

Feedback activation of ferrous 5-lipoxygenase during leukotriene synthesis by coexisting linoleic acid

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Abstract Ferrous lipoxygenases seem to be activated through a feedback control mechanism via FA hydroperoxides generated from PUFAs by partially existing ferric lipoxygenases. However, during leukotriene synthesis, feedback activation of ferrous 5-lipoxygenase in the presence of arachidonic acid (AA) was not observed. In the present study, we examined the feedback activation of ferrous 5-lipoxygenase in the 5-lipoxygenase/AA system in the presence of linoleic acid (LA), which is a predominant component of membrane phospholipids. When potato 5-lipoxygenase was incubated with AA and LA in the presence of nitroxyl radical, 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-*N*-oxyl (CmΔP), one-electron redox cycle reaction between ferric and ferrous 5-lipoxygenase was detected. For each revolution of the cycle, one molecule of PUFA and one molecule of its hydroperoxide were converted into PUFA-allyl radical-CmΔP adduct ([PUFA-H]-CmΔP) and PUFA-epoxyallyl radical-CmΔP adduct ([PUFA-H+O]-CmΔP), respectively. The ratios, [AA-H]-CmΔP/[LA-H]-CmΔP and [AA-H+O]-CmΔP/[LA-H+O]-CmΔP, were estimated to be 1.7 and 0.13, respectively. These facts indicate that ferrous 5-lipoxygenase is activated through feedback control in the presence of LA, and that resulting ferric 5-lipoxygenase catalyzes the stoichiometric synthesis of leukotrienes from AA. **In conclusion, the biosynthesis of leukotrienes is remarkably efficient.**—Takajo, T., K. Tsuchida, K. Ueno, and I. Koshiishi. Feedback activation of ferrous 5-lipoxygenase during leukotriene synthesis by coexisting linoleic acid. *J. Lipid Res.* 2007. 48: 1371–1377.

Supplementary key words isozyme • pseudoperoxidase reaction • hydroperoxides

It is generally accepted that lipoxygenases exist in vivo in an inactive ferrous form (1–3). In earlier reports, it was demonstrated that, in the reaction of soybean lipoxygenase (15-lipoxygenase) with linoleic acid (LA), accumulation of hydroperoxides started just after a specific time lag. Interestingly, the kinetics of soybean lipoxygenase reactions with PUFA was altered by supplementation with

exogenous hydroperoxides, which reduced the initial lag phase of the reaction. Namely, the lipoxygenase reactions in vivo appear to be regulated through feedback control system dependent on resulting hydroperoxides, which were produced by partially existing ferric lipoxygenase (4–6).

5-Lipoxygenase, which catalyzes leukotriene synthesis from arachidonic acid (AA), also exists in an inactive ferrous form in vivo. However, ferrous 5-lipoxygenase is hardly activated by feedback control mechanisms during reactions with AA (7–9). In addition, the pseudoperoxidase (lipo-hydroperoxidase) reaction of ferrous 5-lipoxygenase using 5-hydroperoxyeicosatetraenoic acid (5-HpETE) as a substrate wastes a part of AA that must be stoichiometrically converted to leukotrienes. Namely, feedback control of 5-lipoxygenase activation in the 5-lipoxygenase/AA system may be inefficient for the biosynthesis of the physiologically functional molecule. In contrast, PUFAs, which are essential components of phospholipids in biological membranes, consist of not only AA but also LA (10). LA is a suitable substrate for 5-lipoxygenase. Conclusively, we hypothesized that LA plays a role in the feedback activation of 5-lipoxygenase for efficient leukotriene synthesis.

In our previous reports (11, 12), we established a novel method for converting ferric lipoxygenase into its ferrous form. When soybean ferric lipoxygenase was incubated with PUFAs in the presence of nitroxyl radical, which selectively traps carbon-centered radicals, FA allyl radical on the ferrous lipoxygenase (13–15) was trapped by nitroxyl radical, generating ferrous lipoxygenase. This one-electron reduction of ferric lipoxygenase appeared to be linked to one-electron oxidation of ferrous lipoxygenase (pseudoperoxidase reaction), which converts hydroperoxy FA into FA alkoxy radical. Subsequently, the FA

Abbreviations: AA, arachidonic acid; CmΔP, 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-*N*-oxyl; FA, fatty acid; HpETE, hydroperoxyeicosatetraenoic acid; HpODE, hydroperoxyoctadecadienoic acid; LA, linoleic acid; LC, liquid chromatography; MS/MS, tandem mass spectrometry; PIS, precursor ion scanning; PUFA, polyunsaturated fatty acid; XIC, extracted ion chromatogram.

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alkoxyl radical immediately changes into epoxyallyl radical through intramolecular rearrangement (16, 17), and this carbon-centered radical is also trapped by nitroxyl radical. Therefore, each revolution of the one-electron redox cycle reaction produces a FA allyl radical-nitroxyl radical adduct and a FA epoxyallyl radical-nitroxyl radical adduct. These adducts are able to be quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using the precursor ion scanning (PIS) technique (18).

In the present study, we evaluated a function of LA in the feedback control of leukotriene synthesis, by using potato 5-lipoxygenase and the nitroxyl radical spin-trapping method.

EXPERIMENTAL PROCEDURES

Materials

Potato tuber 5-lipoxygenase and 9-hydroperoxyoctadecadienoic acid (9-HpODE) were obtained from Cayman Chemical (Ann Arbor, MI). Rabbit reticulocyte 15-lipoxygenase was obtained from BIOMOL Research Lab. Inc. Soybean lipoxygenase-1 (Type I-b), LA, and AA were obtained from Sigma Co. (St. Louis, MO). 13-Hydroperoxy-(9Z,11E)-octadecadienoic acid (13-HpODE) was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 3-Carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-*N*-oxyl (CmΔP) was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). The nitroxyl radical was recrystallized in ethanol before use. TSKgel ODS-80Ts QA and TSKguardgel ODS-80Ts were obtained from Tosoh Co. (Tokyo, Japan). Chelex® 100 Resin (100–200 mesh) was obtained from Bio-Rad Lab (Hercules, CA). All other chemicals were reagent grade.

LA and AA were chromatographically purified as follows: 2 ml of 50 mM FA in 50% acetonitrile were passed through two Sep-Pak Plus C18 columns (Waters Co., Milford, MA). FA on the

columns was eluted by water-acetonitrile gradient elution. These operations were performed in a nitrogen atmosphere. Contaminant hydroperoxide was eluted before FA. The FA fraction was evaporated, and the residue was dissolved in ethanol. The concentration was adjusted to 100 mM, and the solution was stored at -80°C .

FA-derived carbon-centered radical trapping by nitroxyl radical

FA-derived carbon-centered radicals generated in the PUFA/lipoxygenase system were trapped by nitroxyl radical, CmΔP, as follows: 20 μl of 2 mM PUFA emulsion in 0.1 M phosphate buffer (pH 7.4, treated with Chelex 100) containing 2% ethanol was mixed with 10 μl of 4 mM nitroxyl radical in 0.1 M phosphate buffer (pH 7.4, treated with Chelex 100) and 10 μl of lipoxygenase in 0.1 M phosphate buffer (pH 7.4, treated with Chelex 100) in a glass vial tube with screw cap (inner volume, 0.5 ml), which was incubated at 25°C for 10 min. The reaction solution was mixed with 160 μl of cold acetonitrile and centrifuged at 10,000 g for 1 min. The supernatant was subjected to LC-MS/MS-PIS.

HPLC analyses of FA-derived carbon-centered radical-nitroxyl radical adducts

The chromatographic conditions for the quantification of FA-derived carbon-centered radical-nitroxyl radical adducts are as follows: column, TSKgel ODS-80Ts QA 4.6 mm i.d. \times 150 mm with guard column, TSKguardgel ODS-80Ts (3.2 mm i.d. \times 15 mm); eluent, 75% acetonitrile containing 0.05% formic acid; flow rate, 1.0 ml/min; column temperature, 25 – 28°C . The on-line LC-MS/MS system consisted of the Agilent1100 HPLC system and API 4000® Triple Quadrupole LC-MS/MS system (Applied Biosystems/MDS Sciex; Concord, ON, Canada) equipped with an electrospray ion source (ESI). MS/MS conditions for API-4000 were as follows: polarity, positive; curtain gas, 50 psi; ion source gas 1, 30 psi; ion source gas 2, 70 psi; ion spray voltage, 5500 V;

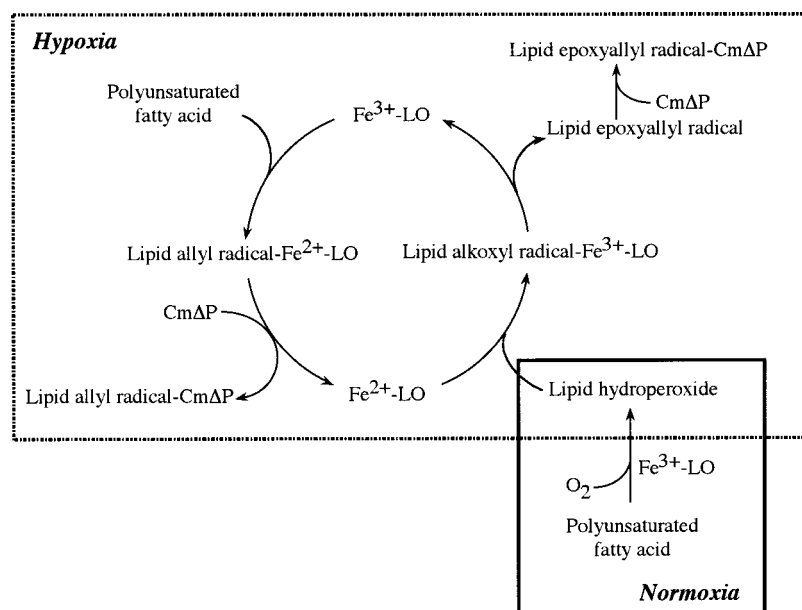


Fig. 1. One-electron redox cycle reaction between ferric lipoxygenase and ferrous one by hydrogen abstraction from polyunsaturated fatty acid as a result of the pseudoperoxidase reaction. CmΔP, 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-*N*-oxyl; LO, lipoxygenase.

temperature, 600°C; collision gas, 1.00; eclustering potential, 81 V; entrance potential, 10 V; collision cell exit potential, 15 V; collision energy, 30 V; channel electron multiplier, 2000 V; deflector, -100 V.

Effects of coexisting hydroperoxides on the generation of lipid-derived radical-nitroxyl radical adducts

Arachidonate allyl radicals generated in the 5-lipoxygenase/AA system containing HpODE were trapped with the nitroxyl radical, CmΔP, as follows: 10 μl of 4 mM AA emulsion in 0.1 M phosphate buffer (pH 7.4, treated with Chelex 100) containing 4% ethanol was mixed with 10 μl of HpODE in 0.1 M phosphate buffer (pH 7.4, treated with Chelex 100), 10 μl of 4 mM CmΔP in 0.1 M phosphate buffer (pH 7.4, treated with Chelex 100) and 10 μl of 5-lipoxygenase solution in a glass vial tube with screw cap (inner volume, 0.5 ml), which was incubated at 25°C for 10 min. The reaction solution was mixed with 160 μl of cold acetonitrile and centrifuged at 10,000 *g* for 1 min at 0°C. The supernatant was subjected to HPLC with ultraviolet (UV)-detection at 234 nm.

RESULTS

When 0.1 M phosphate buffer solution (pH 7.4; 200–250 μM soluble oxygen) containing a concentrated suspension of PUFA and ferric lipoxygenase was incubated in

a sealed glass vial, the ratio of residual FA content to its hydroperoxide content reached 6:4. At the end of the reaction, lipoxygenase may exist in the form of FA allyl radical-ferrous lipoxygenase complex. As shown in **Fig. 1**, coexisting nitroxyl radical (CmΔP) scavenges the FA allyl radical on the lipoxygenase, producing FA allyl radical-nitroxyl radical adducts and ferrous lipoxygenase (11). Subsequently, the ferrous lipoxygenase should be reoxidized to ferric one by cycling hydroperoxides through a pseudoperoxidase reaction, generating a FA alkoxy radical, which may be intramolecularly rearranged by the addition of a double bond to form the FA epoxyallyl radical (carbon-centered radical) (16, 17). Nitroxyl radical can trap FA epoxyallyl radical to the FA epoxyallyl radical-nitroxyl radical adduct through radical-radical conjunction (12). Namely, each revolution of the redox cycle reaction between ferrous lipoxygenase and ferric one produces one FA allyl radical-nitroxyl radical adduct and one FA epoxyallyl radical-nitroxyl radical adduct.

FA-derived carbon-centered radical-CmΔP adducts are commonly cleaved into a hydroxylamine form of CmΔP (molecular mass, 184) and residual cationic FA derivatives (12). Therefore, it is possible to selectively detect adducts by LC-MS/MS with PIS for *m/z* 185.

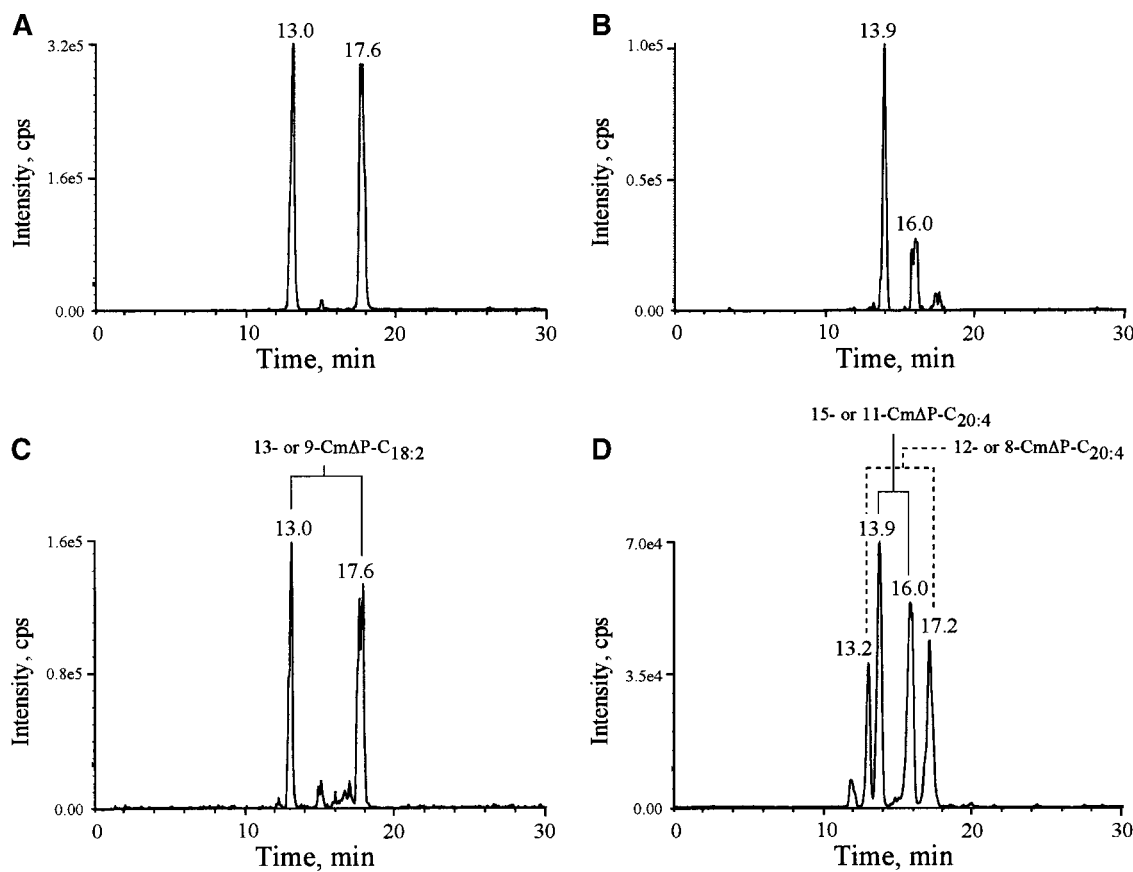


Fig. 2. Total ion chromatograms of CmΔP-spin trapping adducts in polyunsaturated fatty acid/lipoxygenase/CmΔP systems in LC-MS/MS with precursor ion scanning for *m/z* 185. Phosphate buffer solution (pH 7.4) containing 1 mM polyunsaturated fatty acid, 1 mM CmΔP, and lipoxygenase (15-lipoxygenase, 283 units; 12/15-lipoxygenase, 500 units) was incubated at 25°C for 10 min. A: adducts in 15-lipoxygenase/LA/CmΔP system. B: adducts in 15-lipoxygenase/AA/CmΔP system. C: adducts in 12/15-lipoxygenase/LA/CmΔP system. D: adducts in 12/15-lipoxygenase/AA/CmΔP system.

The intermediate carbon-centered *E/Z*-pentadiene radical on lipoxygenase undergoes resonance stabilization into two positionally isomeric pentadiene radicals; $-\text{CH}=\text{CH}-\dot{\text{C}}\text{H}=\text{CH}-$ and $-\text{CH}-\text{CH}=\text{CH}-\dot{\text{C}}\text{H}=\text{CH}-$. Nitroxyl radical can bind to both sides of the pentadiene moiety, generating two regioisomers of FA allyl radical-nitroxyl radical adduct. For example, in the lipoxygenase/LA/nitroxyl radical system, octadecadienoic acid substituted by nitroxyl radical at the C-9 or C-13 position should be generated. In the present study, we used soybean lipoxygenase-1 and rabbit reticulocyte lipoxygenase as 15-lipoxygenase and 12/15-lipoxygenase isozymes, respectively. LA was incubated with soybean 15-lipoxygenase or rabbit reticulocyte 12/15-lipoxygenase in the presence of Cm Δ P in phosphate buffer solution (pH 7.4), and the reaction solution was then subjected to LC-MS/MS-PIS. The extracted ion chromatograms (XIC) of *m/z* 463 corresponding to [LA-H]-Cm Δ P are shown in Fig. 2A, C. These results indicate that linoleate allyl radical-9-Cm Δ P and linoleate allyl radical-13-Cm Δ P were generated for both soybean 15-lipoxygenase and rabbit reticulocyte 12/15-lipoxygenase. It is of note that predominant hydroperoxide produced in the 15- or 12/15-lipoxygenase/LA system is 13-HpODE. Specifically, it was confirmed that the resultant 13-HpODE oxidizes ferrous 15- and 12/15-lipoxygenases to ferric ones through pseudoperoxidase reaction.

In a similar manner, AA was incubated with soybean 15-lipoxygenase or rabbit reticulocyte 12/15-lipoxygenase in the presence of Cm Δ P in phosphate buffer solution (pH 7.4) and the reaction solution was then subjected to LC-MS/MS-PIS. The XIC of *m/z* 487 corresponding to arachidonate allyl radical-Cm Δ P adducts are shown in Fig. 2B, D. As shown in Fig. 2B, two regioisomers corresponding to eicosatetraenoic acid substituted by Cm Δ P at the C-11 or C-15 position were produced in the 15-lipoxygenase/AA/Cm Δ P system. In contrast, in the 12/15-lipoxygenase/AA/Cm Δ P system, four regioisomers corresponding to eicosatetraenoic acid substituted by Cm Δ P at the C-11, C-15, C-8, or C-12 position were produced, because 12/15-lipoxygenase abstracts bisallylic hydrogen at the C-13 or C-10 position. Based on these facts, ferrous 15- and 12/15-lipoxygenase isozymes are activated to the ferric form via resultant 15-HpETE through a pseudoperoxidase reaction during positive feedback control.

In the present study, we used potato tuber 5-lipoxygenase as a representative 5-lipoxygenase. 5-Lipoxygenase dioxygenizes LA at the C-9, but not the C-13 position (19). Figure 3 shows total ion chromatograms (TIC). When the reaction solution of the 5-lipoxygenase/LA/Cm Δ P system was subjected to LC-MS/MS-PIS, linoleate allyl radical-9-Cm Δ P and linoleate allyl radical-13-Cm Δ P adducts were detected at 13.0 and 17.6 min (Fig. 3A). In contrast, in the 5-lipoxygenase/AA/Cm Δ P system, eicosatetraenoic acid substituted by Cm Δ P at the C-5 or C-9 position should be generated. Remarkably, arachidonate allyl radical-Cm Δ P adducts were not detected in the 5-lipoxygenase/AA/Cm Δ P system at all (data not shown). However, when purified LA was added to the 5-lipoxygenase/AA/Cm Δ P system, not only linoleate allyl radical-Cm Δ P adducts but

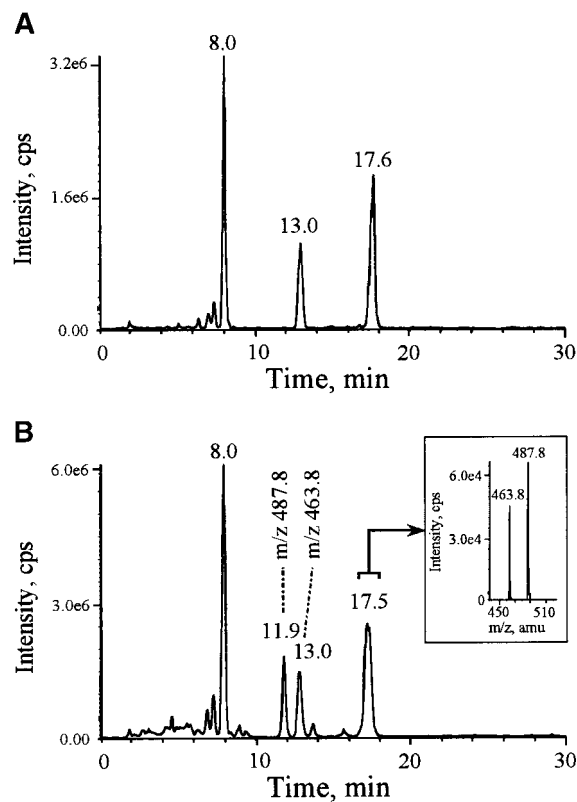


Fig. 3. Total ion chromatograms of Cm Δ P-spin trapping adducts in the polyunsaturated fatty acid/5-lipoxygenase/Cm Δ P systems in LC-MS/MS with precursor ion scanning for *m/z* 185. Phosphate buffer solution (pH 7.4) containing polyunsaturated fatty acid [1 mM LA (A); 0.5 mM linoleic acid and 0.5 mM AA (B)], 1 mM Cm Δ P, and 5-lipoxygenase (85 units) was incubated at 25°C for 10 min. A: adducts in 5-lipoxygenase/LA/Cm Δ P system. B: adducts in 5-lipoxygenase/LA/AA/Cm Δ P system. The inset represents MS spectrum of molecular ions corresponding to [M+H]⁺ of fatty acid allyl radical-Cm Δ P adducts eluted from 17 to 18 min.

also arachidonate allyl radical-Cm Δ P adducts were generated in the reaction solution (Fig. 3B). The cumulative MS spectrum of FA-derived carbon-centered radical-Cm Δ P adducts eluted over the period of 0–30 min and the XIC of *m/z* 487 are shown in Fig. 4. Interestingly, the content of arachidonate epoxyallyl radical-Cm Δ P adducts was less than that of linoleate epoxyallyl radical-Cm Δ P adducts. The ratio of [AA-H+O]-Cm Δ P/[LA-H+O]-Cm Δ P was estimated to be 0.13, whereas the ratio of [AA-H]-Cm Δ P/[LA-H]-Cm Δ P was estimated to be 1.7. Two regioisomers corresponding to eicosatetraenoic acid substituted by Cm Δ P at the C-5 or C-9 position were detected at 11.9 and 17.2 min. Based on these facts, it was elucidated that ferrous 5-lipoxygenase is not activated to ferric 5-lipoxygenase by 5-HpETE through a pseudoperoxidase reaction but is instead dependent on HpODE.

It is generally known that the predominant resulting hydroperoxides in the 15-lipoxygenase/LA system and the 5-lipoxygenase/LA system are 13-HpODE and 9-HpODE, respectively (19). The linoleate epoxyallyl radical-Cm Δ P adducts produced in the 15-lipoxygenase/LA/Cm Δ P system should be two regioisomers corresponding to ^{12,13}Δ-

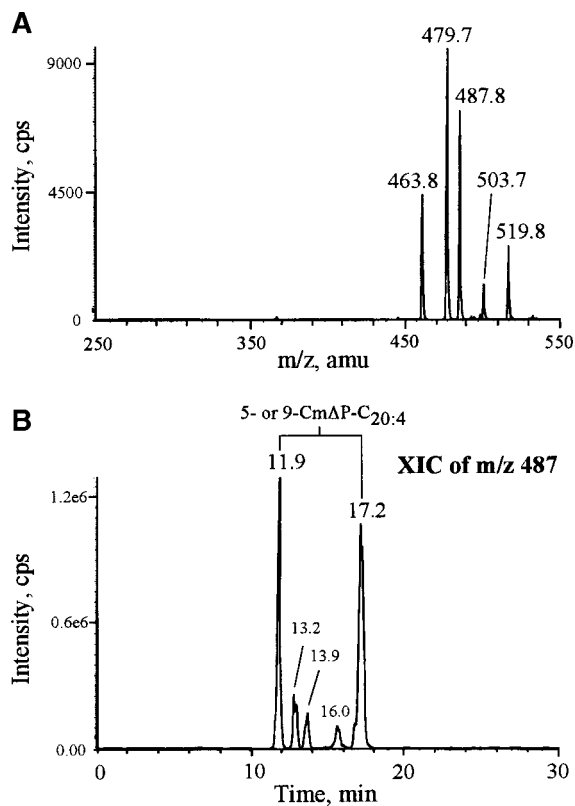


Fig. 4. The cumulative MS spectrum (A) of molecular ions corresponding to $[M+H]^+$ of fatty acid-derived radical-CmΔP adducts eluted from 0 to 30 min, and the extracted ion chromatogram (B) from the total ion chromatogram in LC-MS/MS with precursor ion scanning for m/z 185. Phosphate buffer solution (pH 7.4) containing polyunsaturated fatty acid (0.5 mM linoleic acid and 0.5 mM AA), 1 mM CmΔP, and 5-lipoxygenase (85 units) was incubated at 25°C for 10 min. XIC, extracted ion chromatogram.

epoxy-octadecamonoenoic acid substituted by CmΔP at the C-11 or C-9 position. In contrast, the linoleate epoxyallyl radical-CmΔP adducts produced in the 5-lipoxygenase/LA/CmΔP system should be two regioisomers corresponding to $^{9,10}\Delta$ -epoxy-octadecamonoenoic acid substituted by CmΔP at the C-11 or C-13 position. The proportion of both regioisomers should depend on the difference in the thermodynamic stability of corresponding radicals. **Figure 5** shows the elution profiles of linoleate-derived carbon-centered radical-CmΔP adducts. This result shows that linoleate epoxyallyl radical-CmΔP adducts derived from 13-HpODE are eluted at 6.4 and 7.1 min, whereas those from 9-HpODE are eluted at 8.0 min.

To confirm the activation of ferrous 5-lipoxygenase by HpODE but not HpETE, we examined whether supplementation of the 5-lipoxygenase/AA/CmΔP system with HpODE promotes the generation of arachidonate allyl radical-CmΔP adducts. Results are shown in **Fig. 6**. Both 9-HpODE and 13-HpODE appeared to convert ferrous 5-lipoxygenase into its ferric form, so adducts accumulated in an HpODE content-dependent manner. Approximately 20 μ M HpODE appears to be consumed through oxidation of ferrous lipoxygenase as well as contaminat-

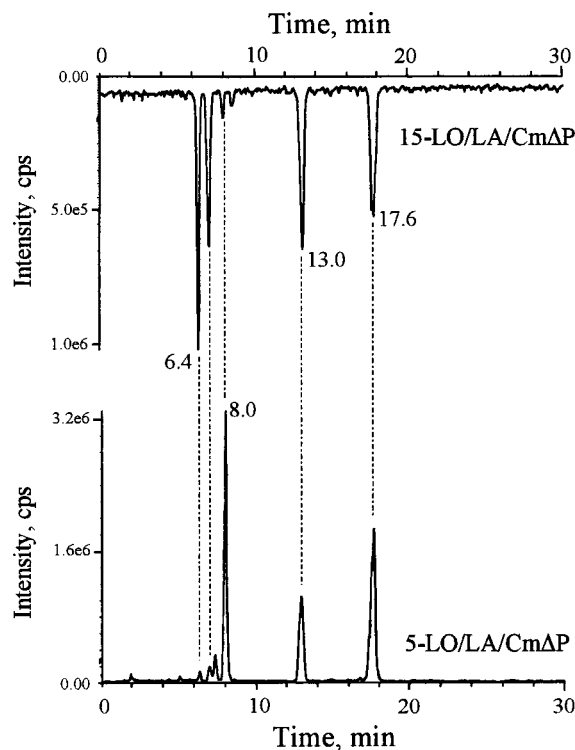


Fig. 5. Comparison of elution profile of linoleate-derived carbon-centered radical-CmΔP adducts in the 15-lipoxygenase/LA/CmΔP system (top) with that in the 5-lipoxygenase/LA/CmΔP system (bottom).

ing transition metals in the enzyme solution. It should be noted here that the HpODE content added was sufficient to convert ferrous 5-lipoxygenase into the ferric one. Therefore, 200–250 μ M 5-HpETE, which corresponds to the oxygen content in the reaction solution, should be generated in the system containing HpODE. These facts indicate that accumulated 5-HpETE cannot promote the

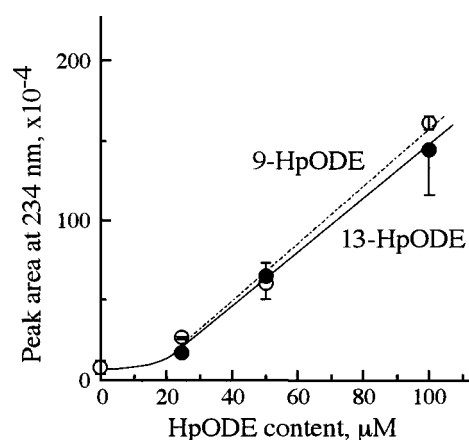


Fig. 6. Effects of coexisting hydroperoxyoctadecadienoic acid (HpODE) (open circle, 9-HpODE; closed circle, 13-HpODE) on arachidonate allyl radical-CmΔP adduct generation in the lipoxygenase/AA/CmΔP system. The arachidonate allyl radical-CmΔP adduct was detected by HPLC with UV (ultraviolet) detection at 234 nm. Results are mean \pm SD for triplicate individual experiments.

one-electron redox cycle reaction between ferrous 5-lipoxygenase and ferric one.

DISCUSSION

It is generally accepted that soybean ferrous lipoxygenase is converted into its ferric form through pseudoperoxidase reaction using linoleate hydroperoxide as a substrate. This is why the hydroperoxide content in the soybean lipoxygenase/LA system rises after a certain time lag. In the present study, we confirmed linoleate alkoxyl radical generation in the reaction of ferrous soybean 15-lipoxygenase with 13-HpODE via the nitroxyl radical spin-trapping method. In addition, we confirmed that mammalian ferrous 12/15-lipoxygenase (rabbit reticulocytes) was similarly activated in the lipoxygenase/LA system through feedback control. Furthermore, we observed feedback activation in both the soybean 15-lipoxygenase/AA system and rabbit reticulocyte 12/15-lipoxygenase/AA system, in which resultant 15-HpETE or 12-HpETE functions as a substrate for the pseudoperoxidase reaction. These facts indicate that substrate-specificity of ferrous 15- or 12/15-lipoxygenase against hydroperoxides is relatively low.

In contrast, feedback activation in the potato 5-lipoxygenase/AA system was not observed in the present study. Similarly, some reports show that a slight amount of 5-HpETE was produced when mammalian leukocyte 5-lipoxygenase was incubated with AA (7–9). In contrast, some investigators demonstrated that supplementation of the 5-lipoxygenase/AA system with exogenous HpETEs including 5-HpETE resulted in a significant increase in 5-HpETE content (8, 9). We hypothesize that this discrepancy is due to a reduced reactivity of 5-lipoxygenase for 5-HpETE. Indeed, Rouzer and Samuelsson (8) showed that the reaction efficiency of human leukocyte ferrous 5-lipoxygenase with 5-HpETE was significantly lower than 15- and 12-HpETE.

Maclouf, De Laclous, and Borgeat (7) demonstrated that coexisting platelets in leukocyte suspensions remarkably promoted the production of 5-HpETE and leukotrienes in the leukocytes. They indicated that 12-HpETE, which was produced in platelets via the 12-lipoxygenase reaction, played a key role not only in feedback activation but also in enhancement of Ca^{2+} -permeability through ionophore-like activity.

Upon stimulation of inflammatory cells including leukocytes, the lipoxygenase cascade is initiated by phospholipase A_2 -mediated PUFA release. Phospholipase A_2 represents a family of esterases that hydrolyze the *sn*-2 ester bond in phospholipids. In leukocytes, the predominant PUFAs at *sn*-2 position of phospholipids are LA and AA (10). Therefore, it is necessary to study feedback control in the 5-lipoxygenase/AA/LA system. It should be noted that potato 5-lipoxygenase was used as an analog of the mammalian enzyme because potato 5-lipoxygenase is similar to mammalian 5-lipoxygenase in terms of both lipoxygenase and leukotriene A_4 synthase activities (20). When 5-lipoxygenase was incubated with AA in the

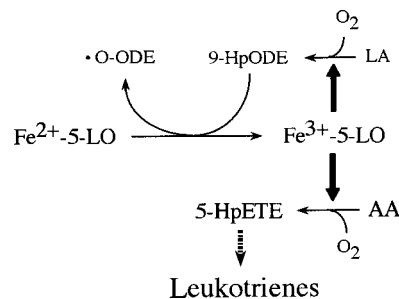


Fig. 7. Possible mechanism for feedback activation of ferrous 5-lipoxygenase in leukotriene synthesis by LA.

presence of equal amount of purified LA, the reactivity of ferric 5-lipoxygenase for AA was greater than LA, whereas the reactivity of ferrous 5-lipoxygenase for 9-HpODE was greater than 5-HpETE. As summarized in **Fig. 7**, partially existing ferric 5-lipoxygenase catalyzes 9-HpODE and 5-HpETE production from LA and AA, respectively. Resultant 9-HpODE specifically converts ferrous 5-lipoxygenase into ferric 5-lipoxygenase, and this conversion results in the promotion of 5-HpETE production, specifically the stoichiometrical production of leukotrienes from AA. **■**

REFERENCES

- Schilstra, M. J., G. A. Veldink, and J. F. G. Vliegthart. 1994. The dioxygenation rate in lipoxygenase catalysis is determined by the amount of iron (III) lipoxygenase in solution. *Biochemistry*. **33**: 3974–3979.
- De Groot, J. J. M. C., G. A. Veldink, J. F. G. Vliegthart, J. Boldingh, R. Wever, and B. F. Van Gelder. 1975. Demonstration by EPR spectroscopy of the functional role of iron in soybean lipoxygenase-1. *Biochim. Biophys. Acta*. **377**: 71–79.
- Ludwig, P., H. G. Holzhütter, A. Colosimo, M. C. Silvestrini, T. Schewe, and S. M. Rapoport. 1987. A kinetic model for lipoxygenases based on experimental data with the lipoxygenase of reticulocytes. *Eur. J. Biochem.* **168**: 325–337.
- Haining, J. L., and B. Axelrod. 1958. Induction period in the lipoxygenase-catalyzed oxidation of linoleic acid and its abolition by substrate peroxide. *J. Biol. Chem.* **232**: 193–202.
- Finazzi-Agro, A., L. Avigliano, G. A. Veldink, J. F. Vliegthart, and J. Boldingh. 1973. The influence of oxygen on the fluorescence of lipoxygenase. *Biochim. Biophys. Acta*. **326**: 462–470.
- Schilstra, M. J., G. A. Veldink, J. Verhagen, and J. F. G. Vliegthart. 1992. Effect of lipid hydroperoxide on lipoxygenase kinetics. *Biochemistry*. **31**: 7692–7699.
- Maclouf, J., B. F. De Laclous, and P. Borgeat. 1982. Stimulation of leukotriene biosynthesis in human blood leukocytes by platelet-derived 12-hydroperoxy-icosatetraenoic acid. *Proc. Natl. Acad. Sci. USA*. **79**: 6042–6046.
- Rouzer, C. A., and B. Samuelsson. 1986. The importance of hydroperoxide activation for the detection and assay of mammalian 5-lipoxygenase. *FEBS Lett.* **204**: 293–296.
- Riendeau, D., D. Denis, L. Y. Choo, and D. J. Nathaniel. 1989. Stimulation of 5-lipoxygenase activity under conditions which promote lipid peroxidation. *Biochem. J.* **263**: 565–572.
- Fisher, M., M. H. Johnson, A. M. Natale, and P. H. Levine. 1987. Linoleic acid levels in white blood cells, platelets, and serum of multiple sclerosis patients. *Acta Neurol. Scand.* **76**: 241–245.
- Koshiishi, I., K. Tsuchida, T. Takajo, and M. Komatsu. 2006. Radical scavenger can scavenge lipid alkyl radicals complexed with lipoxygenase at lower oxygen content. *Biochem. J.* **395**: 303–309.
- Koshiishi, I., K. Tsuchida, T. Takajo, and M. Komatsu. 2005. Quantification of lipid alkyl radicals trapped with nitroxyl radical via

HPLC with postcolumn thermal decomposition. *J. Lipid Res.* **46**: 2506–2513.

13. Nelson, M. J., S. P. Seitz, and R. A. Cowling. 1990. Enzyme-bound pentadienyl and peroxy radicals in purple lipoxygenase. *Biochemistry*. **29**: 6897–6903.
14. Nelson, M. J., R. A. Cowling, and S. P. Seitz. 1994. Structural characterization of alkyl and peroxy radicals in solutions of purple lipoxygenase. *Biochemistry*. **33**: 4966–4973.
15. Nelson, M. J., D. B. Chase, and S. P. Seitz. 1995. Photolysis of “purple” lipoxygenase: implications for the structure of the chromophore. *Biochemistry*. **34**: 6159–6163.
16. Iwahashi, H., C. E., Parker, R. P. Mason, and K. B. Tomer. 1991. Radical adducts of nitrosobenzene and 2-methyl-2-nitrosopropane with 12, 13-epoxylinoleic acid radical, 12,13-epoxylinolenic acid radical and 14,15-epoxyarachidonic acid radical: Identification by h.p.l.c.-e.p.r. and liquid chromatography-thermospray-m.s. *Biochem. J.* **276**: 447–453.
17. Garssen, G. J., G. A. Veldink, J. F. G. Vliegthart, and J. Boldingh. 1976. The formation of three-11-hydroxy-trans-12:13-epoxy-9-cis-octadecanoic acid by enzymatic isomerization of 13-L-hydroperoxy-9-cis,11-trans-octadecadienoic acid by soybean lipoxygenase-1. *Eur. J. Biochem.* **62**: 33–36.
18. Takajo, T., K. Tsuchida, T. Murahashi, K. Ueno, and I. Koshiishi. 2007. Intramolecular rearrangement of linolenate peroxy radicals in lipoxygenase reactions at lower oxygen content. *J. Lipid Res.* **48**: 855–862.
19. Galliard, T., and D. R. Phillips. 1971. Lipoxygenase from potato tubers: Partial purification and properties of an enzyme that specifically oxygenates the 9-position of linoleic acid. *Biochem. J.* **124**: 431–438.
20. Shimizu, T., O. Rådmark, and B. Samuelsson. 1984. Enzyme with dual lipoxygenase activities catalyzes leukotriene A₄ synthesis from arachidonic acid. *Proc. Natl. Acad. Sci. USA.* **81**: 689–693.