Conversion of α-Linolenic Acid to Dihydro(pero)xyoctadecatrienoic Acid Isomers by Soybean and Potato Lipoxygenases

Dai-Eun Sok[†] and Mee Ree Kim*

Department of Food and Nutrition, Chungnam National University, Daejeon 302-764, Korea

Difference in the conversion of α-linolenic acid into dihydroxyoctadecatrienoic acids by plant lipoxygenases was examined. Reduction of the products from the incubation of α-linolenic acid or 9(*S*)-hydroperoxyoctadecatrienoic acid with soybean lipoxygenase 1 or potato lipoxygenase gave rise to the formation of two 12-*cis* isomers and two 12-*trans* isomers of 9(*S*),16-dihydroxyoctadecatrienoic acid on the basis of UV and GC/MS spectra analyses and *cis* → *trans* isomerization analyses. Further studies indicated that 12-*cis* isomers of 9(*S*),16-dihydroxyoctadecatrienoic acid are derived from the reduction of 9(*S*),16-dihydroperoxyoctadecatrienoic acid, a product from another lipoxygenation of 9(*S*)-hydroperoxyoctadecatrienoic acid at C-16, whereas 12-*trans* isomers of 9(*S*),16-dihydroxyoctadecatrienoic acid are supposed to be formed mainly from an epoxide intermediate. In support of the latter assumption, 9(*S*),10-dihydroxyoctadecatrienoic acids were produced from the incubation of α-linolenic acid with potato lipoxygenase, and the homolytic cleavage of 9(*S*)-hydroperoxyoctadecatrienoic acid by hemoglobin was observed to produce two isomers of 12-*trans*-9(*S*),16-dihydroxyoctadecatrienoic acid as major dihydroxy acids. Whereas the exposure of α-linolenic acid to soybean lipoxygenase 1 gave rise to 12-*cis*-9(*S*),16-dihydroperoxyoctadecatrienoic acid, a double lipoxygenation product, as a major product, 12-*trans*-9(*S*),16-dihydroxyoctadecatrienoic acid isomers were obtained as predominant products in the incubation of potato lipoxygenase with α-linolenic acid.

Keywords: *Lipoxygenase; α-linolenic acid; dioxygenation; epoxide; 9-hydroperoxyoctadecatrienoic acid; 9,16-dihydroxyoctadecatrienoic acid*

INTRODUCTION

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) is a dioxygenase that catalyzes the conversion of polyunsaturated fatty acids containing a *cis,cis*-1,4-pentadiene system into hydroperoxydienoic acids (Hamberg et al., 1967). Thus, plant lipoxygenases were reported to oxygenate linoleic acid to form 9- and 13-hydroperoxyoctadecadienoic acids (Roza et al., 1973). Also, arachidonic acid was oxygenated by soybean lipoxygenase to form 15(*S*)-hydroperoxyeicosatetraenoic acid (15-HPETE) (Bild et al., 1977a), which was further converted into 8,15-dihydroperoxyeicosatetraenoic acid (8,15-diHPETE). Interestingly, polymorphonuclear cells were found to possess enzyme activities to convert arachidonic acid into 5-HPETE [5(*S*)-hydroperoxyeicosatetraenoic acid], and 15-HPETE, which were further lipoxygenated to generate various dihydroxy acids (Borgeat et al., 1979; Rådmark et al., 1980).

Whereas the double lipoxygenation of arachidonic acid is well established, the reports concerning that of linolenic acid are limited (Bild et al., 1977b; Kim et al., 1989). Earlier, α- and γ-linolenic acids were reported to be oxygenated by plant lipoxygenases to form 9- and 13-hydroperoxyoctadecatrienoic acids, respectively (Roza et al., 1973; Galliard et al., 1971). Structurally, it is possible that hydroperoxyoctadecatrienoic acid, which contains the 1,4-pentadiene structure, may be subjected to secondary lipoxygenation. Thus, 13(*S*)-hydroperoxy-6,9,11-octadecatrienoic acid was reported to be subjected to further oxygenation by soybean lipoxygenase to produce 6,13(*S*)-dihydro(pero)xy acids (Kim et al., 1989). Subsequently, 9(*S*)-hydroperoxy-10,12,15-octadecatrienoic acid [9(*S*)-HPOT] was shown to be further oxygenated to form 9(*S*),16-dihydroperoxy acids as major products (Sok et al., 1990). Separately, potato lipoxygenase was observed to produce a double hydroperoxidation product of α-linolenic acid, which was designated 11-*cis*-9,16-dihydro(pero)xyoctadecatrienoic acid (Grechkin et al., 1991).

In this publication, we report that α-linolenic acid and 9(*S*)-hydroperoxyoctadecatrienoic acid are transformed into 9(*S*),10-dihydroxyoctadecatrienoic acid [9(*S*),10-diHOT] and 9(*S*),16-dihydro(pero)xyoctadecatrienoic acid [9(*S*),16-diH(P)OT] isomers through both double oxygenation and unstable intermediate pathways. In comparison, it appears that soybean 9(*S*)-hydroperoxyoctadecatrienoic acid was more predominant in the incubation with potato lipoxygenase.

EXPERIMENTAL PROCEDURES

Materials. α-Linolenic acid (99%), linoleic acid (99%), soybean lipoxygenase (types I and V), hemoglobin (type I, bovine, 75% methemoglobin), Detapac, Brij 56, sodium borohydride, and glutathione were products of Sigma Chemical Co. Lithium hydroxide, palladium oxide, hexane, 2-propanol, and methanol of HPLC grade were supplied by Aldrich Chemical Co. Ethyl ether and TLC plates (silica gel, 20 μm, 5 × 20 cm, 254 nm) were from Junsei and Kanto Chemical Co., respectively. [1-^{14}C]-α-Linolenic acid (53.9 mCi/mmol) was a product of Amersham.

Assay of Lipoxygenase Activity. Lipoxygenase activity was determined at 235 nm using a spectrophotometer (Gilford Model 250), as previously described (Schewe et al., 1981; Sok et al., 1990). One unit is expressed as the activity of enzyme forming 1 μmol of oxygenation product/min.

Preparation of Soybean Lipoxygenase 1. Soybean lipoxygenase 1 was prepared by applying 50 mg of soybean lipoxygenase (type I, Sigma) on the DEAE-Sephacel column (Sigma, 1 × 20 cm) and eluting the column with a gradient

[†] Present address: 1-4-5 Daejeon Machine Depot, Daejeon P.O. Box 35, Korea.

0021-8561/94/1442-2703$04.50/0 © 1994 American Chemical Society

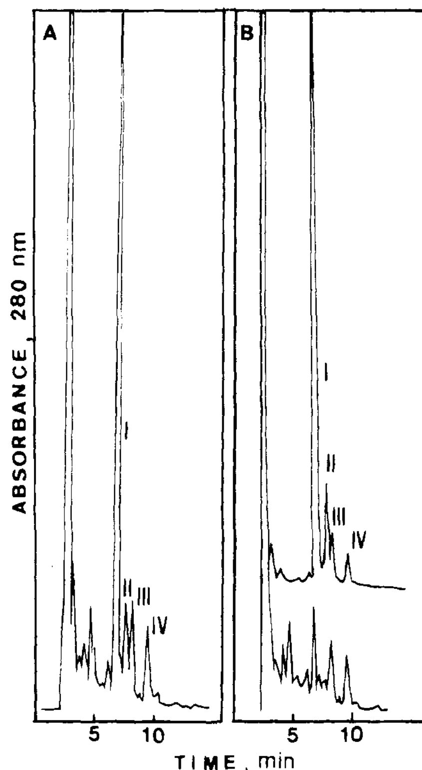


Figure 1. SP-HPLC profile of methyl esters of 9,16-diHOT derived from the incubation of α-linolenic acid with soybean lipoxygenase (type I). Methyl esters were applied onto a μ-Porasil colum (3.9 × 300 mm), which was eluted with hexane/2-propanol/acetic acid (95/5/0.1) at 1.5 mL/min. Compounds **I**, **II**, **III**, and **IV** are derived from peak I, II, III, and IV, respectively (A) The ether extract was reduced with sodium borohydride and methylated. (B) (Upper) The compound (R_f 0.4) from TLC separation of ether extract was reduced and methylated. (Lower) The compound (R_f 0.17–0.23) was methylated without reduction.

concentration (20–220 mM) of phosphate buffer, pH 6.8, as described (Axelrod et al., 1981).

Preparation of Potato Lipoxygenase. Potato lipoxygenase was prepared according to the method of Mulliez et al. (1987). The enzyme purified partially by DEAE-Sephacel chromatography was used as potato lipoxygenase.

Preparation of 9(S)-HPOT. α-Linolenic acid (5 mg) dissolved in 50 μL of ammonium hydroxide was suspended in 50 mL of 0.05 M phosphate buffer (pH 6.5), which was previously cooled to 4 °C. The incubation was started by the addition of soybean lipoxygenase 1 (3 units) or potato lipoxygenase and continued for 20 min at 20 °C. After acidification to pH 3.0, the reaction mixture was extracted with ethyl ether, and 9(*S*)-HPOT was separated and purified as described (Roza et al., 1973; Sok et al., 1990).

Incubation of α-Linolenic Acid with Plant Lipoxygenases. The incubation was started by adding 20 units of potato lipoxygenase to the 0.1 M phosphate buffer, pH 6.0 (50 mL), containing 25 mg of α-linolenic acid and continued for 20 min at 20 °C. The reaction with soybean lipoxygenase (type I, 14 units) was performed in 0.1 M phosphate buffer, pH 6.5 (50 mL) including 5 mg of α-linolenic acid. Separately, the potato lipoxygenase (20 units) was incubated with α-linolenic acid (25 mg) containing [1-^{14}C]-α-linolenic acid (5 μCi). The product from ether extraction was subjected to TLC analysis as described previously (Sok et al., 1990).

Incubation of 9(S)-HPOT with Plant Lipoxygenase or Hemoglobin. 9(*S*)-HPOT (1 mg) was incubated with 14 units of soybean lipoxygenase for 15 min in 25 mL of 0.1 N sodium borate buffer, pH 9.0 at 20 °C. Anaerobic incubation was carried out at 20 °C in the flask containing the above buffer, which was evacuated by water pump, cooled, and then brought to atmosphere by N_2 according to the method of Borgeat et al. (1979). Separately, the incubation of 9(*S*)-HPOT (1 mg) with potato lipoxygenase (20 units) was performed for 15 min in 0.1 M phosphate buffer, pH 6.0 (20 mL). The exposure of 9(*S*)-HPOT (500 μg) to hemoglobin (5 mg) was carried out in 30 mL of 0.1 M phosphate buffer, pH 7.0, as described before. The respective reaction was terminated by acidification (pH 3.5).

Cis → *trans* isomerization of 9,16-diHOT methyl ester was performed according to a thiyl radical reaction as described (Sok et al., 1990).

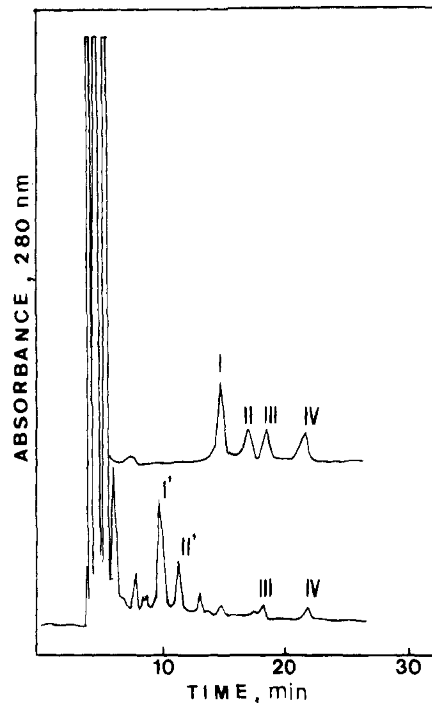


Figure 2. SP-HPLC profile of methyl esters of 9(*S*),16-diHOT produced from the exposure of 9(*S*)-HPOT to soybean lipoxygenase 1. The reaction product was methylated and applied onto a μ-Porasil column (3.9 × 300 mm) before (bottom) or after (top) sodium borohydride treatment. The column was eluted with hexane/2-propanol/acetic acid (95/5/0.1) at 0.8 mL/min.

Separation and Purification. The acidified samples were extracted with ethyl ether twice. The extract was dried by azeotropic evaporation with ethanol. The residue was dissolved in 2 mL of ethanol and reduced with a 10-fold molar excess of sodium borohydride at 4 °C for 30 min. The reduction products were subjected to TLC and HPLC analysis as described in the figure captions.

UV and GC/MS Spectrometry. Spectrometric analyses were performed as reported previously (Sok et al., 1990).

RESULTS

The ether extract from the incubation of α-linolenic acid with soybean lipoxygenase was subjected to TLC analysis, and both an upper zone (R_f 0.4), a peroxide reagent-positive (Bild et al., 1977a), and a downer zone (R_f 0.17–0.23), a peroxide reagent-negative, were shown to contain the compounds (total yield <5%), exhibiting a strong absorption at 267 nm. When the samples from the respective zones were reduced with $NaBH_4$, methylated, and analyzed by SP-HPLC (Figure 1), it was found that peaks I and II are derived mainly from the upper zone on the TLC plate, whereas peaks III and IV are largely from the lower zone. Especially, peak I appeared as a predominant product (60–90%), compared to the other peaks. In the following study (Figure 2), in which soybean lipoxygenase 1 was incubated with 9(*S*)-HPOT, it was found that peaks I′ and II′ were

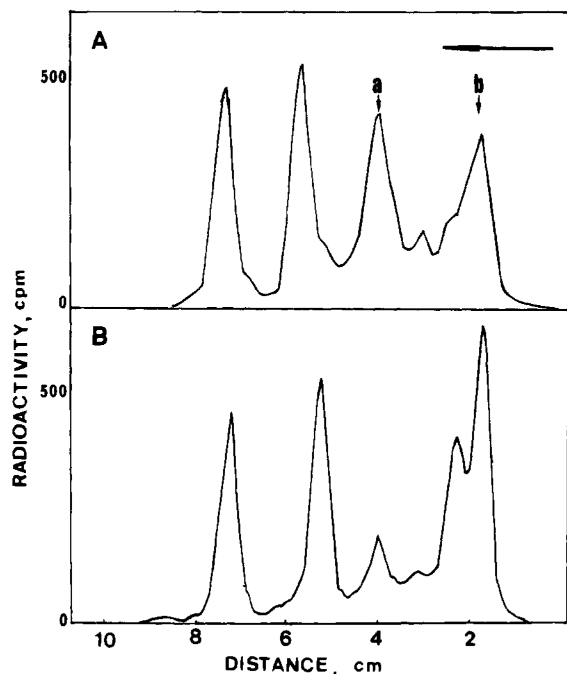


Figure 3. TLC profile of diH(P)OT isomers from the incubation of [1-^{14}C]linolenic acid with potato lipoxygenase preparation. (A) Reaction product was developed on TLC plate in petroleum ether/ethyl ether/acetic acid (50/50/1). (B) Reaction product was reduced with $NaBH_4$ prior to TLC analysis.

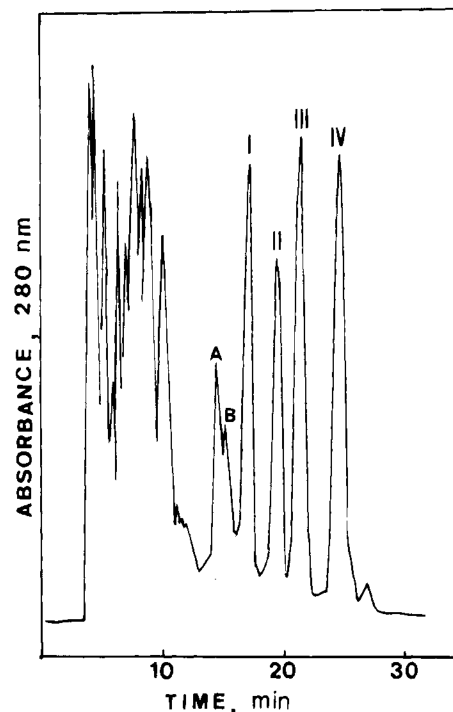


Figure 4. SP-HPLC profile of methyl esters of 9(*S*),16-diHOT derived from the exposure of α-linolenic acid to potato lipoxygenase preparation. The reaction product was reduced, methylated, and applied onto a μ-Porasil column (7.8 × 300 mm) eluted with hexane/2-propanol/acetic acid (95/5/0.1) at 3.0 mL/min.

derived mainly from reduction of peaks I and II, respectively, as reported previously (Sok et al., 1990). However, peaks III and IV, which appeared as major products under anaerobic condition (Sok et al., 1990), were produced by an unidentified pathway, which is supposed to involve an unstable intermediate.

In an attempt to define the latter pathway, we turned to the investigation with potato lipoxygenase, which had been reported to generate an unstable epoxide intermediate from 5(*S*)-hydroperoxyeicosatetraenoic acid (Shimizu et al., 1984). When the ether extract from the incubation of α-linolenic acid with potato lipoxygenase preparation was analyzed by radioactive isotope TLC analysis (Figure 3), the materials (R_f 0.17−0.23 and 0.4) exhibiting the characteristic UV absorption spectra of the conjugated trienoic acid were obtained with a yield of approximately 25%. The material a with an R_f value of 0.4, which gave a positive test on TLC plate with a peroxide-specific reagent, was found to be convertible into the material b with an R_f value of 0.17−0.23 after reduction. These results suggest that the materials from the upper zone (R_f 0.4) correspond to the peroxide forms of the compounds present in the lower zone as observed with soybean lipoxygenase 1. Compared to soybean lipoxygenase 1, potato lipoxygenase was found to produce a higher yield of a product with an R_f value of 0.17−0.23. When the material from ether extraction of the total reaction product was reduced with sodium borohydride, methylated, and subjected to SP-HPLC analysis, the products were resolved into at least six peaks (peaks A, B, I, II, III, and IV) as demonstrated in Figure 4. In comparative studies, peaks I, II, III, and IV were found to correspond to peak I, II, III, and IV, respectively, in the profile (Figure 1) observed with soybean lipoxygenase. Interestingly, peaks A and B appeared as new additional products, and peaks III and IV appeared as major products. In repeated experiments, peaks III and IV were greater than the other peaks.

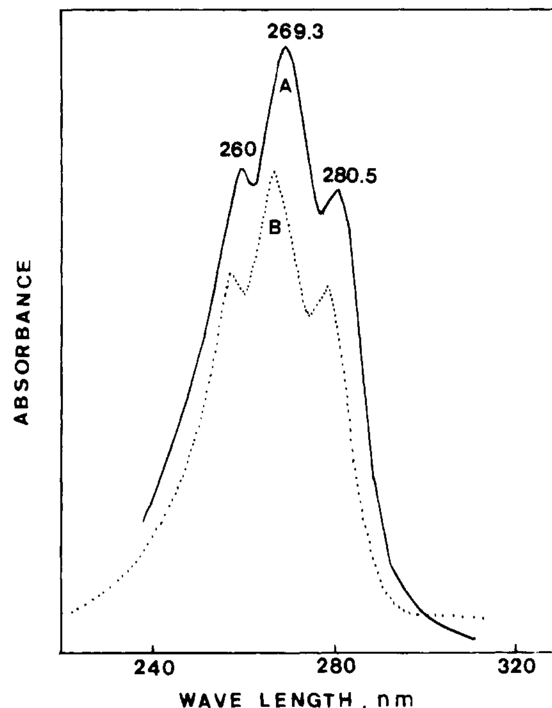


Figure 5. Respective UV spectrum of 9(*S*),10-diHOT methyl ester (A) and 9(*S*),16-diHOT methyl ester (B) purified by SP-HPLC. Spectra were recorded in methanol.

In UV absorption spectrum analysis (Figure 5A), the compounds from peaks A and B were found to possess a maximum absorption wavelength (λ_{max}) at 269.3 nm with shoulders at 280.5 and 260 nm, indicative of the presence of a conjugated triene in the structure. Compounds **A** and **B** were hydrogenated over palladium oxide, converted into the trimethylsilyl ether, and analyzed by GC/MS spectrometry. The mass spectrum

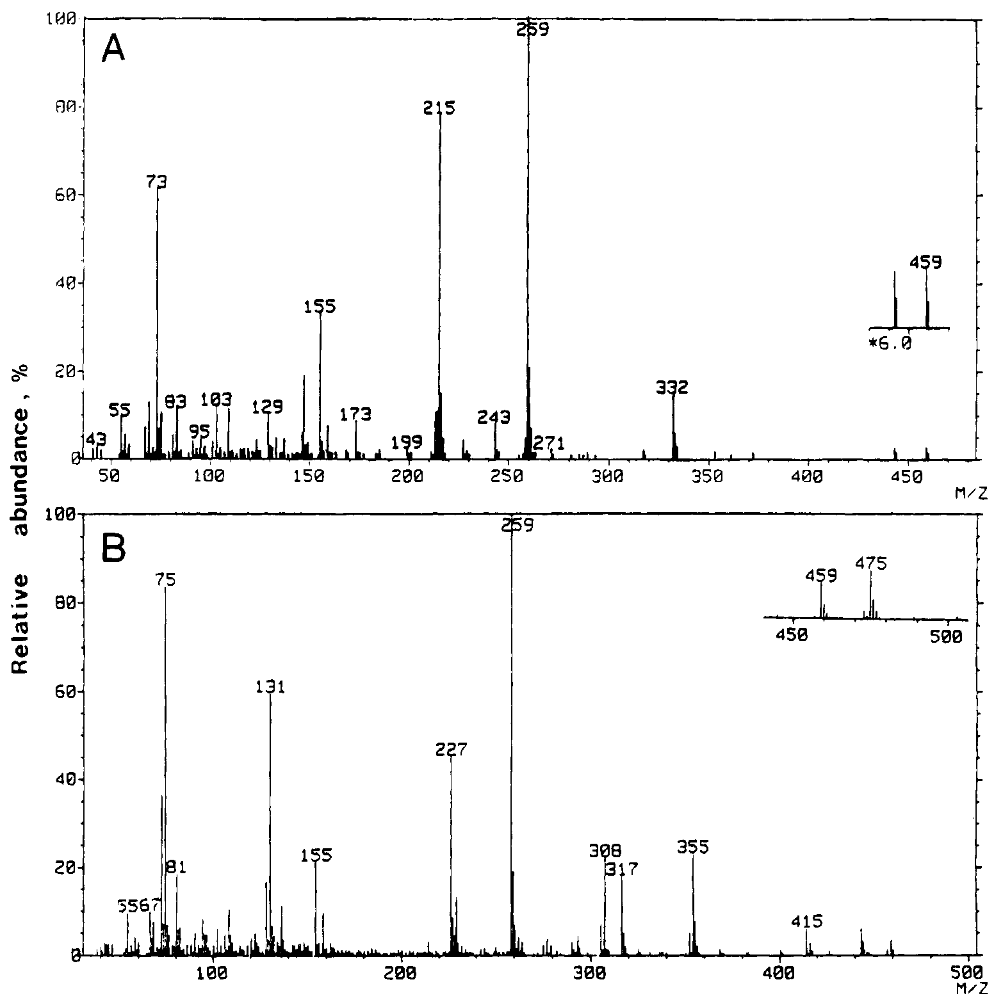


Figure 6. Respective mass spectrum of 9(*S*),10-diHOT methyl ester. An aliquot of peaks A and B (Figure 4) was hydrogenated over palladium oxide, trimethylsilylated, and subjected to GC/MS analysis.

(Figure 6A) showed prominent ions at *m/e* 459 (M − 15), 443 (M − 31), 361 [M − 113; loss of $^{\bullet}(CH_2)_7CH_3$], 259 [$(CH_3)_3SiO{=}CH(CH_2)_7COOCH_3$], 271 [M − (113 + 90)], and 215 [$(CH_3)_3SiO{=}CH(CH_2)_7CH_3$]. This fragmentation pattern is in good agreement with what would be expected for a saturated dihydroxyoctadecanoate. The positions of the hydroxyl groups at C-9 and C-10 were indicated by ions at *m/e* 259 and 215, respectively. On the basis of the UV spectrum and GC/MS analyses, it is suggested that two compounds (peaks A and B) correspond to isomers of 9(*S*),10-diHOT methyl ester. Meanwhile, the UV spectra of compounds **I**–**IV** were quite similar, with a maximum UV absorption at 266.9 nm (Figure 5B). While UV absorption spectra of compounds **I** and **II** showed shoulders at 257.4 and 277.4 nm, compounds **III** and **IV** exhibited shoulders at 257.4 and 278.7 nm, characteristic of a conjugated trienoic acid. When these derivatives were analyzed by GC/MS analyses (Figure 6B) after hydrogenation and trimethylsilylation, those exhibited identical mass spectra with characteristic ions at *m/e* 459 (M − 15), 443 (M − 31), 355 [M − (90 + 29); loss of CH_3SiOH and CH_2CH_3], 353 [M − (90 + 31)], 317 [M − 157; loss of $^{\bullet}(CH_2)_7COOCH_3$], 259 [$(CH_3)_3SiO{=}CH(CH_2)_7COOCH_3$], 227 [M − (157 + 90)], and 131 [$(CH_3)_3SiO{=}CHCH_2CH_3$]. Using CI mode, the ions at *m/e* 474 (M + 1) and 459 (M − 15; loss of $^{\bullet}CH_3$) were also observed. This fragmentation pattern is identical to that of a saturated 9,16-dihydroxyoctadecanoate as reported before (Sok et al., 1990). These data suggest that two isomers of 9(*S*),10-diHOT and four isomers of 9(*S*),16-diHOT are derived from potato lipoxygenase-catalyzed oxygenation of α-linolenic acid.

To elucidate the biosynthetic pathway for the potato lipoxygenase-catalyzed formation of diH(P)OT isomers from α-linolenic acid, the product from the incubation of α-linolenic acid with potato lipoxygenase preparation was methylated without reduction and directly subjected to SP-HPLC analyses (Figure 7, bottom), which showed the presence of additional triene peaks (peaks I′ and II′), compared to the profile (Figure 7, top) obtained after $NaBH_4$ reduction. These results indicate that peaks I′ and II′ are generated from double lipoxygenation of α-linolenic acid as observed in the incubation with soybean lipoxygenase 1. Thus, it is suggested that compounds **I** and **II** correspond to 12-*cis* isomers of 9(*S*),16-diHOT produced from the reduction of 12-*cis*-9(*S*),16-diHPOT isomers. To support the suggestion, compounds **I** and **II** were subjected to the treatment with a mixture of lithium hydroxide and glutathione, which had been utilized to catalyze the *cis* → *trans* isomerization of double bonds. As shown in Figure 8A, HPLC analysis demonstrates that peaks I and II migrated to peaks IV and III, respectively, after treatment with thiyl radicals, which are used for *cis* → *trans* isomerization. Accordingly, it is suggested that peaks III and IV correspond to *trans* isomers of peaks II and I, respectively. In further experiments, 9(*S*)-HPOT was exposed to hemoglobin, which had been reported to form *trans* isomers of dihydroxy acids as major products in

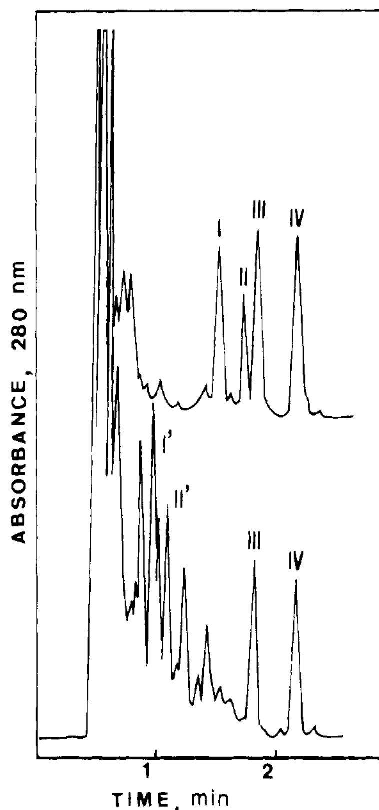


Figure 7. SP-HPLC profile of the reaction products derived from the incubation of α-linolenic acid (5 mg) with potato lipoxygenase. The ether extract was reduced with sodium borohydride, methylated, and applied onto a μ-Porasil column (3.9 × 300 mm) before (bottom) or after (top) sodium borohydride treatment. The elution condition is the same as described in Figure 2.

the homolytic cleavage of hydroperoxy conjugated acids (Sok et al., 1983). As shown in Figure 8B, peaks III and IV turned out to be predominant. These results suggest that peaks III and IV correspond to 12-*trans* isomers of 9(*S*),16-diHOT methyl ester isomers. In the quantitative analysis, it turned out that whereas a major part (60−90%) of peaks III and IV was produced without the $NaBH_4$ treatment, some portion (10−40%) came from the reduction of materials from around peaks I′ and II′. HPLC analyses combined with TLC analyses exhibit that most of peaks III and IV was derived from the material with an R_f value of 0.17−0.23, a peroxide reagent-negative, and that a minor part was derived from the reduction of the material with an R_f value of 0.4. Therefore, it is assumed that a major part of peaks III and IV is formed through an unstable epoxy intermediate, presumably a product from a dehydration of 9(*S*)-HPOT. In support of the assumption, the peaks corresponding to isomers of 9(*S*),10-dihydroxyoctadecatrienoic acid methyl ester were observed in every experiment, although the relative quantity varied according to enzyme preparation.

DISCUSSION

Earlier, the hydroperoxy conjugated acids containing adjacent 1,4-pentadiene structures had been reported to be subjected to further hydrogen abstraction (Bild et al., 1977a,b; Rådmark et al., 1980; Kim et al., 1989) by soybean lipoxygenase 1. In this study, it is shown that potato lipoxygenase transforms α-linolenic acid into 12-*cis* and 12-*trans* isomers of 9(*S*),16-diHOT as achieved with soybean lipoxygenase 1. On the basis of the

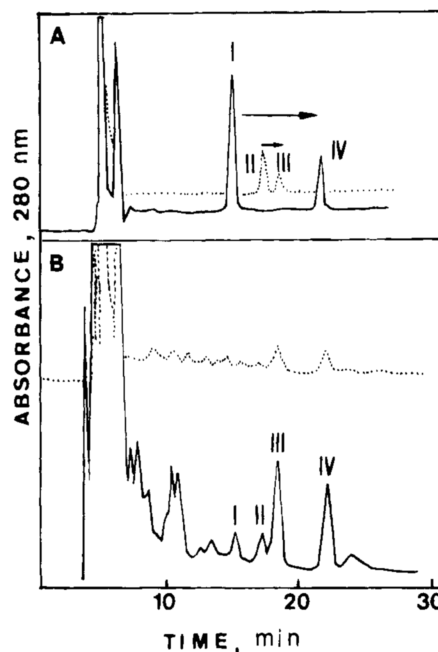
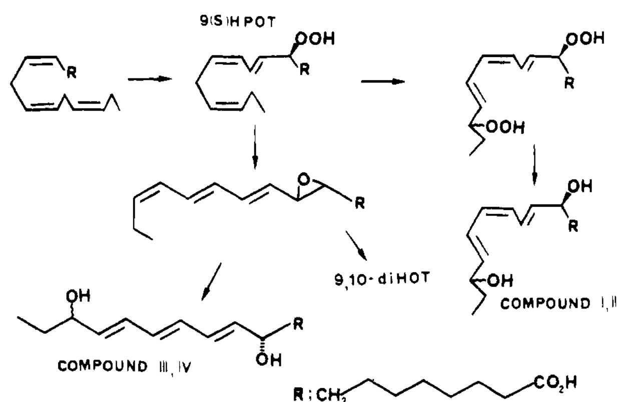


Figure 8. (A) SP-HPLC profile of 9.16-diHOT methyl ester isomers resulting from *cis* → *trans* isomerization. The isomerization product prepared as described under Materials and Methods was applied onto the SP-HPLC column, which was eluted as described in Figure 7. Isomerization product of compound **I** comigrated with compound **IV**, and the product from isomerization of compound **II** does with compound **III**. (B) SP-HPLC profile of diHOT methyl esters prepared from the exposure of 9(*S*)-HPOT to hemoglobin (—). Compound from the zone (R_f 0.17) on TLC plate was methylated and applied (- - -). The chromatography was carried out as described in Figure 7.

chromatographic behavior, UV spectrum, GC/MS spectrometry, and *cis* → *trans* isomerization analysis and in comparison with the profile after hemoglobin treatment, the structures of compounds **I** and **II** (Figure 4) are assigned as isomers of 9(*S*),16-dihydroxy-10(*E*),12-(*Z*),14(*E*)-octadecatrienoic acid [12-*cis*-9(*S*),16-diHOT] methyl ester, and the structures of compounds **III** and **IV** are designated isomers of 9(*S*),16-dihydroxy-10(*E*),-12(*E*),14(*E*)-octadecatrienoic acid [12-*trans*-9(*S*),16-diHOT] methyl ester. Most of the 12-*cis* isomers of 9(*S*),16-diHOT are derived from 12-*cis*-9(*S*),16-diHPOT isomers, which are produced from a secondary oxygenation of 9(*S*)-HPOT at C-16, on the basis of $NaBH_4$ reduction and a peroxide reagent analyses. This notion is consistent with the previous report that a double hydroperoxidation product of α-linolenic acid was 12-*cis*-9(*S*),16-diHPOT (Grechkin et al., 1991). This is further supported by the observation that under anaerobic conditions the formation of compounds **I** and **II** was markedly reduced (Sok et al., 1990). Although the configuration of a hydroxyl group at C-16 of 12-*cis*-9(*S*),-16-diHOT methyl ester was not determined by instrumental analysis, it is likely that compound **I** (Figure 1), which is a major product in the incubation of α-linolenic acid with soybean lipoxygenase 1, corresponds to 9(*S*),-16(*S*)-dihydroxy-10(*E*),12(*Z*),14(*E*)-octadecatrienoic acid, on the basis of the observation that stereospecific oxygenation by soybean lipoxygenase 1 produces almost exclusively the *S* enantiomer, irrespective of the position of the fatty acid carbon, which is oxygenated (Egmond et al., 1975; Van Os et al., 1981). Then, it follows that compound **II**, which behaves similarly to compound **I**, corresponds to 9(*S*),16(*R*)-dihydroxy-10(*E*),12(*Z*),14(*E*)-octadecatrienoic acid. Although 12-*cis*-9(*S*),16-diHOT

Scheme 1. Proposed Pathway for the Enzymatic Formation of 9(S),10- and 9(S),16-Dihydro(pero)xyoctadecatrienoic Acid Isomers from α-Linolenic Acid



isomers, double-lipoxygenation products, had been previously identified in the incubation of α-linolenic acid with soybean lipoxygenase 1 (Sok et al., 1990) or potato lipoxygenase (Grechkin et at., 1991), our findings first show that potato enzyme possesses an additional ability to convert α-linolenic acid to 12-*trans*-9(*S*),16-diHOT isomers and 9(*S*),10-diHOT isomers. Moreover, the predominance of 12-*trans*-9(*S*),16-diHOT isomers over 12-*cis*-9(*S*),16-diHOT isomers is in contrast to the result by Grechkin's group that only *cis* isomers of 9(*S*),16-diHOT were observed in the incubation of potato tuber lipoxygenase with α-linolenic acid. This might be due to a different preparation of lipoxygenase or a different incubation condition.

Meanwhile, it is suggested that 12-*trans* isomers of 9(*S*),16-diHOT methyl ester are derived largely from the unstable intermediate, presumably a 9,10-epoxide intermediate. The possible intermediacy of the epoxide is supported by the isolation of 9(*S*),10-dihydroxyoctadecatrienoic acid methyl ester isomers. Accordingly, it is proposed that potato lipoxygenase preparation may effectively produce 9(*S*),10-epoxy intermediate from 9(*S*)-HPOT, resembling the potato lipoxygenase-catalyzed elimination of D-hydrogen atom of 5(*S*)-HPETE at C-10 to form leukotriene A, an epoxide intermediate (Shimizu et al., 1984). Although the formation of the remaining part (10−40%) of 12-*trans*-9(*S*),16-diHOT isomers was not examined futher, it is possible that the rearrangement of a carbon-centered radical at C-14 can result in the formation of these isomers. Taken together, it is reasonable to deduce that the hydrogen abstraction of 9(*S*)-HPOT at C-14 is a common initiation step in the biosynthesis of 9(*S*),10-diHOT isomers of 9(*S*),16-diHOT isomers (Scheme 1). This mechanism is closely similar to that for mammalian lipoxygenase-catalyzed conversion of 15(*S*)-HPETE into 11-*cis* and 11-*trans* isomers of 8,15-diHETE and isomers of 14,15-diHETE (Rådmark et al., 1980).

The present studies demonstrate that whereas soybean lipoxygenase 1 favors the double lipoxygenation of α-linolenic acid, the dehydration of 9(*S*)-hydroperoxyoctadecatrienoic acid was more predominant in the potato lipoxygenase system. The difference in the biosynthetic pathway might be explained by the stereospecificity of plant lipoxygenases (Hamberg et al., 1967; Roza et al., 1973). Recently, it has been reported that jasmonic acid, a dehydration product of 13(*S*)-HPOT, is involved in wound response (Albrecht et al., 1993). Although the physiological role of these hydroxy acids was not elucidated here, these dihydroxy acid isomers may play a regulatory role in plants.

LITERATURE CITED

Albrecht, T.; Kehlen, A.; Stahl, K.; Knofel, H.-D.; Sembdner, G.; Weiler, E. N. Quantification of rapid, transient increases in jaasmonic acid in wounded plants using a monoclonal antibody. *Planta* **1993**, *191*, 86−94.

Axelrod, B.; Cheesbrough, T. M.; Laakso, S. Lipoxygenase from soybeans. *Methods Enzymol.* **1981**, *71*, 441−45.

Bild, G. S.; Ramadoss, C. S.; Lim, S.; Axelrod, B. Double dioxygenation of arachidonic acid by soybean lipoxygenase-1. *Biochem. Biophys. Res. Commun.* **1977a**, *74*, 949−954.

Bild, G. S.; Ramadoss, C .S.; Axelrod, B. Multiple Dioxygenation by lipoxygenase of lipids containing all-*cis*-1,4,7-octatriene moieties. *Arch. Biochem. Biophys.* **1977b**, *184*, 36−41.

Borgeat, P.; Samuelsson, B. Archidonic acid metabolism in polymorphonuclear leukocytes: Unstable intermediate in formation of dihydroxy acids. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 3213−3217.

Egmond, M. R.; Veldink, G. A.; Vliengenthart, J. F. G.; Boldingh, J. C-11 hydrogen-abstraction from linoleic acid, the rate-limiting step in lipoxygenase catalysis. *Biochim. Biophys. Acta* **1975**, *409* (3), 399−401.

Galliard, T.; Phillips, D. R. Lipoxyganase from potato tubers: Partial purification and properties of an enzyme that specially oxygenates the 9-position of linoleic acid. *Biochem. J.* **1971**, *124*, 431−488.

Grechkin, A. N.; Kuramshin, R. A.; Safonova, E. Y.; Yefremov, Y. J.; Latypov, S. K.; Ilyasov, A. V.; Tarchevsky, I. A. Double hydroperoxidation of α-linoleic acid by potato tuber lipoxygenase. *Biochim. Biophys. Acta* **1991**, *1081*, 79−84.

Hamberg, M.; Samuelsson, B. On the specificity of the oxygenation of unsaturated fatty acids catalyzed by soybean lipoxidase. *J. Biol. Chem.* **1967**, *242*, 5329−5335.

Kim, M. R.; Sok, D.-E. Formation of 6,13-dihydroxyoctadecatrienoic acid isomers from γ-linoleic acid. *Biochem. Biophys. Res. Commun.* **1989**, *157*, 1154−1160.

Mulliez, E.; Leblanc, J. P.; Girerd, J.-j.; Riguad, M.; Chottard, J.-C. 5-Lipoxygenase from potato tubers. Improved purification and physicochemical characteristics. *Biochim. Biophys. Acta* **1987**, *916*, 13−23.

Rådmark, O.; Malmsten, C.; Samuelsson, B. Leukotriene A_4: Enzymatic conversion to leukotriene C_4. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 1679−1687.

Roza, M.; Francke, A. Product specificity of soybean lipoxygenases. *Biochim. Biophys. Acta* **1973**, *316* (1), 76−82.

Schewe, T.; Wiesner, R.; Raport, S. M. Lipoxygenase from rabbit reticucytes. *Methods Enzymol.* **1981**, *71*, 430−431.

Shimizu, T.; Radmark, O.; Samuelsson, B. Enzyme with dual lipoxygenase activities catalyzes leukotriene A_4 synthesis from arachidonic acid. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 689−693.

Sok, D.-E.; Kim, M. R. Enzymatic formation of 9,16-dihydro(pero)xyoctadecatrienoic acid isomers from α-linoleic acid. *Arch. Biochem. Biophys.* **1990**, *277*, 86−93.

Sok, D.-E.; Chung, T.; Sih, C. J. Formation of 8,15-dihydroxyeicosatetraenoic acid isomers. *Biochem. Biophys. Res. Commun.* **1983**, *110*, 273−280.

Van Os, C. P. A.; Rijk-Schilder, G. P. M.; Halbeek, H. V.; Verhagen, J.; Vliegenthart, J. F. G. Double dioxygenation of arachidonic acid by soybean lipoxygenase-1, kinetics and regio-stereo specificities of the reaction step. *Biochim. Biophys. Acta* **1981**, *663*, 177−193.

Received for review March 14, 1994. Revised manuscript received June 28, 1994. Accepted August 30, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1994.