J. Biochem. **133**, 679–685 (2003)
DOI: 10.1093/jb/mvg087

The Formation of the 7-Carboxyheptyl Radical from 13-Hydroperoxy-9,11-Octadecadienoic Acid Catalyzed by Hemoglobin and Myoglobin under Anaerobic Conditions

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Received January 27, 2003; accepted March 11, 2003

Methemoglobin (MetHb), oxyhemoglobin (oxyHb), metmyoglobin (metMb), and oxymyoglobin (oxyMb) catalyze formation of the 7-carboxyheptyl and pentyl radicals from 13-hydroperoxy-9,11-octadecadienoic acid. The relative HPLC-ESR peak height of the pentyl radical to the 7-carboxyheptyl radical was found to depend on the oxygen concentration in the reaction mixture. Under aerobic conditions, the 7-carboxyheptyl radical was predominant for the reaction mixture with ferrous ions (or cytochrome c, metHb, or metMb). On the other hand, under anaerobic conditions, the pentyl radical was predominant for the reaction mixture with ferrous ions (or cytochrome c), but the 7-carboxyheptyl radical was still predominant for the reaction mixture with metHb (or metMb), suggesting that metHb (or metMb) catalyzes the reaction through a mechanism different from that in the case of ferrous ions (or cytochrome c). In order to explain the above results, a mechanism, in which molecular oxygen is not involved, is proposed for the formation of the 7-carboxyheptyl radical in the reaction mixture of 13-HPODE with metHb (or metMb) under anaerobic conditions.

Key words: free radical, hemoglobin, HPLC-ESR-MS, lipid peroxidation, myoglobin.

Abbreviations: 13-HPODE, 13-hydroperoxy-9,11- octadecadienoic acid; 4-POBN, α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone; ESI, electrospray ionization; Hb, hemoglobin; oxyHb, oxyhemoglobin; metHb, methemoglobin; Mb, myoglobin; oxyMb, oxymyoglobin; metMb, metmyoglobin.

Hemoglobin (Hb) and myoglobin (Mb) are not considered to be enzymes, but are rather classified as oxygen carrier proteins. However, Hb and Mb have been implicated in the catalysis of various reactions such as lipid peroxidation (*1–5*), decarboxylation of dopa (*6*, *7*), hydroxylation of aniline (*8–10*), generation of leukotriene intermediates, 5(S), 12-dihydroxy-6,8,10,14-eicosatetraenoic acid and 8, 15(S)-dihydroxy-5,9,11,13-eicosatetraenoic acid (*11*), conversion of 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid to hydroxy epoxide (*12*), and retinoic acid 5,6-epoxidation (*13*).

It is well known that ferryl Mb (or ferryl Hb) resulting from the reaction of H_2O_2 with ferric or ferrous Mb (or Hb) can cause damage to cell membranes, primarily through lipid peroxidation (*2–4*). This lipid peroxidation severely damages the membrane function. Such damage is considered to play a major pathological role in myocardial reperfusion injury after ischaemia. On the other hand, exhaustive exercise also results in increases in the concentrations of lipid peroxidation products (*14*) and serum Mb (*15*, *16*).

The mechanism of degradation of lipid hydroperoxides by hemoproteins has been extensively studied. Many mechanisms have been proposed. One of the most detailed of these involves the conversion, by hematin, of lipid hydroperoxides to alkoxyl radicals, which then go through a series of rearrangements and termination steps (*17–20*) (Scheme 1). The mechanism can be expressed as follows:

$$\text{Hematin}[Fe^{3+}] + \text{LOOH} \rightarrow \text{Hematin}[Fe^{4+}\text{–}OH^{-}]^{3+} + \text{LO}\cdot \quad (1)$$

$$\text{LO}\cdot \rightarrow \text{epoxyL}\cdot \quad (2)$$

$$\text{epoxyL}\cdot + \text{Hematin}[Fe^{4+}\text{–}OH^{-}]^{3+} \rightarrow \text{Hematin}[Fe^{3+}] + \text{epoxyLOH} \quad (3)$$

where Hematin[Fe^{3+}] and LOOH represent hematin and 13-HPODE. The 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radical, LO·, formed is rearranged into the 1-(7-carboxyheptyl)-4,5-epoxy-2-decenyl radical, epoxyL·. The reaction of the ferryl-hydroxo complex, Hematin[Fe^{4+}–$OH^{-}]^{3+}$, with epoxyL· results in the formation of 11-hydroxy-12,13-epoxy-9-octadecenoic acid, epoxyLOH. This mechanism explains some of the major components seen in gas chromatography-MS (GC-MS) studies, and also the loss of the UV signal associated with conjugated diene observed for some unsaturated lipid hydroperoxides such as 13-HPODE. On the other hand, a novel mechanism, in which 13-HPODE reacts with ferric Mb and ferryl Mb to form a redox cycle (Eqs. 4 and 5), has been proposed, based on the results of spectrophotometric, polarographic, and analytical investigation (*21*):

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$$LOOH + Mb(Fe^{III}) \rightarrow LO\cdot + Mb(Fe^{IV}) = O + H^{+} \quad (4)$$
$$Mb(Fe^{IV}) = O + LOOH \rightarrow LOO\cdot + Mb(Fe^{III}) + OH^{-} \quad (5)$$

where $Mb(Fe^{III})$, $Mb(Fe^{IV}) = O$, and $LOO\cdot$ represent metHb, ferryl Mb, and the 1-pentyl-12-carboxy-2,4-dodecadienylperoxyl radical, respectively.

In the present study, we have examined the reaction of 13-HPODE with Hb (or Mb) using ESR, HPLC-ESR (*22–24*), and HPLC-ESR-MS (*25*) with the spin trapping technique. Our results indicate a new path in addition to the mechanisms described above.

EXPERIMENTAL AND METHODS

Materials—α-(4-Pyridyl-1-oxide)-N-tert-butylnitrone (4-POBN) was purchased from Tokyo Kasei Kogyo (Tokyo). Bovine Hb was purchased from Nacalai Tesque (Kyoto). Linoleic acid, soybean lipoxygenase [EC 1.13.11.12] Type V, and equine skeletal muscle Mb were from Sigma Chemical (St. Louis, MO, USA). OxyHb and oxyMb were prepared according to the methods of Mieyal and Blumer (*9*). Ferrous ammonium sulfate was obtained from Kishida Chem. (Osaka). All other chemicals used were commercial products of the highest grade available.

Preparation of 13-Hydroperoxy-9,11-Octadecadienoic Acid—The reaction mixture contained, in a total volume of 25 ml, 1.5 mg/ml linoleic acid, 440 units/ml soybean lipoxygenase, and 0.2 M boric acid (pH 9.0). The reaction was performed at 25°C for 1h under air. The reaction mixture (25 ml) was acidified to pH 2.0 and then extracted with 20 ml diethyl ether. The extract was evaporated under reduced pressure and then subjected to a normal-phase HPLC (*26*). The normal-phase HPLC used consisted of a model 7125 Rheodyne injector (Reodyne, Cotati, CA, USA), a model Hitachi 655A-11 pump with a model L-5000 LC controller (Hitachi, Ibaragi), and a model SPD-M10AVP diode array detector (Shimadzu, Kyoto) with a model CLASS-LC10 LC workstation (Shimadzu, Kyoto). The SPD-M10AVP diode array detector in the HPLC system was operated from 200 nm to 350 nm. HPLC was performed on a Zorbax SIL column (250 mm × 9.4 mm) (Du Pont Company, Wilmington, DE, USA) with an n-hexane-diethyl ether-acetic acid mixture (v/v, 1,000: 150:1) at 20°C. The flow rate was 3 ml/min. When the HPLC profile was monitored at 234 nm, four prominent peaks (P_{46}, P_{56}, P_{60}, and P_{64}) were observed at retention times of 46 min, 56 min, 60 min, and 64 min, respectively. Based on the paper by Teng and Smith (*26*), we assigned the four peaks as follows: P46, 13-hydroperoxy-(9*Z*,11*E*)-octadeca-9,11-dienoic acid; P_{56}, 13-hydroperoxy-(9E,11E)-octadeca-9,11-dienoic acid; P_{60}, 9-hydroperoxy-(10*E*,12*Z*)-octadeca-10,12-dienoic acid; P_{64}, 9-hydroperoxy-(10*E*,12*E*)-octadeca-10,12-dienoic acid. We collected P_{46} and used it for the reactions. The concentration of 13-HPODE was determined from its absorbance at 234 nm (ε = 25,600 $cm^{-1}\,M^{-1}$) (*27*).

ESR Measurements—The ESR spectra were obtained with a model JES-FR30 Free Radical Monitor (JEOL, Tokyo). Samples were each aspirated into a Teflon tube centered in a microwave cavity. The operating conditions for the ESR spectrometer were: power, 4mW; modulation width, 0.1mT; center of magnetic field, 337.000mT; sweep time, 4min; sweep width, 10mT; time constant, 0.3s. Magnetic fields were calculated as the splitting of MnO (ΔH_{3-4} = 8.69 mT).

HPLC-ESR Analysis—The HPLC used for the HPLC-ESR consisted of a model 7125 injector (Reodyne, Cotati, CA, USA) with a 5ml sample loop, and a model 655A-11 pump with a model L-5000 LC controller (Hitachi, Ibaragi). A YMC packed C_{18} semi-preparative column (300 mm × 10 mm) (Yamamura Chemical Laboratry, Kyoto) was used. The column was kept at 20°C throughout the analyses. For the HPLC-ESR analyses, two solvent systems were used: solvent A, 50 mM ammonium acetate; solvent B, 50 mM ammonium acetate/acetonitrile (36:64, v/v). A combination of isocratic and linear gradients was used: 0–40 min, 100% A to 0% A (linear gradient) at a flow rate of 2.0 ml/min; 40–60 min, 0% A (isocratic) at a flow rate of 2.0 ml/min. The eluent was introduced into a model JES-FR30 Free Radical Monitor (JEOL, Tokyo). The ESR spectrometer was connected to the HPLC by a Teflon tube, which passed through the center of the ESR cavity. The operating conditions for the ESR spectrometer were: power, 4 mW; modulation width, 0.2 mT; time constant, 1 s. The magnetic field was fixed at the third ESR peak indicated by an arrow (Fig. 1) throughout the experiments.

HPLC-ESR-MS Analysis—The HPLC-ESR-MS consisted of a model 7125 injector (Reodyne Cotati, CA, USA), a model L-7100 pump (Hitachi, Ibaragi), a Water MicroBondapak C_{18} semi-preparative column (30 mm × 7.8 mm) (Millipore, Milford, MA, USA), a model JES-FR30 Free Radical Monitor (JEOL, Tokyo), and a model M-1200AP LC-MS system with an electrospray ionization (Hitachi, Ibaragi). The HPLC and ESR conditions for the HPLC-ESR-MS analysis were the same as those for the HPLC-ESR analysis. The operating conditions for the mass spectrometer were: nebulizer, 180°C; aperture 1, 120°C; N_2 controller pressure, 2.0 kg/cm^2; drift voltage, 70 V; multiplier voltage, 1,800 V; needle voltage, 3,000 V; polarity, positive; resolution, 48.

The control reaction mixture of 13-HPODE with metHb (or ferrous ions) was applied to the HPLC-ESR-MS. Mass spectra of peaks 1 and 2 were obtained by introducing the eluent from the ESR detector into the LC-MS system just before the respective peaks were eluted. The flow rate was kept at 50 μl/min while the eluent was introduced into the LC-MS system.

Control Reaction Mixtures of 13-HPODE with metHb (or oxyHb, metMb, oxyMb, Ferrous ions, Cytochrome c, or Hematin)—Control reaction mixtures of 13-HPODE contained 140 μM 13-HPODE, 0.1 M 4-POBN, various concentrations of metHb (or oxyHb, metMb, oxyMb, ferrous ions, cytochrome c, or hematin), and 40 mM phosphate buffer (pH 7.4). The reaction was started by adding metHb (or oxyHb, metMb, oxyMb, ferrous ions, cytochrome *c*, or hematin). The reaction was performed for 2 min at 20°C. Anaerobic conditions were obtained with a 3VP-C rotary pump (Hitachi, Tokyo) in Thunberg tubes.

RESULTS

ESR Measurements of the Reaction Mixtures of 13-HPODE with Methemoglobin (metHb)—ESR spectra of the complete reaction mixture of 13-HPODE with metHb, the complete reaction mixture without 13-HPODE, and

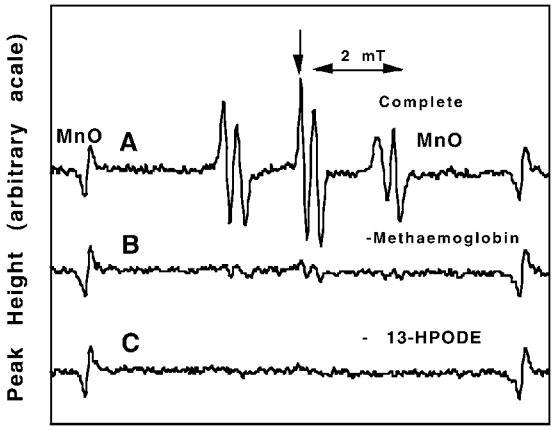


Fig. 1. **ESR spectra of the reaction mixture of 13-HPODE with metHb.** The reaction and ESR conditions were as given under "MATERIALS AND METHODS." The concentration of metHb was 2.1 μM. A, complete reaction mixture of 13-HPODE with metHb; B, without metHb; C, without 13-HPODE.

the complete reaction mixture without metHb were measured (Fig. 1). A six-line ESR spectrum was obtained for the complete reaction mixture of 2.1 μM metHb (Fig. 1A). The six-line ESR spectrum is composed of hyperfine splittings (a^N = 1.58 mT and $a^H\beta$ = 0.26 mT) from the nitrogen and β-hydrogen originating from 4-POBN itself. An ESR spectrum was hardly observed in the absence of metHb (or 13-HPODE) (Fig. 1, B and C).

A prominent ESR spectrum (a^N = 1.58 mT and $a^H\beta$ = 0.26 mT) was also observed for the complete reaction mixture of 2.1 μM oxyHb (or oxyMb or metMb) (data not shown). An ESR spectrum was hardly observed in the absence of 2.1 μM oxyHb (or oxyMb or metMb) (data not shown).

ESR spectra of the complete reaction mixture of 13-HPODE with metHb (or oxyHb, metMb, or oxyMb) were measured with various concentrations of metHb (or oxyHb, metMb, or oxyMb) (Fig. 2). The ESR peak height increased with an increase in the concentration of metHb (or oxyHb, metMb, or oxyMb). The ESR peak height for the reaction mixture of metHb (or oxyHb, metMb, or oxyMb) reached a plateau at 2.1 μM (or 6.6 μM, 6.2 μM, or 11.4 μM). The plateau may have appeared because 13-HPODE in the reaction mixtures was used up.

HPLC-ESR and HPLC-ESR-MS Analyses of the Radicals Formed in the Reaction Mixture of 13-HPODE with metHb (or Ferrous Ions)—HPLC-ESR analyses were performed for the complete reaction mixture of metHb (Fig. 3A), the complete reaction mixture without metHb (Fig. 3B), and the complete reaction mixture without 13-HPODE (Fig. 3C). In the HPLC-ESR elution profile of the complete reaction mixture of metHb, a prominent peak was observed at the retention time of 31.4 ± 0.6 min (peak 1) (Fig. 3A). Peak 1 was not observed in the elution profile of the complete reaction mixture without metHb (or without 13-HPODE) (Fig. 3, B and C). In order to identify the peak 1 compound, HPLC-ESR-MS analysis was performed for the complete reaction mixture of 13-HPODE with metHb (Fig. 4A). HPLC-ESR-MS analysis of the peak 1 compound gave ions at m/z 251 and m/z 338. The ion at m/z 338 corresponds to the protonated molecular

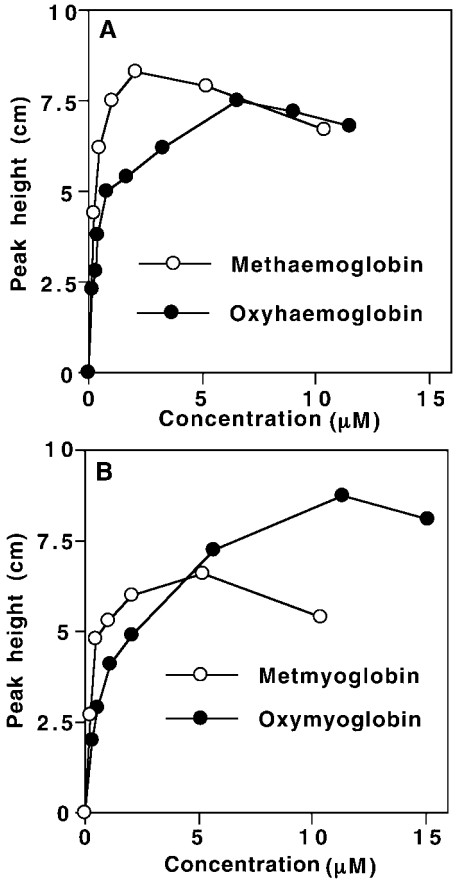


Fig. 2. **Hemoprotein concentration dependence of the ESR peak height.** The reaction and ESR conditions were as given under "MATERIALS AND METHODS." (A) metHb (open circles), oxyHb (solid circles); (B) metMb (open circles), oxyMb (solid circles).

ion of the 4-POBN/7-carboxyheptyl radical adduct $(M + H)^+$. The fragment ion at m/z 251 corresponds to the loss of $[(CH_3)_3C(O)N]$ from the protonated molecular ion. In order to determine the effect of the oxygen concentration on the formation of 13-HPODE derived-radicals, the reaction of 13-HPODE with metHb was performed under anaerobic conditions. A new peak (peak 2) appeared under anaerobic conditions, but peak 1 was still predominant (Fig. 3D).

HPLC-ESR analysis of the reaction mixture of 13-HPODE with ferrous ions was performed. Peak 1 (retention time, 31.6 ± 0.3 min) and peak 2 (retention time, 47.4 ± 0.4 min) were observed for the reaction mixture of ferrous ions with 13-HPODE under aerobic conditions. Peak 1 was predominant under aerobic conditions (Fig. 3E). On the other hand, peak 2 became predominant under anaerobic conditions (Fig. 3F). HPLC-ESR-MS analysis of the peak 2 compound gave ions at m/z 179 and m/z 266 (Fig. 4B). The ion m/z 266 corresponds to the protonated molecular ion of the 4-POBN/pentyl radical adduct $(M + H)^+$. The fragment ion at m/z 179 corresponds to the loss of $[(CH_3)_3C(O)N]$ from the protonated molecular ion.

The relative intensities of peak 1 to peak 2 were determined for the reaction mixtures of ferrous ions, hematin, and various kinds of hemoproteins, such as cytochrome c, metHb, and metMb, under aerobic conditions (or anaero-

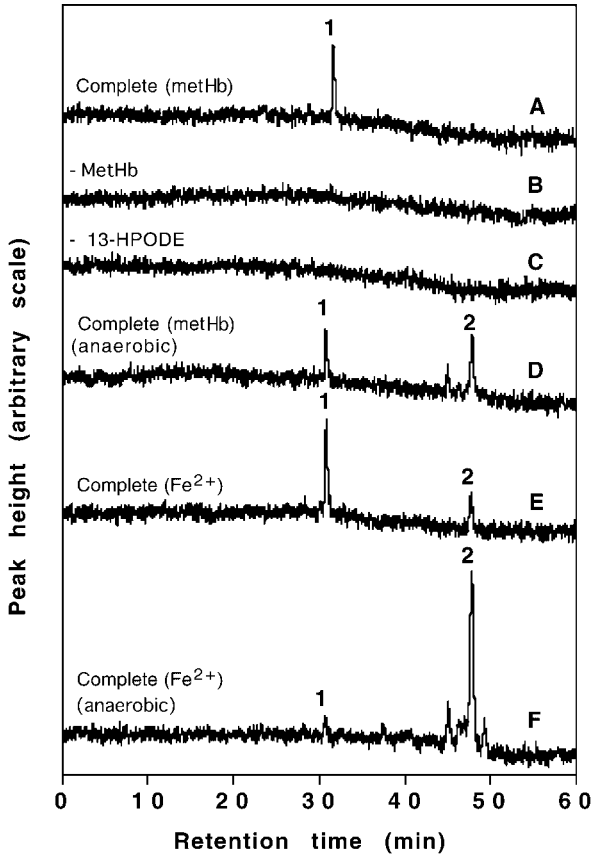


Fig. 3. **HPLC-ESR analyses of reaction mixture of 13-HPODE with metHb (or Fe^{2+}).** The reaction and HPLC-ESR conditions were as given under "MATERIALS AND METHODS." The total volume of the reaction mixture was 2.1 ml. The concentrations of metHb and ferrous ions were 2.1 μM and 67 μM, respectively. A, complete reaction mixture of metHb; B, without metHb; C, without 13-HPODE; D, complete reaction mixture of metHb under anaerobic conditions; E, complete reaction mixture of Fe^{2+}; F, complete reaction mixture of Fe^{2+} under anaerobic conditions.

bic conditions) (Fig. 3 and Table 1). Peak 1 was predominant for all the catalysts under aerobic conditions. Peak 2 was hardly detected for the reaction mixture of metHb (or metMb or hematin) under aerobic conditions (Fig. 3A and Table 1). Peak 2 became predominant for the reaction mixture of cytochrome c (or ferrous ions) under anaerobic conditions (Fig. 3F and Table 1). On the other hand, peak

Table 1. **Relative HPLC-ESR peak heights of peak 1 to peak 2 under aerobic conditions (or anaerobic conditions).**

Catalyst	Relative peak height (%)			
	Aerobic		Anaerobic	
	Peak 1	Peak 2	Peak 1	Peak 2
Fe^{2+}	71 ± 5	30 ± 5	11 ± 5	89 ± 5
Cytochrome *c*	79 ± 4	21 ± 4	20 ± 6	80 ± 6
MetHb	100	0	44 ± 16	47 ± 20
MetMb	95 ± 8	5 ± 8	62 ± 7	37 ± 9
Hematin	97 ± 6	3 ± 6	44 ± 10	56 ± 10

The reaction and HPLC-ESR conditions were as given under "MATERIALS AND METHODS." The concentration of metHb and metMb was 2.1 μM. The concentrations of Fe^{2+}, cytochrome *c*, and hematin were 67 μM, 50 μM, and 5 μM, respectively.

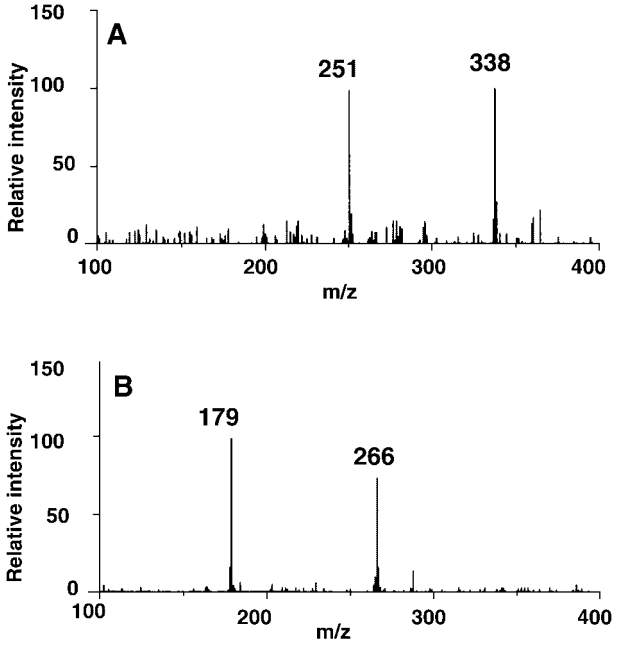


Fig. 4. **HPLC-ESR-MS analysis of the complete reaction mixture of 13-HPODE with metHb (or ferrous ions).** The reaction and HPLC-ESR-MS conditions were as given under "MATERIALS AND METHODS." The total volume of the reaction mixture was 4.2 ml. A, mass spectrum of peak 1 for the reaction mixture of 13-HPODE with metHb; B, mass spectrum of peak 2 for the reaction mixture of 13-HPODE with ferrous ions under anaerobic conditions.

1 was still predominant for the reaction mixture of metHb (or metMb or hematin) under anaerobic conditions (Fig. 3D and Table 1).

DISCUSSION

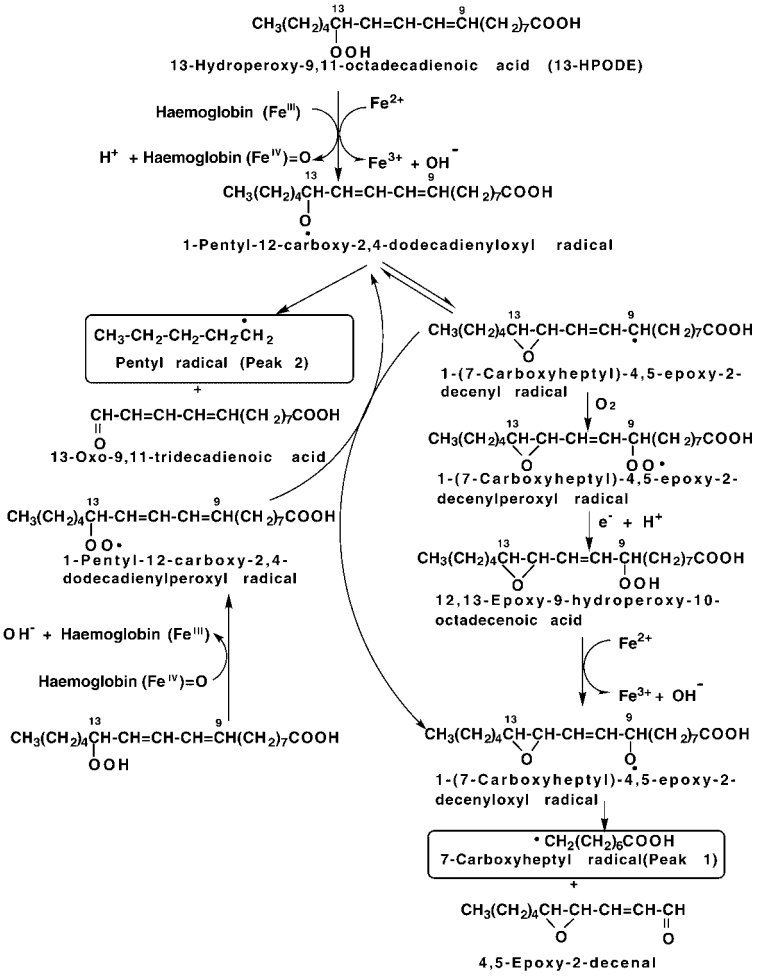
In this study, 4-POBN/7-carboxyheptyl radical and 4-POBN/pentyl radical adducts were detected and identified in a reaction mixture of 13-HPODE with metHb (or oxyHb, metMb, or oxyMb) on ESR, HPLC-ESR, and HPLC-ESR-MS. Possible reaction paths for the formation of these radicals (7-carboxyheptyl and pentyl radicals) are shown in Scheme 1.

Ferrous ions participate in the following reaction Eq. 6 (*28*):

$$LOOH + Fe^{2+} \rightarrow OH^- + LO\cdot + Fe^{3+} \quad (6)$$

where LOOH and LO· represent 13-HPODE (or 12,13-epoxy-9-hydroperoxy-10-octadecenoic acid) and the 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radical [or 1-(7-carboxyheptyl)-4,5-epoxy-2-decenyloxyl radical], respectively.

On the other hand, the 4-POBN/7-carboxyheptyl radical and 4-POBN/pentyl radical adducts were also both detected in the reaction mixture of 13-HPODE with metHb (or metMb or hematin) by HPLC-ESR-MS. As shown by Eq. 7, one electron reductive homolytic decomposition of a lipid hydroperoxide by metHb (or metMb or hematin) results in the formation of the 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radical, LO· (*29–31*).



Scheme 1. **One of the possible reaction paths for formation of the 7-carboxyheptyl radical (peak 1) and pentyl radical (peak 2).**

$$\text{LOOH} + \text{Hb(Fe}^{\text{III}}\text{)} \rightarrow \text{LO}\cdot + \text{Hb(Fe}^{\text{IV}}\text{)} = \text{O} + \text{H}^{+} \quad (7)$$

The pentyl radical is exclusively formed in the reaction mixture of 13-HPODE with ferrous ions under anaerobic conditions, suggesting that O_2 is essential for formation of the 7-carboxyheptyl radical in the reaction mixture of 13-HPODE with ferrous ions (or cytochrome c) (Scheme 1) (*32*, *33*). On the other hand, the 7-carboxyheptyl radical still forms in the reaction mixture of 13-HPODE with metHb (or metMb or Hematin) to some extent under anaerobic conditions. The above results suggest that the 7-carboxyheptyl radical is formed through a path in which molecular oxygen is not involved in the reaction mixture of 13-HPODE with metHb (or metMb or Hematin). We propose a possible reaction path for the formation of the 7-carboxyheptyl radical under anaerobic conditions in the reaction mixture of metHb (or metMb or Hematin) (Scheme 1). The reaction of 13-HPODE with metHb (or metMb or Hematin) results in the formation of ferryl Hb [Hb(Fe^{IV})=O] {or ferryl Mb [Mb(Fe^{IV})=O]} and the 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radical, LO· (Eq. 7). On the other hand, the 1-pentyl-12-carboxy-2,4-dodecadienylperoxyl radical, LOO·, is formed through the reaction of 13-HPODE with ferryl Hb [Hb(Fe^{IV})=O] {or ferryl Mb [Mb(Fe^{IV})=O]} (*21*) (Eq. 8).

$$\text{LOOH} + \text{Hb(Fe}^{\text{IV}}\text{)} = \text{O} \rightarrow \text{LOO}\cdot + \text{Hb(Fe}^{\text{III}}\text{)} + \text{OH}^{-} \quad (8)$$

The 1-pentyl-12-carboxy-2,4-dodecadienylperoxyl radical, LOO·, and the 1-(7-carboxyheptyl)-4,5-epoxy-2-decenyl radical, epoxyL·, formed from the 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radical, LO·, combine to form epoxyLOOL, which is easily decomposed into the 1-(7-carboxyheptyl)-4,5-epoxy-2-decenyloxyl radical, epoxyLO·, and 1-pentyl-12-carboxy-2,4-dodecadienyloxyl radical, LO· (Eqs. 9 and 10) (*34*, *35*).

$$\text{epoxyL}\cdot + \text{LOO}\cdot \rightarrow \text{epoxyLOOL} \quad (9)$$
$$\text{epoxyLOOL} \rightarrow \text{epoxyLO}\cdot + \text{LO}\cdot \quad (10)$$

β scission of the epoxyLO· results in formation of the 7-carboxyheptyl radical. The 1-pentyl-12-carboxy-2,4-dodecadienylperoxyl radical, LOO·, was not detected in this reaction mixture. Dikalov *et al*. recently showed that it is difficult to detect peroxyl radicals using the spin trapping technique (*37*). On the other hand, a lipid peroxyl radical intermediate has been directly detected on ESR spectroscopy with the fast-flow technique (*36*). Table 1 shows that the relative peak 1 intensity is weak for the reaction mixture of ferrous ions (or cytochrome c) under anaerobic conditions compared with in the case of MetHb (or MetMb or Hematin). The formation of the 1-pentyl-12-carboxy-2,4-dodecadienylperoxyl radical, LOO· (Eq. 8), is essential for the formation of peak 1. It may be hard for formation of the 1-pentyl-12-carboxy-2,4-dodecadienylp-

eroxyl radical, LOO· (Eq. 8), to occur in the reaction mixture of ferrous ions (or cytochrome *c*). The high-valence iron species (Fe^{IV})=O may not be generated effectively in a reaction mixture of ferrous ions (or cytochrome *c*) (*38*). A similar result was obtained for the 5,6-epoxydation by haemoproteins (*39*). Both Hb and Mb catalyzed the 5,6-epoxydation of retinoic acid, but neither ferrous ions nor cytochrome *c* did.

The participation of respiratory heme proteins in the oxidation of lipids is widely considered to be a major contributing factor in the pathology of many disorders, such as reperfusion injury, atherosclerosis, and kidney dysfunction associated with rhabdomyolysis (*40–43*). The lipid peroxidation catalyzed by hemoproteins may occur *in vivo* through the mechanism described above.

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