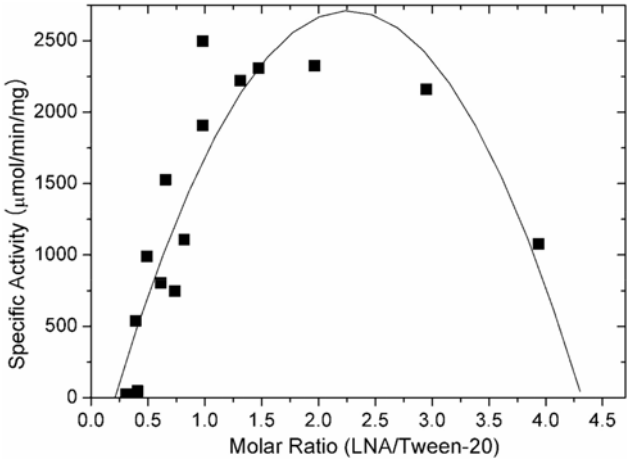

Fig. 1. Dependence of the specific activity of the dual positional specific maize LOX on the molar ratio of linolenic acid to Tween-20. The specific activity was determined using 2.5 mM of linolenic acid as described in Materials and Methods. The molar ratios of linolenic acid to Tween-20 were in a range of Tween-20 concentrations from 0.08-1.22%.

Results and Discussion

Effect of Tween-20 and pH on specific activity. Since PUFA aggregates to form micelles in the aqueous phase, the solubilization of the substrate molecules by detergents is an important factor in the LOX reaction. Several detergents have been employed to stabilize the emulsion of the substrate in LOX studies (Butovich *et al*., 1998). As seen in Fig. 1, the activity of the maize LOX was increased markedly by Tween-20 and the specific activity achieved a maximal flat value at a molar ratio of 1.23-3.07 LNA to Tween-20 (0.10-0.25% of Tween-20 at 2.5 mM LNA). All of the experiments with detergents described herein were conducted in the presence of 0.25% Tween-20, unless otherwise noted.

As ionization of the substrate, as well as the LOX enzyme itself, would be affected by pH of the LOX reaction mixture, the pH dependence of the LOX reaction was examined in the presence of 0.25% Tween-20 or 0.8% ethanol, using LA or LNA as a substrate. As is shown in Figure 2, the optimal pH ranged from 6.8 to 6.9 depending on the substrate and the presence of Tween-20, and the specific activity was generally higher with LA than LNA as a substrate in the presence of Tween-20 or ethanol over the pH range of 6.0-9.0. Butovich *et al*. (1998) suggested that the ionization of the carboxyl group in a substrate molecule is not an essential requirement for the enzymatic reaction to occur in the presence of detergents based on the pH profile of dual positional specific potato LOX with various substrate analogs, and concluded that the rate of enzymatic reaction is primarily controlled by two ionizable groups of the enzyme which have not been identified. If this is the case in the dual positional specific maize LOX, the pH dependence seen in Fig. 2 is the consequence of the ionization of specific amino acids of the maize LOX enzyme.

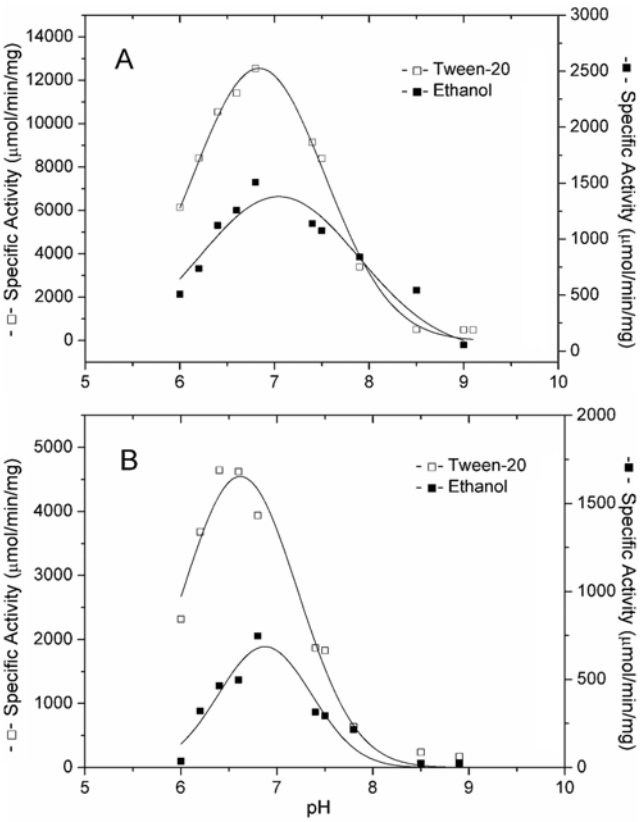

Fig. 2. pH-Dependence of the specific activity of the dual positional specific maize LOX. The activity was measured in the presence of 0.25% Tween-20 or 0.8% ethanol as described in the Materials and Methods. A: LA as a substrate, B: LNA as a substrate.

Kinetic constants. Since the specific activities of the LOX reaction were influenced significantly by the presence of detergent molecules, kinetic experiments with detergents (0.25% Tween-20) and without detergents (0.8% ethanol) were conducted with LA or LNA as a substrate, and their results compared (Table 1). As would be anticipated from Fig. 2, the maize LOX was a more effective catalyst with LA than with LNA as a substrate in terms of both K_m and k_{cat} values. Since the maize LOX was not able to catalyze the oxidation of trilinolein or trilinolenin even in the presence of detergent (Table 1), and could be severely inhibited by β-cyclodextrin, a host molecule for the host-guest complexing with free fatty acids (Park, 2004), the dual positional specific maize LOX seems to utilize only free fatty acid as a substrate. The apparent K_m values of the maize LOX in 0.8% ethanol were smaller than those in 0.25% Tween-20, particularly with using LA as a substrate. This result indicates that substrates as free fatty acids might be more available for the LOX enzyme in the absence of detergents. In other words, the detergent molecule may play a negative role in providing effective free fatty acids for binding with the LOX enzyme even though the detergent helps to solubilize substrate molecules by their

Table 1. Kinetic constants of the nontraditional LOX with dual positional specificity

Plant	LOX name	Substrates	Additives	Kinetic constants		
				K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})
Maize[a]	LOX1-Zm1	LA	0.25% Tween-20	1.6	13.9	8.7
			0.8% Ethanol	0.1	0.3	3.0
		LNA	0.25% Tween-20	2.2	1.0	0.5
			0.8% Ethanol	1.3	0.4	0.3
		Trilinolein	0.25% Tween-20		NR	
		Trilinolenin	0.25% Tween-20		NR	
Potato[b]	LOX1-St2	LA	0.01% Tween-20	0.2	106.0	530.0
Pea[c]	LOX1-Ps2	LA	0.01% Tween-20	0.3	2.0	6.7
	LOX1-Ps3	LA	0.01% Tween-20	6.7	78.0	12.2

[a]This study.
[b]Hughes *et al.* (2001)
[c]Hughes *et al.* (1998)
NR, No reaction.

incorporation of substrate molecules into the micelles. If this is the case, the increased activity of maize LOX by Tween-20 as seen in Fig. 1 could be due to the interaction between the detergent and the LOX enzyme. In fact, the k_{cat} and k_{cat}/K_m values of the maize LOX were higher in 0.25% Tween-20 than those in the 0.8% ethanol (Table 1). These results are consistent with the conclusion drawn from Fig. 2 that the ionization of a carboxyl group in a substrate molecule may not constitute a critical determinant in the reaction rate and that the ionization of specific amino acids of the LOX enzyme is the essential requirement for the enzymatic reaction to occur in the presence of detergents. Actually, the activation of the LOX enzyme by detergents has been observed by others in potato tubers (Butovich *et al*., 2000; 2001). Thus, Tween-20 seems to interact with the maize LOX enzyme and this interaction might be responsible for the increased activity of the maize LOX in the presence of detergents.

Dependence of lagging and initial burst on pH, detergent, and substrate. Kinetic lag phases have been commonly observed at high pH in reactions catalyzed by several LOXs from soybean LOX-1 (Schilstra *et al*., 1993), *T. Claveryi* LOX (Perez-Gilabert *et al*., 2005), and potato tuber LOX (Butovich *et al*., 1998). The induction period attributable to the kinetic lag phase has been explained by the oxidative activation of the enzyme by hydroperoxy fatty acid (Schilstra *et al*., 1993). It is known that the activation associated with lagging includes the oxidation of Fe (II) to Fe (III) in the active site of the LOX enzyme by HPODE, and the lagging period is dependent on the distribution of the Fe (II) and Fe (III) forms of the enzyme (Schilstra *et al*., 1994). As seen in Fig. 3A, the oxidation of LA catalyzed by the dual positional specific maize LOX at pH 7.5 also showed the typical lag phase in the pre-steady state (Fig. 3A). The induction period of the maize LOX was rather long compared with values of other traditional LOXs (Schilstra

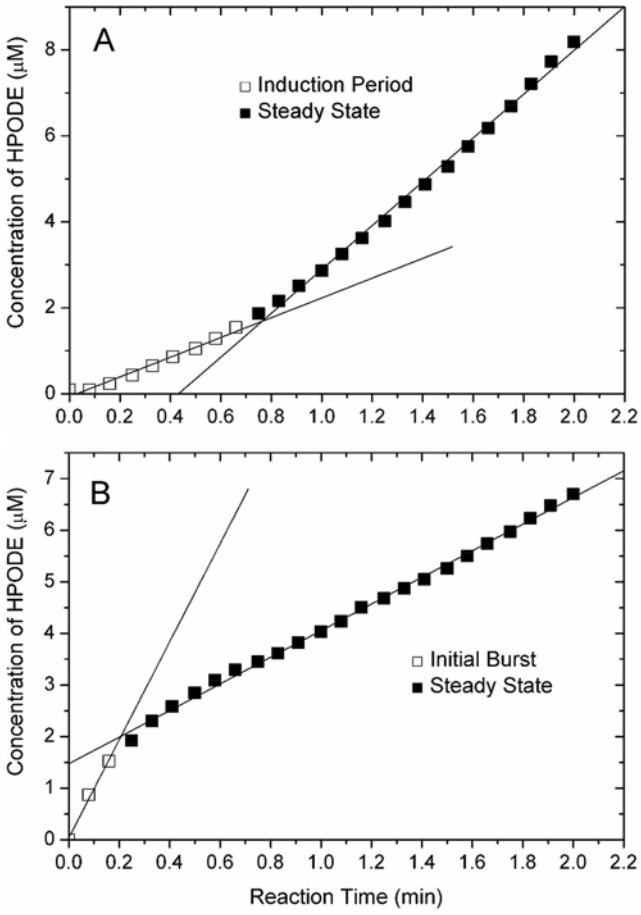

Fig. 3. Dependence of lagging or initial burst on pH in the oxidation of LA catalyzed by the dual positional specific maize LOX. The production of HPODE was monitored at 234 nm as described in the Materials and Methods. A: pH 7.5 in the presence of 0.25% Tween-20, B: pH 6.2 in the presence of 0.8% ethanol.

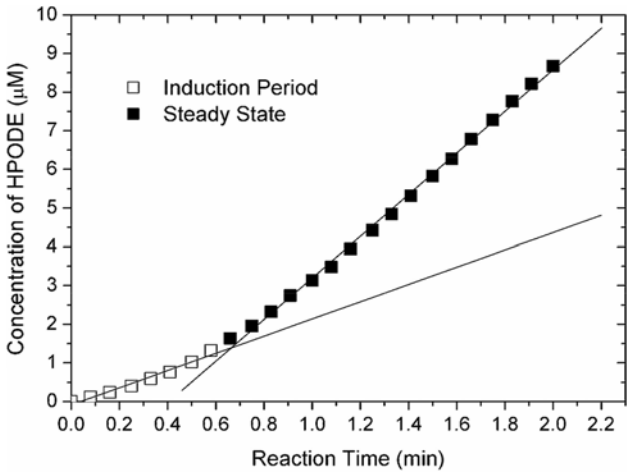

Fig. 4. Lagging phenomenon in the oxidation of LNA catalyzed by the dual positional specific maize LOX. The production of HPOTE was monitored at 234 nm in the presence of 0.8% ethanol at pH 6.2 as described in the Materials and Methods.

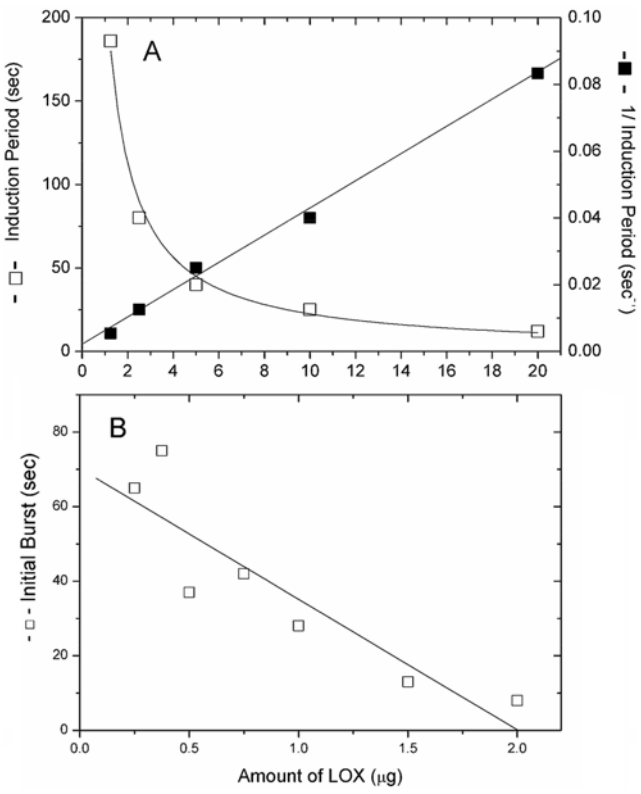

Fig. 5. Dependence of induction period and initial burst period on the amount of enzyme at pH 6.2 in the presence of 0.8% ethanol. The induction period and initial burst period was calculated as described in Materials and Methods. A: LNA as a substrate, B: LA as a substrate.

et al., 1993), which implies that the oxidative activation of Fe (II) to Fe (III) by HPODE in the maize LOX reaction at high pH is so slow that the lag phase can be detected as a prolonged induction period in the pre-steady state. Slow oxidative activation may be functionally correlated to the dual positional specificity of the maize LOX, however, an exact relationship between the dual positional specificity and a mechanistic origin of the slow activation of the maize LOX remains unclear.

Interestingly, the oxidation of LA at pH 6.2 in the absence of detergents (in 0.8% ethanol) showed the typical initial burst (Fig. 3B) which is contrary to the lagging phenomenon observed at pH 7.5 in the presence of 0.25% Tween-20. Since pH and detergents are involved in the ionization and the activation of the maize LOX enzyme as discussed previously in Fig. 2 and Table 1, it is reasonable to assume that the lagging or initial burst associated with the activation of the LOX enzyme would depend upon pH and the presence of detergents.

The initial burst phenomenon illustrated in Fig. 3B can be explained in two ways. One possibility is that the oxidative activation rate of Fe (II) to Fe (III) is so high that the majority of the enzyme is present in the Fe (III) form during the pre-steady state. This situation might be plausible at low pH since the oxidation of the Fe (II) to Fe (III) form of the LOX with the concomitant reduction of hydroperoxide would be accelerated as the pH decreases. However, the initial burst could not be observed at low pH values in the presence of 0.25% Tween-20 (data not shown). Therefore, the initial burst phenomenon can not be explained by the rapid oxidation of Fe (II) to Fe (III) due to the low pH. The other possibility is that a greater quantity of free fatty acids as substrates is available at low pH in the absence of detergents during the pre-steady state since the initial burst can be observed at low pH only in the absence of detergents. This explanation is consistent with our previous speculation that more free fatty acids would be available for binding with the LOX enzyme in the absence of detergents based on the fact that the K_m of LA in 0.8% ethanol was much smaller than that in the presence of 0.25% Tween-20 (Table 1). If K_m values in Table 1 represent the effective concentration of substrates for binding with the LOX enzyme, then free fatty acids as substrates in the oxidation of LNA would be limitedly available since the K_m values of LNA were large (comparable to that of LA in the presence of detergent). This finding predicts that the LNA may not show the initial burst phenomenon, even at low pH, in the absence of detergents. Indeed, a lag phase instead of the initial burst was observed in the oxidation of LNA catalyzed by the maize LOX at pH 6.2 (Fig. 4). Therefore, the initial burst observed in the maize LOX-catalyzed oxidation of LA can be attributed principally to the availability of free fatty acids as substrates at low pH in the absence of detergents.

Dependence of induction period and initial burst period on the amount of enzyme at low pH. Reciprocal relationships between the induction period and the enzyme concentration have been reported in several LOX reactions taking place at

high pH values (Perez-Gilabert *et al*., 2005). The dual positional specific maize LOX also showed a reciprocal correlation between the induction period and the amount of enzyme in the oxidation of LNA even at low pH (Fig. 5A). This reciprocal relationship is consistent with our conclusion that the lagging phenomenon is due to the slow oxidative activation of Fe (II) to Fe (III) by the hydroperoxy fatty acid derivatives. To evaluate the initial burst as a function of enzyme concentration in the oxidation of LA at low pH, the initial burst period was measured at a pH of 6.2 with 0.8% ethanol, and plotted against the quantity of enzyme present within the reaction mixture. The result shown in Fig. 5B indicates that the initial burst period was inversely proportional to the quantity of enzyme in the reaction mixture. This inverse correlation is concordant with our presumption that the initial burst observed in maize LOX-catalyzed LA oxidation is due primarily to the availability of free fatty acids as substrates at low pH.

In conclusion, our kinetic data and the characterization of initial burst or lagging phenomenon of the dual positional maize LOX reaction indicate that detergent molecules are involved in both the interaction with the LOX enzyme and the solubilization of substrates. The solubilization of substrate involves the incorporation of substrate molecules into the detergent micelles, which leads to decreased availability of effective free fatty acids for binding with the LOX enzyme. The unique initial burst phenomenon of the maize LOX observed in the oxidation of LA at low pH may also be attributed to the availability of free fatty acids as substrates.

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References

Agrawal, G. K., Jwa, N., Shibato, J., Han, O., Iwahashi, H. and Rakwal, R. (2003) Diverse environmental cues transiently regulate OsOPR1 of the octadecanoid pathway reveling its importance in rice defense/stress and development. *Biochem. Biophy. Res. Commun*. **310**, 1081-1090.

Agrawal, G. K., Tamogami, S., Han, O., Iwahashi, H. and Rakwal, R. (2004) Rice octadecanoid pathway. *Biochem. Biophys. Res. Commun*. **317**, 1-15.

Brash, A. R. (1999) Lipoxygenases: occurrence, function, catalysis, and acquisition of substrate. *J. Biol. Chem*. **274**, 23679-23682.

Bryant, R. W., Bailey, J. M., Schewe, T. and Rapport, S. M. (1982) Positional specificity of a reticulocyte lipoxygenase specificity. Conversion of arachidonic acid to 15S-hydroperoxy-eicosatetraenoic acid. *J. Biol. Chem*. **257**, 6050-6055.

Butovich, I. A., Lukyanova, S. M. and Reddy, C. C. (1998) Oxidation of linoleyl alcohol by potato tuber lipoxygenase: Possible mechanism and the role of carboxylic group in substrate binding. *Biochem. Biophy. Res. Commun*. **249**, 344-349.

Butovich, I. A., Lukyanova, S. M. and Reddy, C. C. (2000) Oxidation of linoleyl alcohol by potato tuber lipoxygenase: Kinetics and positional, stereo, and geometrical (cis, trans) specificity of the reaction. *Arch. Biochem.Biophys*. **378**, 65-77.

Butovich, I. A. and Reddy, C. C. (2001) Enzyme-catalyzed and enzyme-triggered pathway in dioxygenation of 1-monolinoleoyl-rac-glycerol by potato tuber lipoxygenase. *Biochim. Biophys. Acta* **1546**, 379-398.

Coffa, G., Schneider, C. and Brash, A. R. (2005) A comprehensive model of positional and stereo control in lipoxygenase. *Biochem. Biophys. Res. Commun*. **338**, 87-92.

Egmond, M. R., Vliegenthart, J. F. G. and Boldingh, J. (1972) Stereospecificity of the hydrogen abstraction at carbon atom n-8 in the oxygenation of linoleic acid by lipoxygenases from corn germs and soya beans. *Biochem. Biophys. Res. Commun*. **48**, 1055-1060.

Feussner, I. and Wasternack, C. (2002) The lipoxygenase pathway. *Annu. Rev. Plant Biol*. **53**, 275-297.

Göbel, C., Feussner, I. and Rosahl, S. (2003) Lipid peroxidation during the hypersensitive response in potato in the absence of 9-lipoxygenase. *J. Biol. Chem*. **278**, 52834-52840.

Hughes R. K., Wu Z., Robinson D. S., Hardy D., West S. I., Fairhurst S. A. and Casey R. (1998) Characterization of authentic recombinant pea-seed lipoxygenases with distinct properties and reaction mechanism. *Biochem. J*. **333**. 33-43.

Hughes, R. K., West, S. I., Hornostaj, A. R., Lawson, D. M., Fairhurst, S. A., Sanchez, R. O., Hough, P., Robinson, B. H. and Casey, R. (2001) Probing a novel potato lipoxygenase with dual positional specificity reveals primary determinants of substrate binding and requirements for a surface hydrophobic loop and has implications for the role of lipoxygenases in tubers. *Biochem. J*. **353**, 345-355.

Jalloul, A., Montillet, J. L., Assigbetse, K., Agnel, J. P., Delannoy, E., Triantaphylides C., Daniel, J. F., Marmey, P., Geiger, J. P. and Nicole, M. (2002) Lipid peroxidation in cotton: *Xanthomonas* interactions and the role of lipoxygenases during hypersensitive reaction. *Plant J*. **32**, 1-12.

Kim, E., Back, K., Baik, M., Choi, E. and Han, O. (2001) Sequence of wound-responsive lipoxygenase gene from maize seedlings. *J. Biochem. Mol. Biol*. **34**, 259-261.

Kim, E., Choi, E., Kim, Y., Cho, K., Lee, A., Shim, J., Rakwal, R., Agrawal, G. K. and Han, O. (2003) Dual positional specificity and expression of nontraditional lipoxygenase induced by wounding and methyl jasmonate in maize seedlings. *Plant Mol. Biol*. **52**, 1203-1213.

Kim, E., Kim, H., Park, R., Lee, Y. and Han, O. (2002) Dual positional specificity of wound-responsive lipoxygenase from maize seedlings. *J. Plant Physiol*. **159**, 1263-1265.

Lee, A., Cho, K., Jang, S., Rakwal, R., Iwahashi, H., Agrawal, G. K., Shim, J. and Han, O. (2004) Inverse correlation between jasmonic acid and salicylic acid during early wound response in rice. *Biochem. Biophys. Res. Commun*. **318**, 734-738.

Lee, S. H., Ahn, S. J., Im, Y. J., Cho, K., Chung, G. C., Cho, B. H. and Han, O. (2005) Differential impact of low temperature on fatty acid unsaturation and lipoxygenase activity in figleafgourd and cucumber roots. *Biochem. Biophys. Res. Commun*. **330**, 1194-1198.

Montillet, J. L., Chamnongpol, S., Rusterucci, C., Dat, J., Cotte, B., Agnel, J. P., Battesti, C., Inze, D., Breusegem, F. V. and Triantaphylides, C. (2005) Fatty acid hydroperoxides and H_2O_2 in the execution of hypersensitive cell death in tobacco leaves.

Plant Physiol. **138**, 1516-1526.

Park, J. (2004) MS Theses, The effects of micelle formation in the reaction mechanism of lipoxygenase (AF 271849), Chonnam National University.

Perez-Gilabert, M., Sanchez-Felipe, I., Morte, A. and Garcia-Carmona, F. (2005) Kinetic properties of lipoxygenase from desert truffle (Terfezia claveryi chatin) ascocarps: Effect of inhibitors and activators. *J. Agrc. Food Chem.* **53**, 6140-6145.

Reymond, P. and Framer, E. E. (1998) Jasmonate and salicylate as global signals for defense gene expression. *Curr. Opin. Plant Biol.* **1**, 404-411.

Schilstra, M. J., Veldink, G. A. and Vliegenthart, J. F. G. (1993) Kinetic analysis of the induction period in lipoxygenase catalysis. *Biochemistry* **32**. 7686-7691.

Schilstra, M. J., Veldink, G. A. and Vliegenthart, J. F. G. (1994) The dioxygenation rate in lipoxygenase catalysis is determined by the amount of iron (III) lipoxygenase in solution. *Biochemistry* **33**. 3974-3979.

Siedow, J. N. (1991) Plant lipoxygenases : Structure and function. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**, 145-188.

Slappendel, S., Malmström, B. G., Peterson, L., Eherenberg, A., Veldink, G. A. and Vliegenthart, J. F. G. (1982) On the spin and valence state of iron in native soybean lipoxygenase-1. *Biochem. Biophys. Res. Commun.* **108**. 673-677.

Smith, W. L.and Lands, W. E. M. (1972) Oxygenation of unsaturated fatty acids by soybean lipoxygenase. *J. Biol. Chem.* **247**. 1038-1047.

Surrey, K. (1964) Spectrophotometric method for determination of lipoxidase activity. *Plant Physiol.* **39**. 65-70.