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Effects of Polyunsaturated Free Fatty Acids and Esterified Linoleoyl Derivatives on Oxygen Consumption and C_6 Aldehyde Formation with Soybean Seed Homogenates

Hong Zhuang, David F. Hildebrand, Roger A. Andersen, and Thomas R. Hamilton-Kemp*

Departments of Agronomy and Horticulture, University of Kentucky, Lexington, Kentucky 40546

We investigated the effects of the form of fatty acid substrate with specific lipoxygenase (LOX) isozymes on O_2 uptake and C_6 compound formation using a soybean seed model system at pH 6.8. In most cases lipid-dependent O_2 consumption with different unsaturated free fatty acids and 18:2 derivatives was qualitatively similar to C_6 aldehyde formation. LOX 1 activity was higher with arachidonic acid and linolenic acid than linoleic acid, but with LOX 1 and/or LOX 2, linoleic acid yielded the highest C_6 aldehyde production. The O_2 uptake and C_6 aldehyde production varied considerably among the esterified forms of linoleic acid. LOX 1 was relatively more effective in the lipid-dependent O_2 uptake of free fatty acids compared to esterified linoleoyl derivatives, whereas LOX 2 or 3 was relatively more effective with esterified linoleic acid than LOX 1. LOX 2 gave the highest C_6 aldehyde production with all substrates except with linoleoyl coenzyme A, where LOX 1 was most effective. The C_6 aldehyde formation was reduced with all substrates when LOX 3 was present in the seed homogenates.

INTRODUCTION

Lipoxygenases (linoleate:oxygen oxidoreductase, EC 1.13.11.12) (LOX) catalyze the peroxidation of certain unsaturated fatty acids, in which a *cis,cis*-1,4-pentadiene structure is converted to a conjugated hydroperoxide by molecular oxygen (Axelrod et al., 1981). Soybean seed is an especially rich source of lipoxygenases. Three LOX isozymes have been isolated from soybean seeds and are designated LOX 1, LOX 2, and LOX 3. With linoleic acid (18:2) as the substrate LOX 1 has maximal activity at pH 9.0, LOX 2 has a peak of activity at pH 6.8, and LOX 3 exhibits a broad pH activity profile centered on pH 7.0 (Axelrod et al., 1981).

LOX activity can lead to formation of intracellular free radicals and fatty acid hydroperoxides, which may be involved in plant senescence (Leshem et al., 1981) and synthesis of some growth regulatory compounds (Kacperska and Kubacka-Zebalska, 1985; Vick and Zimmerman, 1983; Zimmerman and Coudron, 1979). The cleavage products, C_6 aldehydes, resulting from activity of hydroperoxide lyase on fatty acid hydroperoxides are important in the flavor of many food products (Arai et al., 1970; Axelrod, 1974, Buttery et al., 1969; Eskin et al., 1977) and might also be responsible in part for the pest resistance of some plants (Lyr and Banasiak, 1983; Gardner et al., 1990).

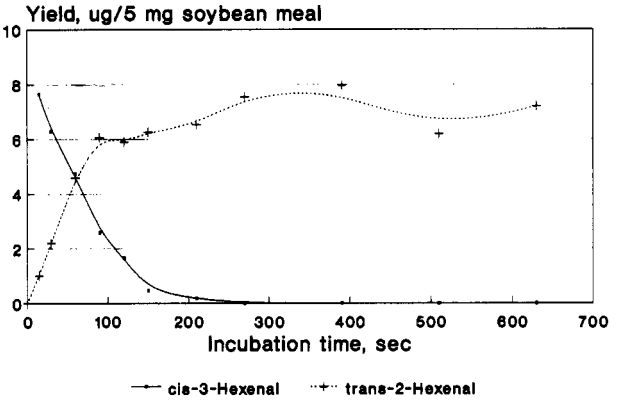

Figure 1. Amounts of *cis*-3-hexenal and *trans*-2-hexenal over time in headspace from a homogenate of soybean seed meal mutant for LOX 3 (-L3). Details for this measurement system are given under Materials and Methods.

Even though the three abundant LOX isozymes found in mature soybean seeds have been well characterized biochemically and the LOX pathway has been largely elucidated (Hatanaka et al., 1987; Vick and Zimmerman,

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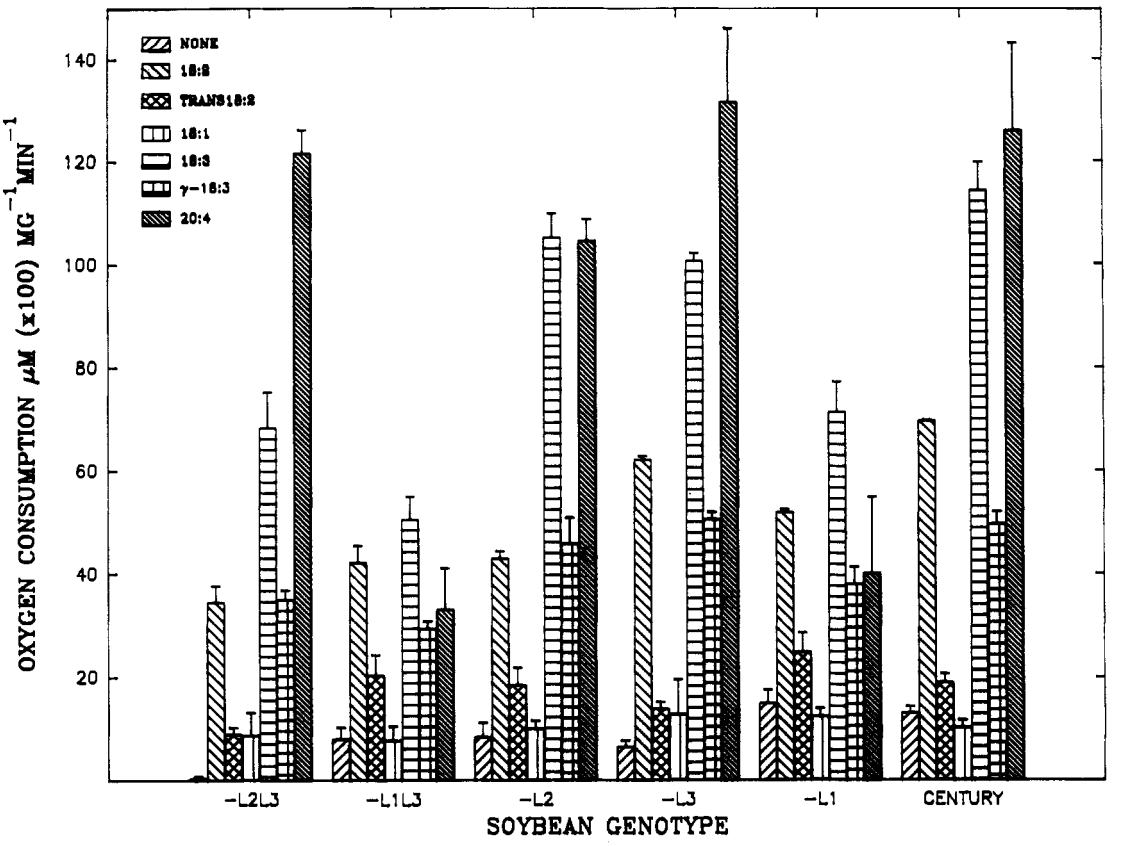

Figure 2. Effect of free fatty acids on lipid-dependent O_2 consumption with soybean seed homogenates. O_2 consumption is reported per milligram of seed meal.

1987; Hildebrand, 1989), the actual molecular structures of in vivo substrates for LOXs in plants are unknown. The majority of cellular fatty acids are esterified to phospholipids, glycolipids, and triacylglycerols (Harwood, 1980). In soybean seeds, for example, more than 90% of the 18:2 and linolenate (18:3) is esterified in triglyceride. Most of the remaining 18:2 and 18:3 is esterified in phospholipids. Fresh, high-quality mature soybean seeds generally contain only a few tenths of a percent of free fatty acids (FFA) (Frankel et al., 1987). It is thought that FFA may be the principal substrate for LOXs (Gardner et al., 1990). However, there are several reports in the literature which indicated that fatty acids esterified to phospholipids (Brash et al., 1987; Eskola and Laakso, 1983) and glycolipids (Koch et al., 1958; Guss et al., 1968; Yamauchi et al., 1985) can act as substrates for LOXs. Moreover, different LOX isozymes make variable contributions to C_6 aldehyde formation. With 18:2 as a substrate LOX 2 has the largest effect in generation of *n*-hexanal in soybean seed homogenates (Matoba et al., 1985), but LOX 3 reduces hexanal production (Hildebrand et al., 1990). In our present studies we have investigated various FFA (18:1, *trans*-18:2, 18:2, 18:3, γ-18:3, and 20:4) and 18:2 derivatives to gain further information about the actual in vivo substrates for LOXs and hydroperoxide lyases and the specificity of particular LOX isozymes for the substrates.

MATERIALS AND METHODS

Soybean Cultivars and Their Seed Homogenates. The soybean lines with genotypes designated -L2L3, -L1L3, -L2, -L3, and -L1 were kindly provided by Dr. Niels Nielsen, Purdue University, and Century is a commercial cultivar (Davies and Nielsen, 1987). These mutants are lines back-crossed to Century that are homozygous recessive null (or very low) for the alleles indicated. For example, -L2L3 is a null for LOX 2 and LOX 3 (but has the normal wild-type level of LOX 1). Century seeds contain LOXs 1, 2, and 3 (as do all widely grown commercial soybean cultivars). Harvest mature (dry) seeds were collected from plants grown concomitantly in a greenhouse with illumination supplemented to 13 h with high-intensity sodium halide lamps. The seed meal was made from lyophilized seeds ground to a fine powder in a coffee grinder and passed through a 60-mesh sieve.

Reagents. Oleic acid, linoleic acid, *trans*-linoleic acid, linolenic acid, γ-linolenic acid, arachidonic acid, linoleoyl coenzyme, propyl linoleate, methyl linoleate, 1-monolinoleoyl-*rac*-glycerol, monogalactosyldiacylglycerol (from whole wheat flour having about 88% 18:2, 12% 16:0, and 4% FFA), L-α-phosphatidylcholine dilinoleoyl, dilinolein (1,3-dilinoleoylglycerol), trilinolein, and Tween 20 were purchased from Sigma Chemical Co.

Substrate Preparation. One millimolar substrate suspensions were prepared by mixing the lipids in water with a Tissuemizer (Tekmar SDT-1810) for about 1 min (C_6 aldehyde measurements) or 25 mM phosphate buffer (oxygen uptake study). The potassium linoleate (18:2K) solution and 18:2K solution with Tween 20 were dissolved by stirring.

Preparation of Soybean Extracts for Oxygen Consumption Analysis. Proteins were extracted from the soybean seed meal with 20 volumes (per weight) of cold Milli-Q water. The extract was centrifuged (12000*g*, 15 min, 4 °C), and the supernatant was used to measure lipid-dependent O_2 consumption.

Measurement of Oxygen Consumption. Fatty acid dependent O_2 consumption was determined by using an oxygen electrode in the presence of a 1-mL sonicates of 1 mM lipid substrate in 25 mM phosphate buffer with pH 6.8 at 25 °C. The pH of 6.8 was used instead of pH 9.0 since it is the optimum pH of LOX 2, which is reported to be most significant in the generation of *n*-hexanal (Matoba et al., 1985) and the hydroperoxide lyase of germinating soybean seedlings has its maximal activity in the range pH 6–7 (Olias et al., 1990).

Determination of C_6 Aldehydes. Analyses of C_6 aldehyde production were performed in 1.8-mL screw-top vials with gastight septa. To each vial were added 5 mg of seed meal, 50 μL of 0.1 M sodium phosphate, pH 6.8, and 150 μL of 1 mM substrate homogenates in water. Samples were stirred for 30 s with a magnetic stir bar and then incubated at 30 °C for 10 min, after which time 250-μL headspace vapor samples were analyzed by

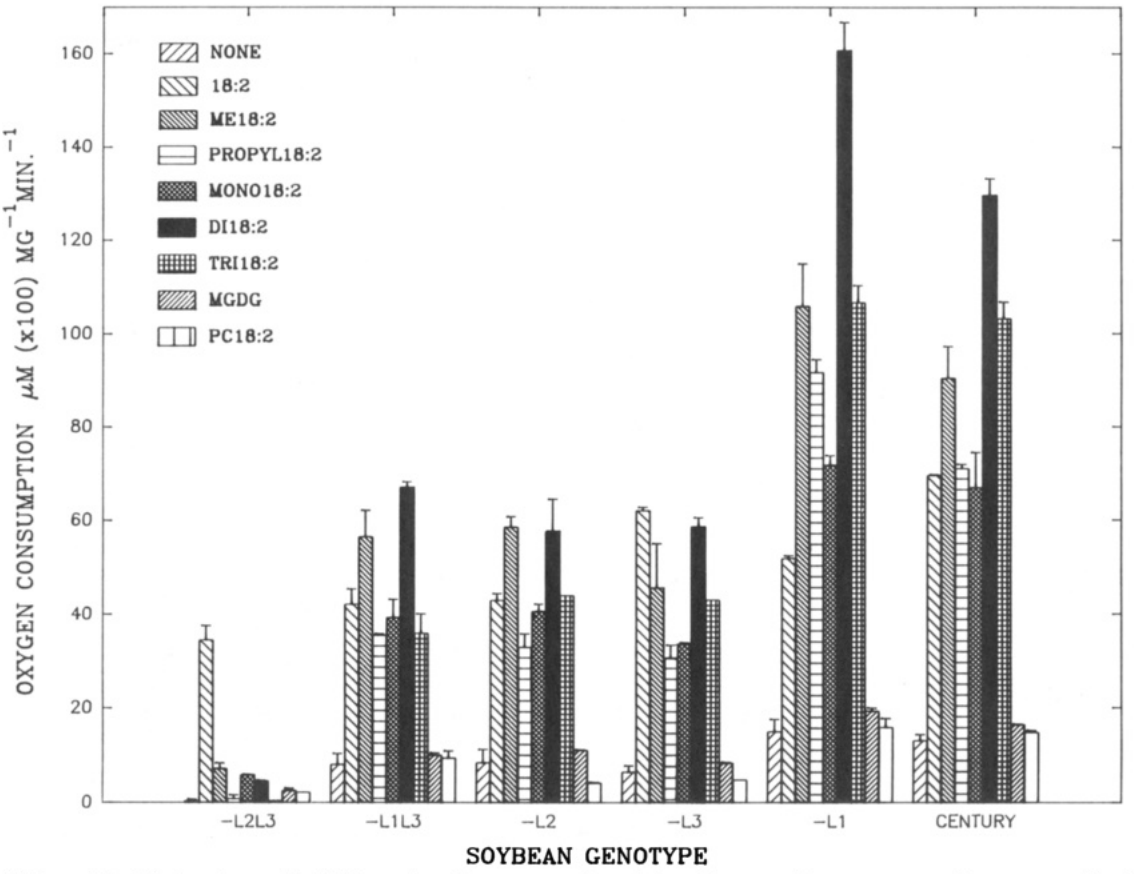

Figure 3. Effect of 18:2 derivatives on lipid-dependent O_2 consumption with soybean seed homogenates. O_2 consumption is reported per milligram of seed meal.

gas chromatography using direct injection onto a 30 m × 0.54 mm DB-5 (methyl silicone) fused silica column. The column oven was held isothermal at 50 °C for 3 min, and then the temperature was programmed at 5 °C/min to 200 °C. Two replications of each experiment were performed, and the entire set of experiments was repeated.

ω − 6 fatty acids such as 18:2 and derivatives, γ-18:3, and 20:4 yield hexanal as the volatile product of the LOX-lyase reaction, whereas the ω − 3 fatty acid, linolenic acid, yields *cis*-3-hexenal, which rearranges to *trans*-2-hexenal. Essentially 100% of the C_6 aldehyde in the vapor phase formed by the soybean meal homogenates at pH 6.8 and 30 °C is *trans*-2-hexenal by 5 min even though in the initial seconds after the reaction is started it is mostly *cis*-3-hexenal (Figure 1). Therefore, in this study, C_6 aldehyde yield was quantified by comparison to known quantities of hexanal and *trans*-2-hexenal in the vapor phase. The measured values of the different C_6 aldehydes, hexanal and *trans*-2-hexenal, were converted to nanograms per milliliter on the basis of GC response factors for these compounds which were determined in the vapor phase as recommended by the U.S. EPA by using a 2-L static dilution battle (Tekmar Co.). The difference in vapor pressure between these two compounds was therefore taken into account. However, it would be expected that the quantity of hexanal portioned into the vapor phase from this aqueous solution would be greater than that of *trans*-2-hexenal. Therefore, C_6 aldehyde yield with 18:3 as the LOX/lyase substrate was probably underestimated.

RESULTS

Fatty Acid Dependent Oxygen Consumption of Soybean Extracts and Unsaturated FFA. We investigated the effects of different unsaturated FFA, 18:1, 18:2, *trans*-18:2, 18:3, γ-18:3, and 20:4, on lipid-dependent O_2 consumption to compare the relationship between lipid-dependent O_2 consumption and C_6 aldehyde formation under the same conditions. Lipid-dependent O_2 consumption was measured with 1 mM lipid dispersions in phosphate buffer (pH 6.8) at 25 °C after addition of the soybean mutant seed extracts. Century, with wild-type levels of LOXs, consumed the largest amount with 20:4, followed by 18:3, 18:2, γ-18:3, and *trans*-18:2 under these experimental conditions. Lipid-dependent O_2 consumption was lowest with 18:1. These properties were still observed with mutants containing LOX 1. In the mutants without LOX 1 the largest amount of O_2 was consumed with 18:3 (Figure 2).

Substrate Selectivity of the LOX Isozymes. The absence of LOX 1 in mutants apparently reduced O_2 consumption with 18:3 and 20:4 substrates (Figure 2). The presence of LOX 2 and LOX 3 had relatively smaller effects on the O_2 consumption with the FFA substrates tested as illustrated by comparing Century to the -L1 line and -L1 to -L1L3. In contrast, most of the O_2 uptake with the nonpolar 18:2 derivatives was due to LOX 2 and LOX 3 (Figure 3). LOX 1 had little activity except with the polar 18:2 derivative CoA18:2 (data not shown).

Effects of Different Esterified 18:2 Derivatives on Lipid-Dependent O_2 Consumption. With Century soybeans, among the range of esterified 18:2 derivatives examined, including those occurring abundantly in plants, di18:2 gave the largest O_2 uptake. Tri18:2 and me18:2 followed, higher than 18:2, then propyl18:2 and mono18:2, both of which were similar to 18:2 (Figure 3). The O_2 consumption utilizing MGDG and PC18:2 was relatively small, similar to that with the control (no substrate added) (Figure 3). The O_2 consumption showed considerable differences among the mutant lines compared to 18:2. The -L2L3 line consumed much less O_2 with the esterified substrates than the FFA.

C_6 Aldehyde Generation from Soybean Seed Homogenates with Unsaturated FFA during Incubation

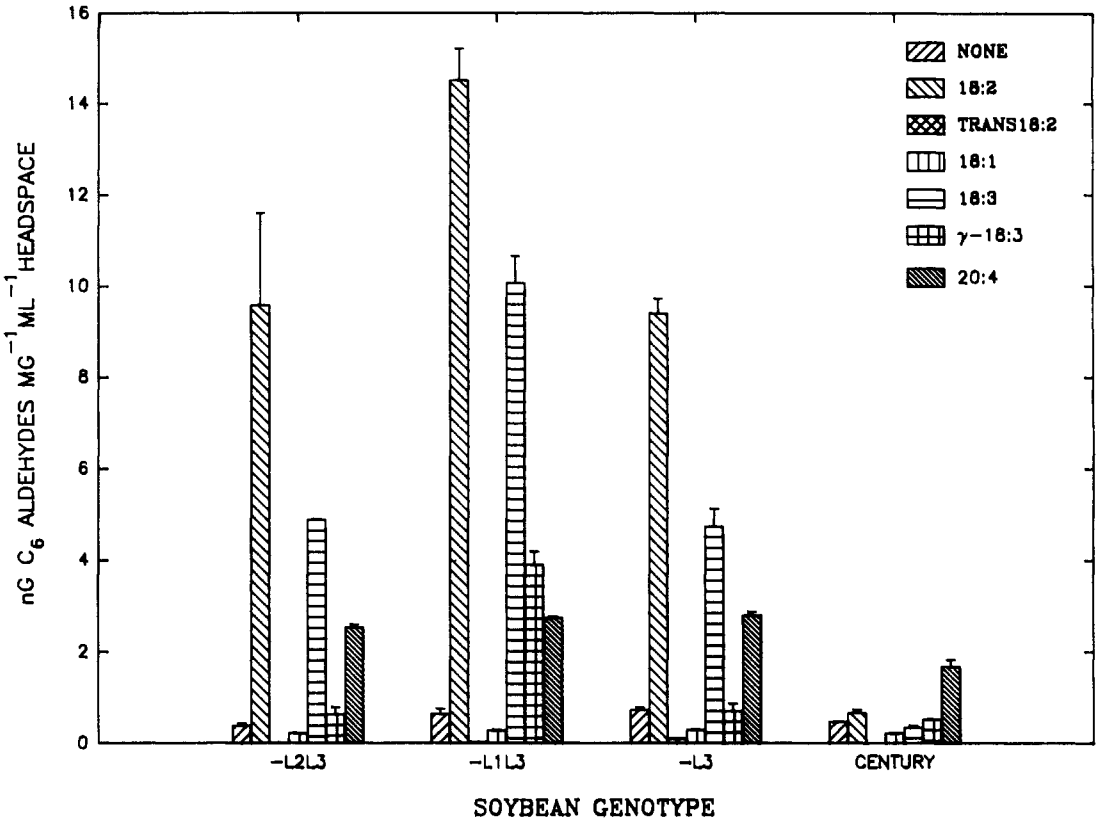

Figure 4. Effect of free fatty acids on C_6 aldehyde formation with soybean seed homogenates. C_6 aldehyde production is reported per milligram of seed meal. Hexanal was produced with all substrates except 18:3, where *cis*-3-hexenal and *trans*-2-hexenal were formed. The results presented here for 18:3 are *trans*-2-hexenal since after the 10-min incubation essentially 100% of the C_6 aldehyde in the headspace is *trans*-2-hexenal (see Figure 1).

at 30 °C. Homogenates of Century and -L3, -L1L3, and -L2L3 null mutant seeds were incubated in phosphate buffer at pH 6.8 at 30 °C for 10 min together with unsaturated FFA, and C_6 aldehyde levels in the headspace were determined (Figure 4). Near maximum C_6 aldehyde levels were reached within 15 s after the start of the reaction, which reached an equilibrium that was constant for at least 40 min (Figure 1 and unpublished data). Most of the C_6 aldehydes produced in this system from 18:3 was *cis*-3-hexenal initially, but by 5 min of incubation essentially 100% of the C_6 aldehyde seen in the headspace was *trans*-2-hexenal (Figure 1). The level of C_6 aldehydes was highest in the homogenates of -L2L3, -L1L3, and -L3 mutants, with 18:2, followed by 18:3 (Figure 4). Addition of 20:4 increased hexanal production with all seed homogenates from lines with all combinations of LOXs, whereas significantly enhanced hexanal formation with γ-18:3 was only seen with the seed only containing LOX 2 (Figure 4). The presence of LOX 3 reduced or prevented the increase in C_6 aldehyde formation seen upon addition of all substrates, although the effect was small with 20:4 (Figure 4). The addition of *trans*-18:2 and oleic acid reduced C_6 compound formation with all homogenates (illustrated by gap following 18:2 bar in Figure 4).

Effects of the 18:2 Ester Derivatives on Hexanal Formation. Figure 5 shows the results obtained when the different esterified 18:2 derivatives were used as substrates for hexanal production by the same method mentioned above. With the mutant line without L1L3 (high only in LOX 2 activity), hexanal formed from free 18:2 was much higher than any of its ester derivatives. Among the derivatives, me18:2 resulted in the highest hexanal formation followed by mono18:2, CoA18:2, MGDG, and propyl18:2. The soybean line with LOX 3, Century, showed a reduction in hexanal formation with the esterified derivatives as was the case with FFA. Upon addition of tri18:2 or di18:2, no net hexanal generation was observed.

Different LOX Mutants Vary Considerably in Their Effect on C_6 Aldehyde Formation. LOX 2 always gave the largest formation of C_6 aldehydes except for with both CoA 18:2 and 20:4 (Figures 4 and 5). For those substrates where LOX 2 promoted formation of C_6 aldehydes, the presence of LOX 1 also increased production of these compounds. With 20:4, LOX 1 and LOX 2 generated the same amounts of C_6 aldehydes. A reduction of C_6 aldehyde production in the presence of LOX 3 (Century) was clearly observed with all C_6 aldehyde forming substrates compared to in the absence of LOX 3 (-L3 line) (Figures 4 and 5).

Relationship between Fatty Acid Dependent O_2 Consumption and C_6 Aldehdye Formation. Lipid-dependent O_2 uptake was compared with C_6 compound formation so as to gain some information concerning the relationship between LOX specificity and lyase specificity for various substrates as well as between LOX activity and C_6 aldehyde generation. Among the FFA, 20:4 yielded the largest O_2 consumption, and among the esterified 18:2 derivatives, di18:2 and tri18:2 yielded the largest O_2 consumption. However, free 18:2 gave highest levels of C_6 aldehyde formation. Generally, the results for both O_2 uptake and C_6 aldehyde formation were similar. We found that 20:4, 18:3, γ-18:3, 18:2, MGDG, me18:2, mono18:2, and propyl18:2 promoted O_2 uptake and C_6 aldehyde production. Contrariwise, PC18:2, *trans*-18:2, and 18:1 did not promote O_2 consumption and also did not promote C_6 compound formation. Tri18:2 and di18:2 were exceptions. They gave very high O_2 uptake but did not promote C_6 aldehyde formation.

Effect of Different Substrate Forms and Tween on O_2 Consumption and C_6 Aldehyde Formation with

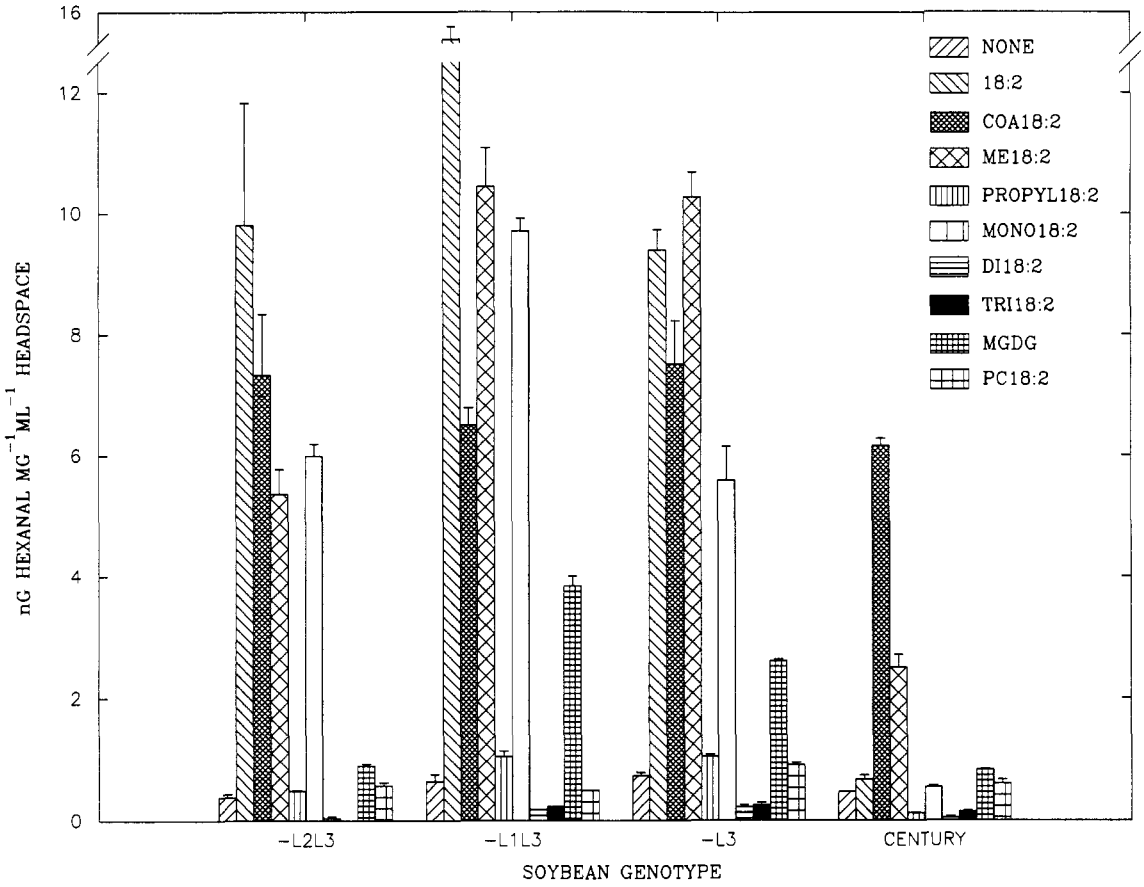

Figure 5. Effect of 18:2 derivatives on hexanal formation with soybean seed homogenates. The rest is as in the legend to Figure 3.

18:2. Substrate solutions were prepared in water to make the conditions more clearly related to in vivo or food-processing situations. However, most measurements of LOX activity reported in the literature were carried out with potassium linoleate (K18:2) in Tween 20 solutions. Therefore, the effects of the different substrate forms on activities of LOXs and lyase were studied at pH 6.8. The results indicate that substrates containing 1 mM KOH (yielding 18:2K) gave the largest O_2 uptake and C_6 aldehyde formation in most cases (Figures 6 and 7). In the presence of Tween 20, the O_2 uptake in the lines containing LOX 1 increased, whereas activity resulting from LOX 2 and LOX 3 was reduced compared to water. The solution without KOH and Tween 20 exhibited decreased C_6 aldehyde formation. Tween 20 increased C_6 aldehyde formation in the mutant lines with LOX 3.

DISCUSSION

Studies of the substrate specificity of LOX isozymes 1, 2, and 3 are useful for understanding the mechanism and enzymatic specificity of LOX action. Although several studies have been reported on this subject, the research has been directed toward two additional aspects including the position of the 1,4-pentadiene in the fatty acid chains. A series of isomeric octadecadienoic acids with the pentadiene system in different positions (Hamberg and Samuelsson, 1965, 1967; Holman et al., 1969; St. Angelo and Ory, 1984) and different chains with the pentadiene system in the same position (Hatanaka et al., 1989) were used to understand the effects of fatty acid structure on LOX activity. The other research area is the effects of esterified forms of the substrate on enzyme activity (Bild et al., 1977; Buss et al., 1968; Koch et al., 1958; Brash et al., 1987; Eskola and Laakso, 1983; Hatanaka et al., 1987; Yamauchi et al., 1985). It was shown that esterified 18:2 and 20:4 and γ-18:3 might be substrates of LOXs in addition to the FFA 18:2 and 18:3. In our experiments reported here we focused on the latter.

The higher the degree of unsaturation in the natural free fatty acid substrates, the greater the O_2 consumption. For example, with Century and all mutant lines used, the O_2 consumption with 20:4 was larger than with 18:3, and that with 18:3 larger than with 18:2. These may result from double dioxygenation or polydioxygenation (Van Os et al., 1981; Yamamoto, 1989). The monounsaturated fatty acid 18:1 was not found to be a substrate. St. Angelo and Ory (1984) also reported that monomeric fatty acids, including 18:1, could inhibit soybean and peanut LOXs. The artificial isomer of 18:2, *trans*-18:2, was not a substrate for LOX, and the uncommon plant fatty acid, γ-18:3, was relatively less effective.

The best substrates among 18:2 ester derivatives for LOXs in soybean seeds with wild-type LOXs are the di- and triunsaturated fatty acids. That triacylglycerol can be a substrate of LOX has long been known, and Koch et al. (1958) suggested the name triglyceride lipoxidase for the LOXs from soybean seeds. Mono18:2 was similar to free 18:2 as a substrate when LOX 2 or LOX 3 was present. The data with Century showed that lipid-dependent O_2 consumption with di- and tri18:2 was higher than with free 18:2 or mono18:2. This indicates that the additional 18:2 moieties esterified to glycerol can act as substrates for LOX 2 and LOX 3. Other esterified 18:2 derivatives such as me18:2, mono18:2, and propyl18:2 were also good substrates for soybean LOXs, similar to results reported with tea leaves (Hatanaka et al., 1987). O_2 uptake was very low in the presence of MGDG and PC. There are some reports that PC, MGDG, and DGDG are substrates for LOX, but all reactions were under special conditions that may affect the chemical or physical form of the

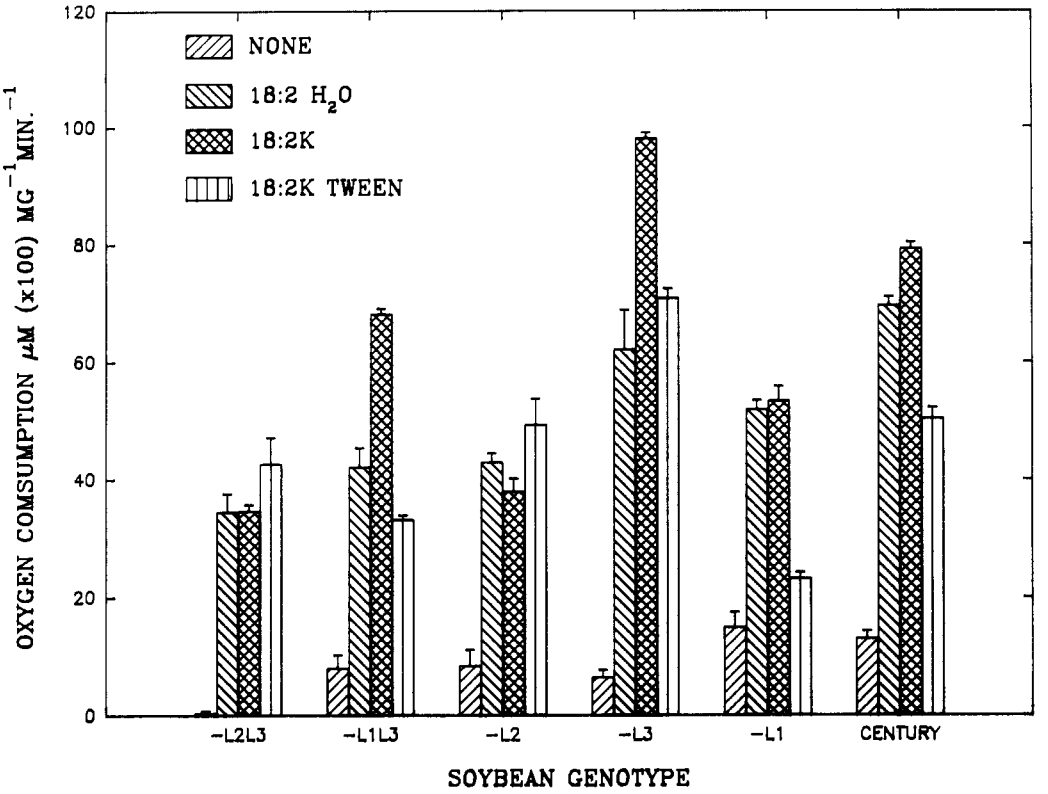

Figure 6. Effect of different 18:2 substrate forms on lipid-dependent O_2 consumption with soybean seed homogenates.

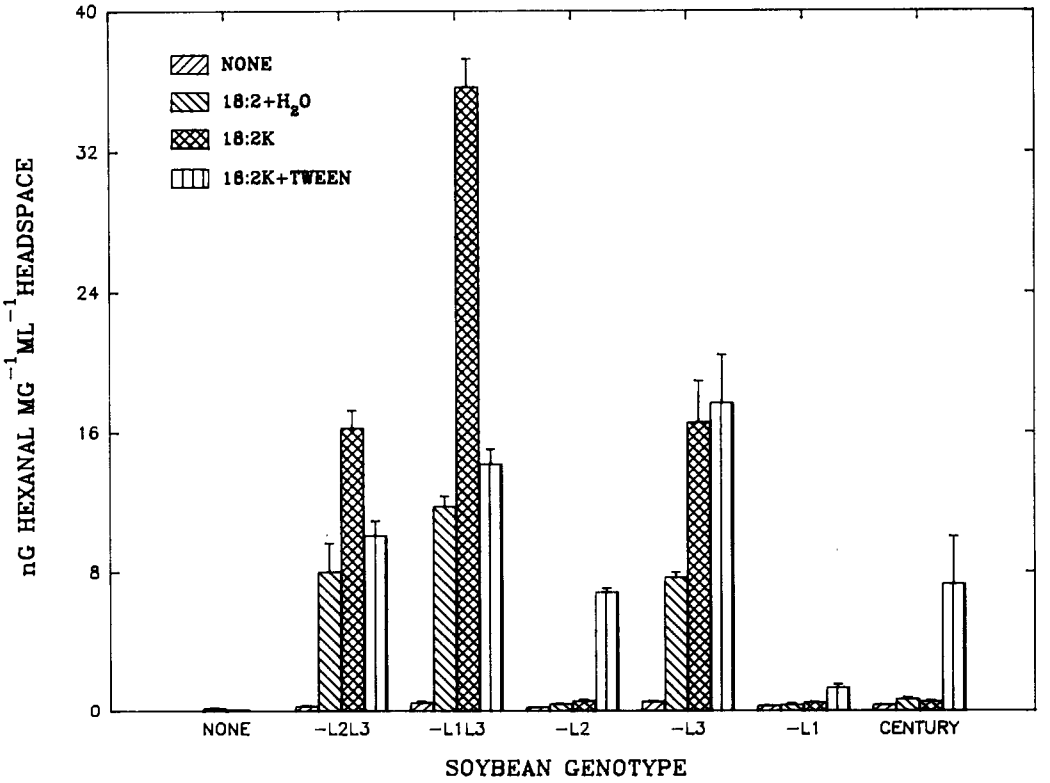

Figure 7. Effect of different 18:2 substrate forms on hexanal formation with soybean seed homogenates.

substrate (Brash et al., 1987; Guss et al., 1968; Eskola and Laakso, 1983; Yamauchi et al., 1985). Our results (Figures 6 and 7) show that the method of substrate preparation, and therefore the available form of substrate, had a large effect on lipid-dependent O_2 consumption.

LOX 1 is most effective in the hydroperoxidation of FFA, whereas LOX 2 and LOX 3 are relatively more effective in the hydroperoxidation of esterified or neutral fatty acids. Bild et al. (1977), to characterize isoenzymes of soybeans, examined the effect of substrate polarity with 18:2, 18:2 sulfate, and esterified 18:2 derivatives. They found that LOX 1 was most active when presented with charged substrates, but LOXs 2 and 3 were more effective with nonpolar substrates. The same phenomenon is also clear in our results (Figures 2 and 3). The presence of LOX 2 and LOX 3 in the soybean lines greatly increased the O_2 uptake with esterified 18:2 derivatives as substrates, whereas LOX 1 gave the largest O_2 consumption with FFA.

Free 18:2 was the best substrate for C_6 aldehyde production with LOX 1 and LOX 2, and diacylglycerol, triacylglycerol, phospholipid, *trans*-18:2, and 18:1 did not promote formation of C_6 compounds with the system used here. The highest formation of C_6 aldehydes among FFA and esterified 18:2 derivatives was with free 18:2 (Figures 4 and 5). When these results are compared with those from O_2 uptake, it appears that FFA with only one hydroperoxy group might be the best substrate for hydroperoxide lyase.

LOX 2 was largely responsible for the generation of C_6 aldehydes with most of the substrates tested, and the line with LOX 3 reduced C_6 aldehyde formation. Matoba et al. (1985) reported that with 18:2 as the substrate, LOX 2 resulted in the highest yield of *n*-hexanal. In our present experiments we found similar results for other polyunsaturated FFA and esterified 18:2 derivatives except for CoA18:2, where LOX 1 was most effective, and 20:4, where LOX 1 had the same activity as LOX 2. LOX 3 was found to reduce C_6 aldehyde formation with all substrates tested. This finding is consistent with the study of Hildebrand et al. (1990), where LOX 3 was discovered to reduce hexanal production using free 18:2 and endogenous substrates.

Oxygen consumption was qualitatively similar to C_6 aldehyde production except for tri18:2 and di18:2 derivatives. However, the relationship between these chemical processes appears to be complex. As mentioned above, most of the lipid compounds used in our experiments caused lipid-dependent O_2 uptake and formation of C_6 aldehydes with the homogenates from mature soybean seeds. This indicates that polyunsaturated long-chain FFA with *cis,cis*-1,4-pentadiene structures and some of their esterified derivatives can be substrates for LOXs and that the hydroperoxides formed are substrates for lyase. Nevertheless, there exists a clear difference, especially in the relative quantity of C_6 aldehyde formation and lipid-dependent O_2 consumption with 18:2, 20:4, di18:2, and tri18:2. Our results show that while free 18:2 yielded the highest relative levels of C_6 aldehyde with LOX 2, its lipid-dependent O_2 uptake was lower than or similar to that of many other compounds (Figures 2 and 3). The highest O_2 uptake was seen with 20:4 and particularly tri18:2 and di18:2 derivatives, but much lower or no hexanal formation was observed. In addition, the presence of LOX 2 resulted in the largest yield of C_6 aldehydes, while the presence of LOX 3 resulted in significantly less yield of these compounds. In contrast, seeds containing LOX 3 exhibited increased O_2 consumption. It appears that LOX 3 competes with hydroperoxide lyase for the fatty acid hydroperoxides produced in the primary LOX reactions resulting in products unavailable for C_6 aldehyde generation (Hildebrand et al., 1990). LOX 3 may also reduce C_6 aldehyde production by converting a proportion of the fatty acid substrates into 9- rather than 13-hydroperoxides. The results reported here are biased toward effects of LOX 2 and LOX 3 since we were operating at or near the pH optima of these isozymes. At higher pHs, the highest C_6 aldehyde formation was associated with the presence of LOX 1 rather LOX 2 (data not shown).

These results indicate that a number of unsaturated fatty acids and their derivatives might be potential in vivo substrates for LOXs and may be involved in lipid-dependent O_2 consumption and C_6 aldehyde formation in plant tissues. However, the relationship between LOX and lyase in C_6 aldehyde formation is complex, and further studies are needed to elucidate the roles of these two groups of enzymes in C_6 aldehyde formation utilizing various lipid substrates. Further work is also needed to determine what the actual in vivo substrates for formation of C_6 aldehydes are.

ABBREVIATIONS USED

LOX, lipoxygenase; FFA, free fatty acid; 18:1, oleic acid; 18:2, linoleic acid; *trans*-18:2, *trans,trans*-linoleic acid; 18:3, α-linolenic acid; γ-18:3, γ-linolenic acid; 20:4 arachidonic acid; CoA18:2, linoleoyl coenzyme A; me18:2, methyl linoleate; propyl18:2, propyl linoleate; mono18:2, monolinolein; di18:2, dilinolein; tri18:2, trilinolein; MGDG, monogalactosyldiacylglycerol; PC18:2, L-α-dilinolein–phosphatidylcholine; None, only phosphate buffer with pH 6.8; 18:2 H_2O, 18:2 dissolved in water; 18:2K, 18:2 in 1 mM KOH; 18:2K + Tween, 18:2 in 1 mM KOH with 0.08% Tween 20 (polyoxylene–sorbitan monolaurate).

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