Lipoxygenases have been proposed to be a possible factor that is responsible for the pathology of certain diseases, including ischaemic injury. In the peroxidation process of linoleic acid by lipoxygenase, the \( E,Z \)-linoleate allyl radical–lipoxygenase complex seems to be generated as an intermediate. In the present study, we evaluated whether \( E,Z \)-linoleate allyl radicals on the enzyme are scavenged by radical scavengers. Linoleic acid, the content of which was greater than the dissolved oxygen content, was treated with soya bean lipoxygenase-1 (ferric form) in the presence of radical scavenger, CmP (3-carbamoyl-2,2,5,5-tetramethylpyrroline-\( N \)-oxyl). The reaction rate between oxygen and lipid allyl radical is comparatively faster than that between CmP and lipid allyl radical. Therefore a reaction between lipoxygenase allyl radical and CmP was not observed while the dioxygenation of linoleic acid was ongoing. After the dissolved oxygen was depleted, CmP stoichiometrically trapped linoleate-allyl radicals. Accompanied by this one-electron redox reaction, the resulting ferrous lipoxygenase was re-oxidized to the ferric form by hydroperoxylinoleate. Through the adduct assay via LC (liquid chromatography)–MS/MS (tandem MS), four \( E,Z \)-linoleate allyl radical–CmP adducts corresponding to regio- and diastereo-isomers were detected in the linoleate/lipoxygenase system, whereas \( E,E \)-linoleate allyl radical–CmP adducts were not detected at all. If \( E,Z \)-linoleate allyl radical is liberated from the enzyme, the \( E/Z \)-isomer has to reach equilibrium with the thermodynamically favoured \( E/E \)-isomer. These data suggested that the \( E,Z \)-linoleate allyl radicals were not liberated from the active site of lipoxygenase before being trapped by CmP. Consequently, we concluded that the lipid allyl radicals complexed with lipoxygenase could be scavenged by radical scavengers at lower oxygen content.

Key words: ischaemic injury, lipid allyl radical, lipid epoxyallyl radical, lipoxygenase, polyunsaturated fatty acid (PUFA), radical scavenger.

INTRODUCTION

PLA2 (phospholipase A\(_2\)) and lipoxygenase appear to be involved in ischaemic injury. Both enzymes play key roles in producing hydroperoxy fatty acids. In particular, upon stimulation of inflammatory cells, including macrophages, mast cells and neutrophilic granulocytes, elevation of cytosolic Ca\(^{2+}\) content results in the translocation of cytosolic 5-lipoxygenase and cytosolic PLA2 to the nuclear membrane. The lipoxygenase cascade is initiated by PLA2-mediated PUFA (polyunsaturated fatty acid) release. PLA2s represent a family of esterases that hydrolyse the sn-2 ester bond in phospholipids. The \textit{in vivo} experiments using selective inhibitors against PLA2s or PLA2\(^{-/-}\) mice indicated that Ca\(^{2+}\)-dependent cytosolic PLA2 plays a key role in ischaemic tissue injury [1–3]. Subsequently, PUFAs accumulated in ischaemic lesions are converted into cytotoxic metabolites by lipoxygenases just after reperfusion. The involvement of lipoxygenases in ischaemic injury was elucidated in the \textit{in vivo} experiments using selective inhibitors against lipoxygenase or lipoxygenase\(^{-/-}\) mice [4–6].

Lipoxygenases are non-haem iron dioxygenases that stereospecifically insert molecular oxygen into PUFAs, resulting in the formation of hydroperoxy fatty acids. Lipoxygenases have been thought to generate carbon-centred radicals during turnover. At the initial stage of lipid peroxidation by lipoxygenase, allylic hydrogen should be abstracted from PUFA accompanied by reduction of ferric lipoxygenase to the ferrous form. Subsequently, the resulting lipid allyl radicals on the enzyme encounter oxygen molecules. On this pathway, it has been speculated that lipoxygenase should exist in the form of the lipid allyl radical–lipoxygenase complex at lower oxygen content. In fact, EPR spectra revealed the existence of lipid allyl radicals that are bound to the enzyme and may be intermediates of the catalytic reaction [7–9].

Through these phenomena, it was thought that radical scavengers, which can scavenge the lipid allyl radicals complexed with ferrous lipoxygenase, should become prime candidates for drugs against ischaemic injury. This radical-scavenging reaction prevents the conversion of PUFAs into cytotoxic metabolites and transforms active ferrous lipoxygenase into the inactive ferrous form. Nitroxyl radicals, which have been used as terminators for the radical chain reaction in industrial polymer synthesis, possess an ability to scavenge carbon-centred radicals, producing \( O\)\text{-}alkyl aminoxyl [10]. Furthermore, the nitroxyl radicals were conveniently used as an indicator for \textit{in vivo} redox balance or free oxygen radical generation [11–13]. Since the reaction between lipid allyl radical and molecular oxygen (\( O\)\text{-}O\textsuperscript{•} \textit{is} radical–radical conjunction, nitroxyl radical (\( > N\)-O\textsuperscript{•}) seems to compete with molecular oxygen for lipid allyl radicals. In the present study, we evaluated a possibility for the nitroxyl radical as a radical scavenger against lipid allyl radicals on the enzyme by using a linoleic acid/soya bean lipoxygenase-1 system.

Abbreviations used: AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); CmP, 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrrolidine-\( N \)-oxyl; CmP, 3-carbamoyl-2,2,5,5-tetramethylpyrroline-N-oxyl; ESI, electrospray ionization; [LA], linoleic acid concentration; [LA-OOH], hydroperoxylinoleic acid concentration; LC, liquid chromatography; MS/MS, tandem MS; PLA2, phospholipase A\(_2\); PUFA, polyunsaturated fatty acid; TIC, total ion chromatogram; XIC, extracted ion chromatogram.

\(^1\) To whom correspondence should be addressed (email ikoshi@nichiyaku.ac.jp).
**EXPERIMENTAL**

**Materials**

Soya bean lipoxygenase-1 (Type I-b) and linoleic acid were purchased from Sigma. CmP (3-carbamoyl-2,2,5,5-tetramethylpyrroline-N-oxyl) and CmAP (3-carbamoyl-2,2,5,5-tetramethylpyrroline-N-oxyl) were purchased from Aldrich Chemical Co. TSKgel ODS-80Ts QA and TSKguardgel ODS-80Ts were purchased from Tosoh. Chelex® 100 resin (100–200 mesh) was purchased from Bio-Rad Laboratories. All other chemicals were of reagent grade.

**Lipid-derived radical trapping by nitroxyl radical**

Lipid-derived radicals generated in the linoleate/lipoxygenase system were trapped with CmP as follows. A 20 µl volume of 2 mM linoleic acid 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 CmP in 0.1 M phosphate buffer (pH 7.4; treated with Chelex® 100) and 10 µl of 4 µM soya bean lipoxygenase-1 in 0.1 M phosphate buffer (pH 7.4; treated with Chelex® 100) in a glass vial tube with a screw cap (inner volume, 0.5 ml), and then the solution was left to stand at room temperature (25–28°C) for 10 min. The reaction solution was mixed with 160 µl of cold acetonitrile and was centrifuged at 10 000 g for 5 min. The supernatant was then subjected to HPLC.

Lipid-derived radicals were chemically generated from linoleic acid or linolealaidic acid via lipophilic radical initiator, AMVN′-azobis(2,4-dimethylvaleronitrile), and the resulting lipid-derived radicals were trapped with CmP as follows. A 1 ml solution of acetonitrile containing 10 mM linoleic acid or linolealaidic acid, 10 mM CmP and 10 mM AMVN was left to stand in a glass vial with a screw cap (inner volume, 2 ml) at 37°C for 48 h. The reaction solutions were diluted 20 times with acetonitrile, and the solutions were subjected to HPLC.

**HPLC and LC-MS/MS (liquid chromatography-tandem MS) analyses of lipid-derived radical–nitroxyl radical adducts**

The HPLC assembly consisted of a HPLC pump (PU-2080; Jasco), a sample injector (7725; Rhodyne), a UV/visible spectrophotometer (L-2420; Hitachi), and a chromatointegrator (Chromatorder 21; SIC). The chromatographic conditions for the quantification of lipid-derived radical–nitroxyl radical adduct were as follows: column, TSKgel ODS-80Ts QA (4.6 mm internal diameter × 150 mm) with guard column, TSKguardgel ODS-80Ts (3.2 mm internal diameter × 15 mm); eluent, 0.05% methanolic (formic) acid containing 75% acetonitrile; flow rate, 1.0 ml/min; column temperature, 25–28°C; detection at 210 nm or at 234 nm.

The online LC–MS/MS system consisted of the Agilent1100 HPLC system and Q TRAP® LC–MS/MS system (Applied Biosystems/MDS Sciex) equipped with an ESI (electrospray ionization) source. The LC flow rate (1.0 ml/min) into the mass spectrometer inlet was adjusted to 200 µl/min with a splitter. Chromatographic conditions identical with those used for the HPLC with UV detection described above were used.

**EPR measurements of nitroxyl radical**

The disappearance of nitroxyl radicals was monitored using a spectrometer (JES-RE-1X; JEOL) operating at X-band (9.43 GHz), a microwave power of 10 mW, 100 kHz field modulation and 0.2 mT modulation width. A range of external magnetic field of 15 mT was swept at a scan rate of 3.75 mT/min. The amplitude of EPR spin signal (centre magnetic field) was used to estimate the reaction of nitroxyl radical with lipid-derived radicals.

A 20 µl volume of 2 mM linoleic acid 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 CmP in 0.1 M phosphate buffer (pH 7.4; treated with Chelex® 100). To the mixture, 10 µl of 4 µM soya bean lipoxygenase-1 in 0.1 M phosphate buffer (pH 7.4; treated with Chelex® 100) was added. The solution was aspirated into a disposable micropipette (100 µl; Drummond Scientific), and it was sealed with Terumo Seal (Terumo). The micropipette was subjected to X-band EPR.

**Quantification of lipid-derived carbon-centred radical–CmP adducts using the thermal decomposition method**

The reaction solution containing lipid-derived radical–CmP adducts was diluted twice with acetonitrile. The solution was transferred into a glass vial with a screw cap and was heated at 100°C. The solutions were subjected to EPR spectrometry. The conditions for the EPR measurements were as follows: microwave power, 10 mW; frequency, 9.430 GHz; magnetic field, 339.5 ± 7.5 mT; modulation, 100 kHz, 0.2 mT; time constant, 0.3 s.

**RESULTS AND DISCUSSION**

**Evaluation of nitroxyl radical–spin trapping for lipid allyl radicals complexed with ferrous lipoxygenase**

Soya bean lipoxygenase-1 has high sequence identity with all mammalian lipoxygenases, and its X-ray crystal structure reveals similar structural details [14–16]. For this reason, soya bean lipoxygenase-1 has been commonly used as an analogue of the mammalian enzymes. Qian et al. [17–19] examined the free radical generation in the reaction between soya bean lipoxygenase-1 and linoleic acid by using nitrotronic spin-trapping agents including DMPO [5,5-dimethyl-1-pyrroline-N-oxide] and POBN [α-(4-pyrydyl-1-oxide)-N-t-butyl-nitronate]. In the present study, we set the lipoxygenase system according to the approach achieved by Qian et al. [17–19] as follows: lipoxygenase content, 1.0 µM; linoleic acid content, 1.0 mM; solvent, 0.1 M phosphate buffer (pH 7.4; treated with Chelex® 100). In this system, the oxygen molecule should be completely depleted through the dioxygenation of linoleic acid catalysed by lipoxygenase, and the amount of resulting hydroperoxy linoleic acid correlates with the initially dissolved oxygen content in the reaction system.

CmP (partition coefficient between octanol and water, 0.90) is a well-examined and convenient nitroxyl radical as an indicator for redox balance and free radical reactions [11–13,20]. To examine whether nitroxyl radical competes with the oxygen molecule for the lipid allyl radical on the enzyme, the reaction between lipid-derived radicals and CmP was evaluated by monitoring the decrease in the EPR signal intensity of CmP. The EPR signal decay in the system at lower lipoxygenase content is shown in Figure 1(A). It is remarkable that initiation of signal decay was observed after a certain lag period. On the other hand, dioxygenation of linoleic acid started just after addition of lipoxygenase, and then the level of hydroperoxylinoleic acid reached a plateau. Oxygen content in the reaction system seems to contribute to the resulting hydroperoxylinoleic acid content. A theoretical peroxidation period was extrapolated from the time course of hydroperoxylinoleic acid content for each lipoxygenase content, as shown in Figure 1(B). Interestingly, there is a correlation...
Nitroxyl radical scavenges lipid allyl radical on lipoxygenase

between the lag period and the peroxidation period, as shown in Figure 1(C). This indicates that the reaction of CmP with the lipid-derived radicals proceeds rigorously under anaerobic conditions, where oxygen is depleted by dioxygenation of linoleic acid via lipoxygenase.

Furthermore, we examined whether the nitroxyl radical loss in the linoleate/lipoxygenase system is independent of the dioxygenation process. The time between mixing of lipoxygenase and that of CmP was left to stand in a glass vial with a screw cap (inner volume, 0.5 ml) at 25 °C. The EPR signal decay was monitored by X-band EPR. Hydroperoxylinoleic acid was determined by HPLC with UV detection. The lag period and peroxidation period were extrapolated from the EPR signal decay curve (A) and from the time course of hydroperoxylinoleic acid content (B) respectively. (C) Correlation between the lag period and the peroxidation period. Symbols in (A) and (B): ○, 0.1 µM lipoxygenase; ●, 0.2 µM lipoxygenase; ◦, 0.3 µM lipoxygenase; ◊, 0.5 µM lipoxygenase. Results in (C) are means ± S.D. for five individual experiments.

Identification of lipid-derived radical--CmP adducts by LC--MS/MS

To identify the linoleate allyl radical--CmP adducts (molecular mass 464 Da), the HPLC--MS/MS was employed. Soft ionization methods such as ESI have been shown to be powerful techniques to obtain molecular ions of precursor compounds. Figure 4(A)
I. Koshiishi and others

Figure 3 Chromatograms of linoleate allyl radical–CmP adducts and linoleate allyl radical–CmΔP adducts in the linoleate/lipoxygenase system

Phosphate buffer (0.1 M, pH 7.4; treated with Chelex® 100) containing 1.0 mM linoleic acid, 1 µM soya bean lipoxygenase-1 and 1.0 mM nitroxyl radical with CmP (A) or CmΔP (B) was left to stand at 25 °C for 10 min, and then the reaction solution was subjected to HPLC with UV detection. AU, absorbance units; LA-OOH, hydroperoxylinoleic acid.

shows the XIC (extracted ion chromatogram) of m/z 465 from the TIC (total ion chromatogram) of EPI (enhanced product ion scanning). Four distinct peaks were detected, at 8.8 min, 9.6 min, 11.2 min and 13.1 min, corresponding to the regioisomers and diastereoisomers of linoleate allyl radical–CmP adducts.

Scavenging the linoleate allyl radicals on the ferrous lipoxygenase by CmP results in the release of the inactive ferrous lipoxygenase. It is generally known that lipid hydroperoxides play a role in conversion of ferrous lipoxygenase into active ferric lipoxygenase, generating lipid alkoxyl radicals [22–24]. De Groot et al. [22] proposed that equal amounts of lipid allyl radical and lipid alkoxyl radical might be generated in an anaerobic PUFA/hydroperoxy fatty acid/lipoxygenase system. In the present system, the ferrous lipoxygenase–ferric lipoxygenase cyclic reaction in the coexistence of linoleic acid and hydroperoxylinoleic acid under anaerobic conditions should produce equimolar amounts of the linoleate allyl radical and the linoleate alkoxyl radical. Therefore the resulting nitroxyl radical spin-trapping adducts should consist of linoleate alkoxyl radical–CmP adducts (molecular mass 480 Da) as well as the linoleate allyl radical–CmP adducts. Figure 4(B) shows the XIC of m/z 481 from TIC of EPI. Three major peaks were detected, at 5.1 min, 5.5 min and 6.1 min (their peak areas were in the proportion 1:2:1). However, these adducts did not possess the conjugated diene moiety as shown in Figure 3(A). Furthermore, when these adducts were left to stand in the acidic conditions (acidified by methanoic acid), these adducts spontaneously changed into [M + H₂O + H]^+ (m/z 499) (results not shown). It was known that linoleate alkoxyl radical possibly rearranges intramolecularly by the addition of a double bond to cause the formation of the linoleate epoxyallyl radical [25,26]. Through these facts, we speculated that these adducts with m/z 481 may be linoleate epoxyallyl radical–CmP adducts. The possible overall paths are shown in Scheme 1.

Scheme 1 Possible reaction paths in the linoleic acid/lipoxygenase/CmP system

An excess amount of linoleic acid over oxygen content is present in this system. LxG, lipoxygenase.
Lipoxygenases catalyse the dioxygenation of PUFAs in the presence of molecular oxygen. When excess amount of PUFAs over oxygen content was left to stand with lipoxygenase in a sealed vial, a restricted amount of PUFAs was dioxidized to hydroperoxides in the dependence on oxygen content. In the linoleate/lipoxygenase system, [LA] (linoleic acid concentration) and [LA-OOH] (hydroperoxylidic acid concentration) reached a plateau, and the ratio, [LA]/[LA-OOH], was estimated to be approx. 3:2 with independence of lipoxygenase activity as shown in Figure 5. The total content of linoleic acid and its hydroperoxide, [LA]+[LA-OOH], was equal to the initial content of linoleic acid, [LA]0. On the other hand, the existence of the nitroxyl radical contributed to the disappearance of linoleic acid and its hydroperoxide, as shown in Figure 6(A). The content of the disappeared [LA]+[LA-OOH], [LA]0−([LA]+[LA-OOH]), was estimated to be <450 µM.

In general, adducts of carbon-centred radicals with the nitroxy radial are labile at higher temperature, degrading into the nitroxy radical via homolysis [27]. When the linoleate-derived carbon-centred radical–CmP adducts were heated in a glass vial with a screw cap at 100°C for 2 h, the adducts released >97% of CmP (results not shown). Consequently, it become possible to quantify the linoleate-derived carbon-centred radical–CmP adducts by the thermal decomposition method as described in the Experimental section. The linoleate allyl radical–CmP adducts with m/z 465 (8.0–14 min) and the linoleate epoxyallyl radical–CmP adducts with m/z 481 (4.5–8.0 min) in the linoleate/lipoxygenase/CmP system were respectively fractionated through HPLC. Each fraction was subjected to the quantification of lipid-derived carbon-centred radical–CmP adducts by the thermal decomposition method with UV detection. The time courses of linoleate allyl radical–CmP and linoleate epoxyallyl radical–CmP adduct contents were shown in Figure 6(B). These data indicate that the ferrous lipoxigenase–ferric lipoxigenase cyclic reaction in this system produced equimolar amounts of the linoleate allyl radical and the linoleate alkoxyl radical. In addition, it appeared that >90% of the disappeared [LA]+[LA-OOH] was recovered as the lipid-derived carbon-centred radical–CmP adducts. This finding indicates that CmP stoichiometrically traps the lipid-derived radicals in the linoleate/lipoxygenase/CmP system.

Stereospecificity of linoleate allyl radical–CmP adducts generated in the linoleate/lipoxygenase/CmP system

Lipid allyl radicals, which were chemically generated from linoleic acid (9Z,12Z-C18:2) or linolealaidic acid (9E,12E-C18:2) via AMVN (half-life at 37°C of 90 h [28]), were trapped by CmP. The resulting solutions were subjected to HPLC with UV detection. The chromatograms are shown in Figure 7. Four distinct adducts (m/z 465) were detected in both systems, and the chromatographic patterns were almost the same as each other. The intermediate carbon-centred E/Z-pentadiene radical undergoes resonance stabilization into two positionally isomeric pentadiene radicals, C-9 and C-13 positions. In this step, the E/Z isomers reach equilibrium with the thermodynamically favoured E/E isomers [29]. Hence, the reactions between free linoleate allyl radicals and CmP in solution led to equal distribution of 9-CmP-10E,12E-C18:2 and 13-CmP-9E,11E-C18:2. Consequently, despite the difference in stereospecificity of precursor fatty acids, the stereospecificity of resulting lipid allyl radical–CmP adducts was identical.

On the other hand, stereo-selective hydrogen abstraction from a doubly allylic methylene by lipoxigenase led to the formation of lipid allyl radicals. It is important to stress that the radical intermediates largely remain enzyme-bound [7–9]. If nitroxy radical attacks the radical intermediates on the enzyme, the stereospecificity should be maintained. Figure 8 shows the correspondence of the retention time of the linoleate allyl radical–CmP adducts generated chemically and enzymatically. The E/E-linoleate allyl radical–CmP adducts were not detected in the linoleate/lipoxygenase system at all. Through these data, it appeared that the stereospecificity of E/Z-linoleate allyl radical–CmP adducts generated in the linoleate/lipoxygenase/CmP system is rigorously maintained.
Figure 7 Chromatographic analyses of stereospecificity of lipid allyl radical–CmP adducts generated from linoleic acid (LA: 9Z,12Z-C18:2) and linolelaidic acid (LLA: 9E,12E-C18:2) via radical initiator, AMVN

Acetonitrile solution containing 10 mM CmP, 10 mM AMVN and 10 mM linoleic acid (A) or linolelaidic acid (B) was left to stand at 37°C for 48 h. One vol. of the reaction solution was mixed with 19 vol. of acetonitrile, and a 10 µl portion of the solution was subjected to HPLC with UV detection. AU, absorbance units.

Figure 8 Comparison of elution profile of linoleate allyl radical–CmP adducts in the linoleate/lipoxygenase/CmP system with that of linoleate allyl radical–CmP adducts in the linoleate/AMVN/CmP system via LC–MS/MS with XIC of m/z 465

cps, counts per second.

Nitroxyl radical-spin trapping for lipid allyl radicals generated from linolenic acid and arachidonic acid via lipoxygenase

Lipoxygenase abstracts hydrogen atom from a doubly allylic methylene of PUFAs including linoleic acid, linolenic acid and arachidonic acid. If linolenate allyl radicals and arachidonate allyl radicals were released from the active site of lipoxygenase, the allyl radicals seem to exhibit polydiversity in their structure through intramolecular hydrogen abstraction from another doubly allylic methylene. So, the reaction solutions in the linolenate/lipoxygenase/CmP system and in the arachidonate/lipoxygenase/CmP system were subjected to HPLC with UV detection (234 nm). As shown in Figure 9, four distinct peaks corresponding to regioisomers and diastereoisomers of the lipid allyl radical–CmP adducts were respectively detected in both systems. On the contrary, neither linolenate allyl radical–CmP adduct nor arachidonate allyl radical–CmP adduct was detected in the linolenate/AMVN/CmP and the arachidonate/AMVN/CmP systems respectively (results not shown). These facts indicate that nitroxyl radical traps lipid allyl radicals on the lipoxygenase without intramolecular rearrangement of these allyl radicals.

Through the study on the nitroxyl radical-spin trapping in the PUFAs/lipoxygenase system, we elucidated the following: (i) the nitroxyl radical trapped lipid allyl radicals under anaerobic conditions, (ii) scavenging of lipid allyl radicals by the nitroxyl radical promoted the loss of PUFA and its hydroperoxide, (iii) nitroxyl radical stoichiometrically trapped lipid allyl radicals, (iv) the generation of the linoleate allyl radical–CmP adducts was accompanied with that of linoleate alkoxyl radical–derived carbon-centred radical–CmP adducts (possibly linoleate epoxyallyl radical–CmP adducts), (v) the stereospecificity of E/Z-pentadiene radical was held in the lipid allyl radical–CmP adducts, and (vi) the regiospecificity and diastereospecificity of the lipid allyl radical–CmP adducts were rigorously maintained in the PUFA/lipoxygenase/CmP systems. Based on these facts, we concluded that nitroxyl radical scavenges the lipid allyl radicals complexed with lipoxygenase at lower oxygen content.

At lower oxygen concentration, it appeared that both regiospecificity and stereospecificity of conjugated diene moiety in lipoxygenase-catalysed lipid hydroperoxidation are drastically lost. Berry et al. [30] elucidated the lower regiospecificity of hydroperoxycadadienoic acids generated from linoleic acid via soya bean lipoxygenase-1 at low oxygen concentration. In
their study, they hypothesized that the radical intermediate escapes from the active site of the enzyme [30]. To prove their hypothesis, they used nitroxyl radical to prevent the lowering of the regiospecificity [30]. Their experiments were based on the assumption that nitroxyl radicals never react with radical intermediate in the active site of the enzyme [30]. However, our results indicate that nitroxyl radical facilitates the dissociation of the radical intermediate from the active site of the enzyme by converting it into the nitroxyl radical–lipid allyl radical adduct.

We are indebted to Professor Hideo Utsumi (Kyushu University, Fukuoka, Japan) for initiating us into EPR spectrometry.

REFERENCES


© 2006 Biochemical Society

Received 28 September 2005/21 December 2005; accepted 6 January 2006
Published as BJ Immediate Publication 6 January 2006, doi:10.1042/BJ20051595