REVIEW

Proton-Coupled Electron Transfer of Unsaturated Fatty Acids and Mechanistic Insight into Lipoxygenase

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Dedicated to the memory of Professor *Hanns Fischer*

A proton-coupled electron transfer (PCET) process plays an important role in the initial step of lipoxygenases to produce lipid radicals which can be oxygenated by reaction with O_2 to yield the hydroperoxides stereoselectively. The EPR spectroscopic detection of free lipid radicals and the oxygenated radicals (peroxyl radicals) together with the analysis of the EPR spectra has revealed the origin of the stereo- and regiochemistry of the reaction between O_2 and linoleyl (=(2*Z*)-10-carboxy-1-[(1*Z*)-hept-1-enyl]dec-2-enyl) radical in lipoxygenases. The direct determination of the absolute rates of H-atom-transfer reactions from a series of unsaturated fatty acids to the cumylperoxyl (=(1-methyl-1-phenylethyl)dioxy) radical by use of time-resolved EPR at low temperatures together with detailed kinetic investigations on both photoinduced and thermal electron-transfer oxidation of unsaturated fatty acids provides the solid energetic basis for the postulated PCET process in lipoxygenases. A strong interaction between linoleic acid (=(9*Z*,12*Z*)-octadeca-9,12-dienoic acid) and the reactive center of the lipoxygenases (Fe^{III}–OH) is suggested to be involved to make a PCET process to occur efficiently, when an inner-sphere electron transfer from linoleic acid to the Fe^{III} state is strongly coupled with the proton transfer to the OH group.

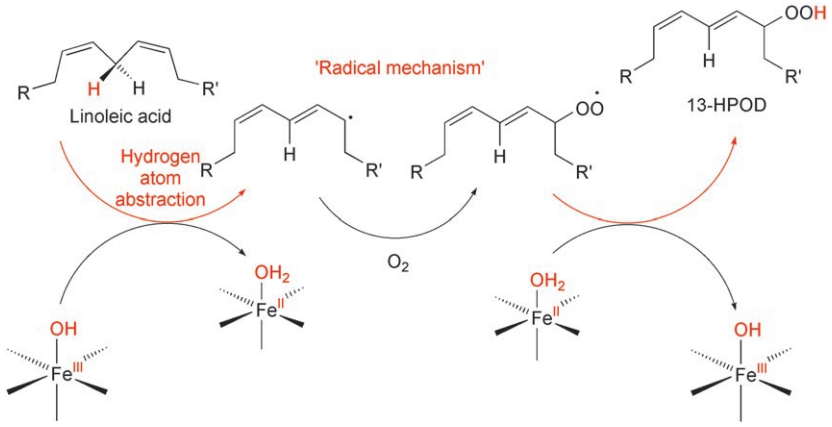
1. Introduction. – Many biological redox reactions involve transfers of electrons and protons, in which most reactions involving cleavage of C–H bonds are discussed in terms of hydrogen-atom transfer (HAT), with concerted proton and electron transfer, referred to as proton-coupled electron transfer (PCET) [1][2]. HAT is an important area of chemistry that has been widely studied in the contexts of combustion, halogenation, antioxidant oxidation, and other processes. HAT is typically defined as a process in which a H-atom moves directly between two groups. There are also PCET reactions in which the proton and electron are somehow separated in the reactants, products, or at the transition state, but they may move at the same time. Such PCET reactions are often defined as concerted proton/electron transfer that is not HAT, but this distinction has been difficult to make in practice, especially when metals are involved like in many enzymatic processes. A typical example is seen in the case of lipoxygenases.

Lipoxygenases (LOs) are mononuclear non-heme iron enzymes that catalyze the regio- and stereospecific peroxidation of polyunsaturated fatty acids and fatty acid esters containing the (1*Z*,4*Z*)-1,4-diene moiety to the corresponding (2*E*,4*Z*)-1-hydroperoxy-2,4-diene [3][4]. For example, linoleic acid (=(9*Z*,12*Z*)-octadeca-9,12-dienoic acid), which is a natural substrate for most plant LOs, is oxygenated with positional stereospecificity to produce (9*Z*,11*E*)-13-hydroperoxyoctadeca-9,11-dienoic acid (13-

HPOD), completing the first step in the biosynthesis of the growth-regulation substance jasmonic acid and of the wound-healing compounds traumatin and traumatic acid [5]. In contrast, autoxidation of linoleic acid radicals in the absence of enzyme results in multiple regio- and stereoisomeric hydroperoxy-substituted products [6]. Soybean lipoxygenase-I also catalyzes the oxygenation of monounsaturated acids to enones [7].

Although the details of how LO oxidizes fatty acids have been a matter of debate[1]), the generally accepted mechanism for the lipoxygenases involves H-atom abstraction at C(3) of the (1*Z*,4*Z*)-1,4-diene in the substrate (linoleic acid), followed by subsequent trapping of linoleyl (=(2*Z*)-10-carboxy-1-[(1*Z*)-hept-1-enyl]dec-2-enyl) radical by oxygen to form the hydroperoxy-substituted product (*Scheme 1*) [9][10]. The H-atom abstraction for soybean lipoxygenase has been reported to proceed through a quantum-mechanical tunneling pathway [11][12].

Scheme 1



To clarify the H-abstraction mechanism of lipoxygenases, lipoxygenase model iron complexes have been synthesized and the reactivity of H-atom abstraction from substrate model compounds containing a (1*Z*,4*Z*)-penta-1,4-diene subunit such as cyclohexa-1,4-diene has been examined [13]. However, there have been no lipoxygenase model complexes that can oxygenate linoleic acid, and the H-abstraction mechanism of lipoxygenases has yet to be clarified. Understanding the H-abstraction process certainly requires knowledge of the thermodynamics and kinetics of the possible HAT, electron-transfer, and proton-transfer steps. It is also important to detect the radical intermediates involved in lipoxygenases, *i.e.*, pentadienyl radicals and subsequent peroxyl radicals derived from unsaturated fatty acids by electron paramagnetic resonance (EPR) spectroscopy, which provides valuable information of the radicals. Time-resolved EPR is particularly useful to quantify the reactivity of radical species as demonstrated by excellent works by *Fischer* and co-workers [14][15].

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[1]) The role of iron(III) (ferric ion Fe^{III}) has been suggested to form a σ-organoiron complex with the substrate, and dioxygen inserts into the Fe–C bond, see [8].

This review is intended to clarify the role of the PCET process in lipoxygenases. First, the position of O_2 addition to 'linoleyl' radical is discussed in relation with the lipoxygenase mechanism based on the EPR detection of 'linoleyl' radical and the peroxyl radicals. Then, the HAT reactivity of unsaturated fatty acids is discussed in relation with the lipoxygenase reactivity, based on determination of the absolute rates of HAT reactions from a series of unsaturated fatty acids to cumylperoxyl (=(1-methyl-1-phenylethyl)dioxy) radical by use of time-resolved EPR at low temperatures. Finally, the energetic basis for the PCET process in lipoxygenases is presented based on the detailed kinetic investigations on both photoinduced and thermal electron-transfer oxidation of unsaturated fatty acids.

2. EPR Detection of Intermediate Radicals in Lipid Peroxidation. – The most widely accepted mechanism of LO involves H-atom abstraction from the unsaturated fatty acid substrate with concomitant reduction of the $Fe^{III}-OH$ reaction center and formation of the pentadienyl radical (*Scheme 1*) [9][10]. The trapping of the radical by O_2 to produce the peroxyl radical, followed by oxidation of the (water) iron(II) complex by the peroxyl radical completes the catalytic cycle.

The pentadienyl radical derived from H-atom abstraction of linoleic acid by *tert*-butoxyl radical (^{t}BuO$^\bullet$), which is generated by the homolytic photocleavage of the O–O bond of ^{t}BuOO^{t}Bu [16][17], has been detected by EPR as shown in *Fig. 1*,*a* [18][19]. The *g* value (2.0024) of the EPR signal in *Fig. 1*,*a*, which is close to the free spin value (2.0023), is typical for a C-centered radical [20]. The well-resolved EPR spectrum (*Fig. 1*,*a*) is reproduced completely by using the hyperfine coupling constants (*hfc*) due to one proton at C(11) (a(H–C(11))=1.13 mT), two equivalent protons at C(9) and C(13) (a(H–C(9))=a(H–C(13))=0.99 mT), two equivalent protons at C(10) and C(12) (a(H–C(10))=a(H–C(12))=0.33 mT), and four equivalent CH_2 protons at C(8) and C(14) (a(H–C(8))=a(H–C(14))=0.825 mT). The computer-simulation spectrum calculated with these *hfc* values (*Fig. 1*,*b*) agrees well with the observed EPR spectrum (*Fig. 1*,*a*). The deuterium substitution of two H-atoms at C(11) of linoleic acid ([11,11-$^2H_2$]linoleic acid) has confirmed the *hfc* assignment in *Fig. 1*,*b* [18], since the observed EPR spectrum agrees with the computer-simulation spectrum calculated with the same *hfc* values, except for the value of the deuterium (I=1) at C(11), which is reduced by the magnetogyric ratio of proton to deuterium (0.153) [20]. The *hfc* values indicate that the spin density is largest at C(11) of the pentadienyl radical. The *hfc* assignment is also supported by the DFT calculation (see the calculated *hfc* values in parentheses in *Fig. 1*,*c*) [18]. Thus, the H-atom abstraction of lipids containing the (1*Z*,4*Z*)-penta-1,4-diene subunit by ^{t}BuO$^\bullet$, which is produced by the photocleavage of the O–O bond of ^{t}BuOO^{t}Bu, produces the pentadienyl radical in which the spin density is largest at C(11), as shown in *Scheme 2* [18].

When the photoirradiation was carried out with an O_2-saturated solution containing linoleic acid and ^{t}BuOO^{t}Bu, no EPR signal due to the pentadienyl radical was observed, but instead a broad *doublet* EPR signal due to the peroxyl radical was observed at g=2.0152 as shown in *Fig. 2*,*a*. The *g* value and the hyperfine coupling constant (a(H)=0.406 mT) is diagnostic of secondary alkylperoxyl radicals [21][22]. The observed a(H) value agrees with the value of 11-HPO$^\bullet$ (0.47 mT) estimated by DFT calculation by using B3LYP/3-21G* basis set [18].

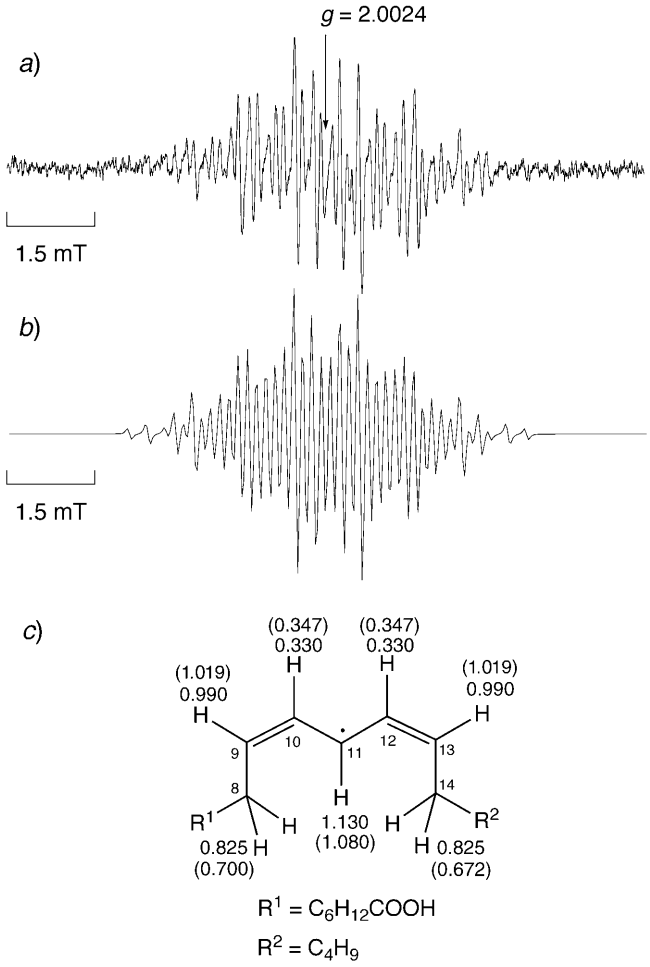
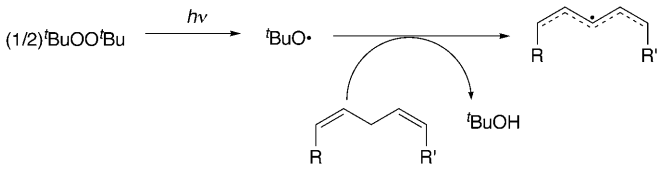


Fig. 1. a) *EPR spectrum of 'linoleyl' radical observed under photoirradiation of a deaerated* ^{t}*BuOO*^{t}*Bu* (neat) *solution containing linoleic acid* (1.3M) *with a high-pressure Hg lamp at 273 K.* b) *Computer-simulation spectrum of pentadienyl radical derived from linoleic acid with* c) *the hfc values.* Values in parenthesis were obtained from the DFT calculation (B3LYP/6-311G** basis set)

Scheme 2



When the photoirradiation was carried out with an air-saturated solution under otherwise the same experimental conditions, the EPR signal due to the pentadienyl radical ($g = 2.0024$) derived from linoleic acid was observed together with the peroxyl radical

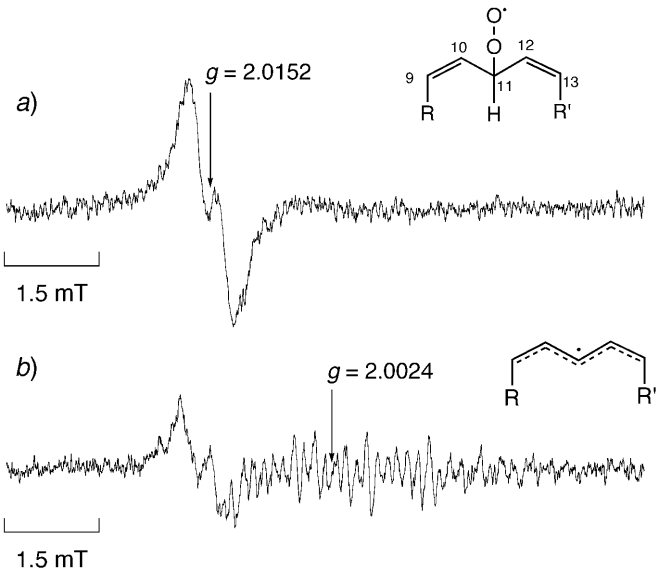


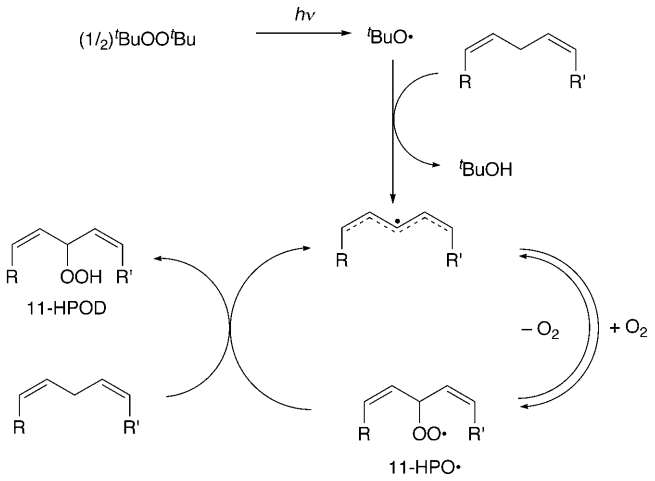
Fig. 2. a) *EPR spectrum of peroxyl radical observed under photoirradiation of an O_2-saturated* ^{t}*BuOO*^{t}*Bu* (neat) *solution containing linoleic acid* (1.3M) *with a high-pressure Hg lamp at 253 K.* b) *EPR spectrum of 'linoleyl' radical and peroxyl radical observed under photoirradiation of an air-saturated* ^{t}*BuOO*^{t}*Bu* (neat) *solution containing linoleic acid* (1.3M) *with a high-pressure Hg lamp at 253 K.* R = $C_7H_{14}COOH$, R′ = C_5H_{11}.

($g = 2.0152$) as shown in *Fig. 2, b*. This indicates that the O_2 addition to 'linoleyl' radical derived from linoleic acid to produce the peroxyl radical is a reversible process.

Oxygen may be preferably added to C(11) of the pentadienyl radical to produce 11-HPO•, which can abstract a H-atom from linoleic acid to form 11-HPOD, accompanied by regeneration of the pentadienyl radical as shown in *Scheme 3*. This is indicated by observation of a single-line EPR signal, when linoleic acid is replaced by [11,11-$^2H_2$]linoleic acid [18]. The disappearance of the hyperfine splitting results from the decrease in the hyperfine coupling constant by the D substitution at C(11), because the hyperfine pattern would be changed from a *doublet* signal of the peroxyl radical derived from linoleic acid to a *triplet* signal of that derived from [11,11-$^2H_2$]linoleic acid due to one deuteron splitting ($I = 1$) at C(11), and the a(D) value should decrease by the magnetogyric ratio of proton to deuterium (0.153). In such a case, the hyperfine structure would be hidden within the line width of the EPR signal to afford a single-line EPR signal. The preferable addition of O_2 at C(11) rather than at C(9) and C(13) may result from the largest spin density of the pentadienyl radical at C(11) (*Fig. 1*).

The same EPR spectrum as observed in *Fig. 2, a* was obtained when (9*Z*,11*E*,13*S*)-13-hydroperoxyoctadeca-9,11-dienoic acid (13-HPOD) was used instead of linoleic acid for the photoirradiation. In the case of H-atom abstraction of 13-HPOD by the *tert*-butoxyl radical, the peroxyl radical (13-HPO•) in which O_2 is attached at C(13) must be initially produced by the H-atom abstraction from the hydroperoxide moiety of 13-HPOD by ^{t}BuO•. If the observed EPR spectrum comes from 13-HPO•, the deuterium substitution of two H-atoms at C(11) of 13-HPOD would not affect the ESR

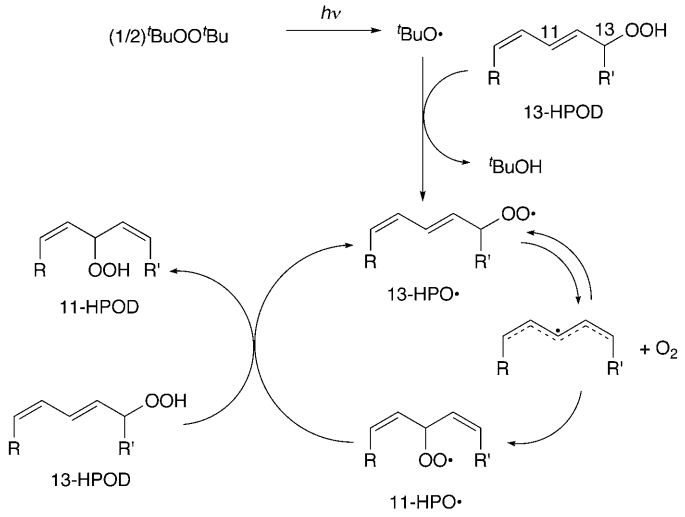
Scheme 3



spectrum, since the hyperfine splitting results from H–C(13). However, the EPR spectrum of the peroxyl radical observed under photoirradiation of a ^{t}BuOO^{t}Bu solution containing [11-^{2}H]-13-HPOD at 253 K exhibits no hyperfine structure [18]. This indicates that the peroxyl radical 13-HPO•, which is initially formed by the H-atom abstraction from the hydroperoxide moiety of 13-HPOD by ^{t}BuO•, isomerizes at least partially to 11-HPO•, *via* removal of O_2 from 13-HPO• and addition of O_2 to 'linoleyl' radical to produce 11-HPO•, as shown in *Scheme 4*. Thus, the C–O bond cleavage in 13-HPO• results in the formation of 'linoleyl' radical to which O_2 may be added mainly at C(11) rather than at C(9) and C(13), since the spin density of 'linoleyl' radical is largest at C(11) (*Scheme 4*).

The preferable addition of O_2 at C(11) of the 'linoleyl' radical derived from linoleic acid, where the spin density is largest, provides valuable insight into the mechanism of the regioselective oxygenation of linoleic acid by LO. If O_2 has free access to the pentadienyl radical derived from linoleic acid, O_2 would add mainly to C(11) to produce the 11-HPO• radical. Even if 13-HPO• is formed, it would be readily isomerized to 11-HPO•, if the peroxyl radical can move freely in the enzyme. Thus, the regio- and stereoselective addition of O_2 at C(13) in LO may result from the enzyme protein environment, which limits O_2 access to the pentadienyl radical bound to the enzyme. The X-ray crystal structure of soybean lipoxygenase-1 (SLO) reveals a side channel intersecting the substrate pocket near the reactive C(11) of linoleic acid [23–25], which is proposed to be the O_2 access channel [26]. This channel is constricted at the Fe^{III}–OH by the side chains of Leu^{546} and Leu^{754}. *Klinman* and co-workers [9] have reported that steady-state kinetics and product distribution data from single-point mutants of SLO show that Leu^{546} and Leu^{754} grant selectivity for (13*S*)-13-HPOD by blocking O_2 access to C(9) of linoleic acid and that O_2 enters the active site *via* the postulated side channel. The reactivity of O_2 with SLO has also been examined by using a range of kinetic evidences to rule out diffusional encounter of O_2 with protein, an outer-sphere electron transfer to O_2, and proton transfer as rate-limiting steps [27]. Either a radical combination of O_2

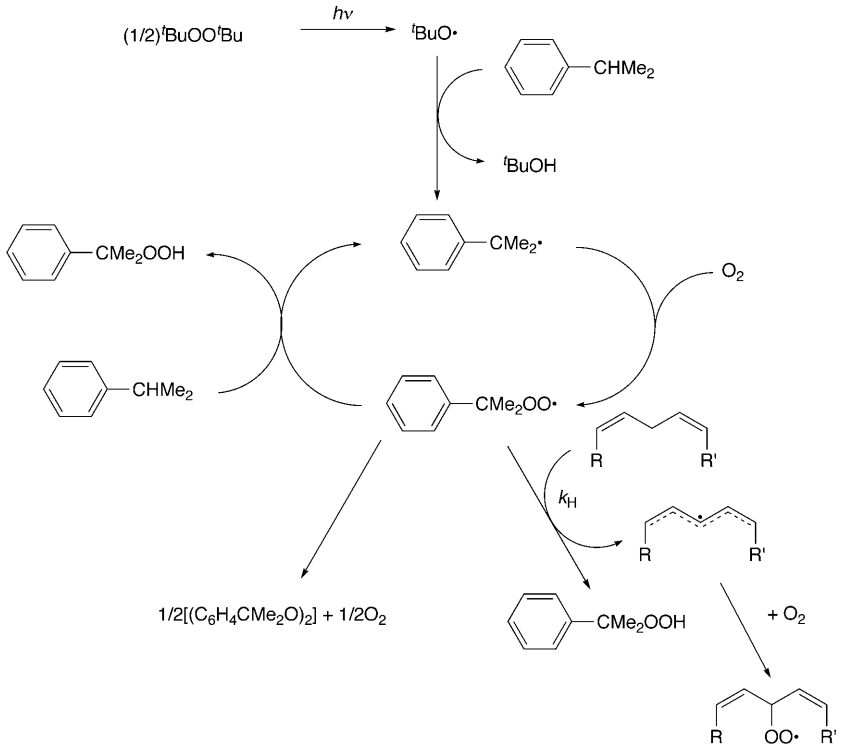
Scheme 4



with 'linoleyl' radical or a subsequent slow conformational change is suggested to be the rate-determining step [27]. The primary role of the Fe^{III} cofactor is, therefore, to generate an enzyme-bound radical, while the protein controls the stereo- and regiochemistry of O_2 encounter with this radical [27].

3. Hydrogen-Atom-Transfer (HAT) Reactivity of Unsaturated Fatty Acids. – The rates of HAT from a series of unsaturated fatty acids to the cumylperoxyl radical have been determined in propanenitrile (EtCN) at various temperatures by means of time-resolved EPR [28]. The cumylperoxyl radical is formed *via* a radical chain process as shown in *Scheme 5* [28]. The photoirradiation of ^{t}BuO^{t}Bu results in the homolytic cleavage of the O–O bond to produce ^{t}BuO•, which abstracts a H-atom from cumene to give the cumyl (=1-methyl-1-phenylethyl) radical, followed by the facile addition of oxygen to the cumyl radical to produce the cumylperoxyl radical. The cumylperoxyl radical abstracts a H-atom from cumene in the propagation step to yield cumyl hydroperoxide, accompanied by regeneration of cumyl radical (*Scheme 5*). When the light is cut off, the EPR-signal intensity due to cumylperoxyl radical fades slowly due to the bimolecular reaction. The decay rate is accelerated by the presence of unsaturated fatty acids due to HAT from unsaturated fatty acids to cumylperoxyl radicals [28]. The decay rate in the presence of unsaturated fatty acids obeys pseudo-first-order kinetics, and the pseudo-first-order rate constants increase linearly with an increase in concentrations of unsaturated fatty acids. The rate constants (k_H) of HAT from a series of unsaturated fatty acids to cumylperoxyl radical were determined from the slopes of linear plots of the pseudo-first-order rate constants *vs.* concentrations of unsaturated fatty acids. The results are summarized in *Fig. 3*, where the k_H values at different temperatures are linearly correlated with the number n of active H-atoms of the penta-1,4-diene subunit of unsaturated fatty acids. Such linear correlations in *Fig. 3* demonstrate that a simple additive rule holds in the HAT reactions as given by *Eqn. 1*, where k_{H1} is

Scheme 5



the HAT rate constant of one H-atom of a penta-1,4-diene subunit (linoleic acid, linolenic acid, or arachidonic acid) and k_{H0} is the H-atom-transfer rate constant of the allylic subunit (oleic acid). *Eyring* plots of the k_H and k_D values are shown in *Fig. 4*, and the activation parameters are given in *Table 1* [28]. There is a significant difference in the activation enthalpy between unsaturated fatty acids containing penta-1,4-diene subunits (linoleic acid, linolenic acid, and arachidonic acid) and that containing only an allylic subunit (oleic acid). Such a difference in the activation enthalpy is consistent with the calculated energy differences between H-atom-abstracted radicals and unsaturated fatty acids (ΔE): the ΔE values of the allyl-type radicals, which are nearly the same among the unsaturated fatty acids, are by 50 ± 2 kJ·mol^{-1} larger than those of the corresponding pentadienyl type radicals derived from the same unsaturated fatty acids [28].

$$k_H = k_{H0} + nk_{H1} \quad (1)$$

When linoleic acid is replaced by [11,11-$^2H_2$]linoleic acid, the k_D value becomes smaller than that of linoleic acid, to exhibit the kinetic isotope effect (KIE). The KIE values of one active H-atom of a pentadiene subunit were determined as 6.1 ± 0.3 from the k_H, k_D, and k_{H0} values by using the relation, $k_{H1}/k_{D1} = (k_H - k_{H0})/(k_D - k_{H0})$ as shown in *Table 2*. The k_{H1}/k_{D1} value is significantly smaller than those

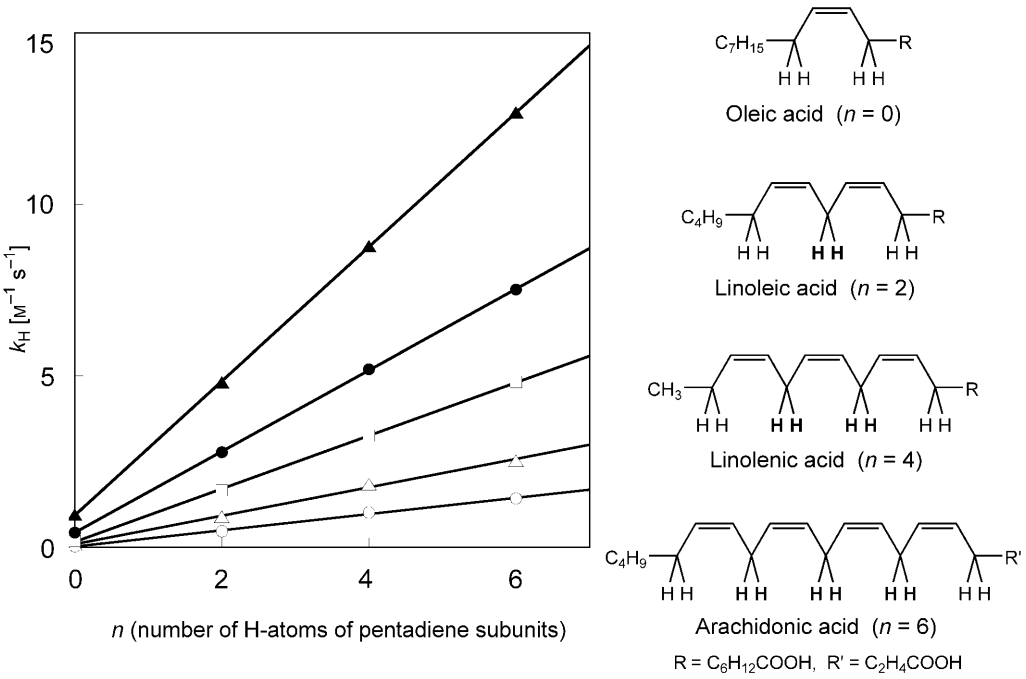


Fig. 3. *Plots of rate constants* k_H vs. *the number of H-atoms* n *of the penta-1,4-diene subunits for H-atom transfer from unsaturated fatty acids* (*n* values in parentheses) *to cumylperoxyl radical in O_2-saturated EtCN at 203 K* (○), *213 K* (△), *223 K* (□), *233 K* (●), *and 243 K* (▲). R = $C_6H_{12}COOH$, R′ = C_2H_4COOH.

Table 1. *Activation Parameters for H-Atom Transfer from Unsaturated Fatty Acids to Cumylperoxyl Radical in O_2-Saturated EtCN*

Unsaturated fatty acid[a])	$\Delta H^{\neq}$ [kJ·mol^{-1}]	$\Delta S^{\neq}$ [J·K^{-1}·mol^{-1}]
Oleic acid	34.7 ± 0.3	−101 ± 1
Linoleic acid	21.9 ± 0.3	−140 ± 1
Linolenic acid	20.1 ± 0.3	−143 ± 1
Arachidonic acid	20.6 ± 0.5	−137 ± 2
[11,11-$^2H_2$]Linoleic acid	25.5 ± 1.3	−135 ± 6

[a]) Oleic acid = (9*Z*)-octadec-9-enoic acid; linoleic acid = (9*Z*,12*Z*)-octadeca-9,12-dienoic acid; linolenic acid = (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid; arachidonic acid = (5*Z*,8*Z*,11*Z*,14*Z*)-eicosa-5,8,11,14-tetraenoic acid.

observed in SLO (81) [9–11], exhibiting no significant tunneling effect in HAT from linoleic acid to cumylperoxyl radical. In addition, the H-atom-transfer reactivity of oleic acid is only five times smaller than that of linoleic acid at 243 K (*Table 1*), whereas the rate of SLO-catalyzed oxygenation of oleic acid is 10^5 slower than that of linoleic acid [7].

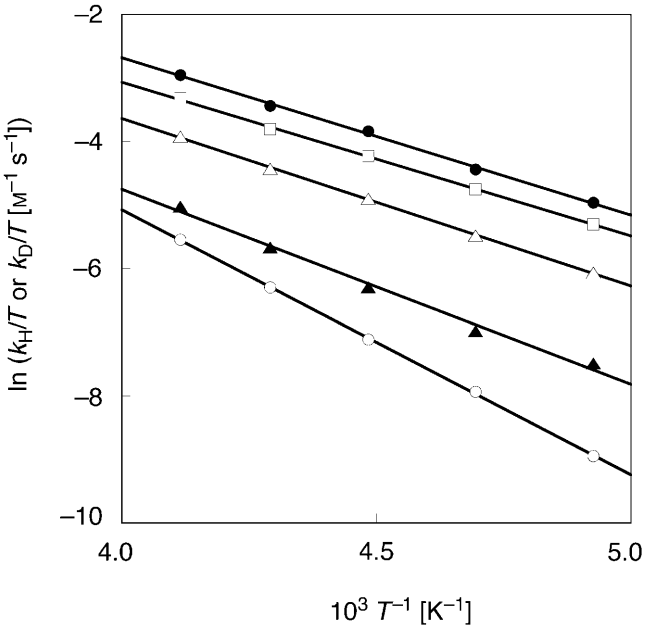


Fig. 4. Eyring *plots of* k_H *for H-atom transfer from oleic acid* (○), *linoleic acid* (△), *linolenic acid* (□), *arachidonic acid* (●), *and [11,11-*$^2H_2$*]linoleic acid* (▲) *to cumylperoxyl radical in* O_2*-saturated EtCN*

Table 2. *Rate Constants* (k_{H1} and k_{D1}) *and Kinetic Isotope Effect* (k_{H1}/k_{D1}) *for H-Atom Transfer From Linoleic Acid ([11,11-*$^2H_2$*]Linoleic Acid) to Cumylperoxyl Radical*

T [K]	k_{H1}[a]) [M^{-1} s^{-1}]	k_{D1}[b]) [M^{-1} s^{-1}]	k_{H1}/k_{D1}
203	$(4.5\pm0.3)\cdot10^{-1}$	$(8.7\pm0.1)\cdot10^{-2}$	5.2 ± 0.4
213	$(8.1\pm0.2)\cdot10^{-1}$	$(1.2\pm0.1)\cdot10^{-1}$	6.8 ± 0.6
223	1.5 ± 0.1	$(2.3\pm0.2)\cdot10^{-1}$	6.5 ± 0.6
233	2.3 ± 0.2	$(3.7\pm0.5)\cdot10^{-1}$	6.2 ± 1.0
243	3.9 ± 0.1	$(6.5\pm0.5)\cdot10^{-1}$	6.0 ± 0.5

[a]) $k_{H1}=k_H-k_{H0}$; [b]) $k_{D1}=k_D-k_{H0}$.

Significant differences in the relative reactivities and KIE values of unsaturated fatty acids in HAT reactions with cumylperoxyl radical from those in lipoxygenases indicate that the simple HAT from unsaturated fatty acids to radical species is quite different from the tunneling H-atom transfer (PCET) in lipoxygenases, where an electron and a proton may be transferred at the same time, but separately to the Fe^{III} site and the OH site, respectively [29].

4. Electron-Transfer Oxidation Properties of Unsaturated Fatty Acids. – Since the relative reactivities and KIE values of unsaturated fatty acids in simple HAT reactions are quite different from those in lipoxygenases (*vide supra*), we have examined the

rates of photoinduced electron-transfer oxidation of unsaturated fatty acids with a series of photosensitizers from which the fundamental one-electron-oxidation properties of unsaturated fatty acids can be deduced [30].

A number of rate constants (k_{et}) of photoinduced electron transfer from unsaturated fatty acids (linoleic acid, methyl linoleate, linolenic acid, arachidonic acid, and oleic acid) to the singlet excited states of a series of electron acceptors, *i.e.*, 1-methyl-3-cyanoquinolinium ion ($CNQuH^+$), 1-methylquinolinium ion (QuH^+), naphthalene-1,4-dicarbonitrile (NDC), 10-methylacridinium ion ($AcrH^+$), 10-methyl-9-phenylacridinum ion ($AcrPh^+$), 9-isopropyl-10-methylacridinium ion (Acr^iPr^+), and anthracene-9,10-dicarbonitrile (ADC), were determined by fluorescence quenching in MeCN at 298 K [30]. The k_{et} values thus determined were plotted against the one-electron reduction potentials (E^*_{red}) of the singlet excited states of electron acceptors [31][32] in *Fig. 5*. The k_{et} values increase with increasing E^*_{red} values to reach a diffusion-limited value. This is a typical driving-force dependence of the rate constant of photoinduced electron-transfer reactions [33][34]. The unknown values of the one-electron oxidation potentials of unsaturated fatty acids (E_{ox}) and the intrinsic barrier of electron transfer ($\Delta G^{\neq}$) are determined from the driving-force dependence of the rate constant of photoinduced electron transfer as reported in [33][34]. The E_{ox} and $\Delta G^{\neq}$ values are listed in *Table 3* [30].

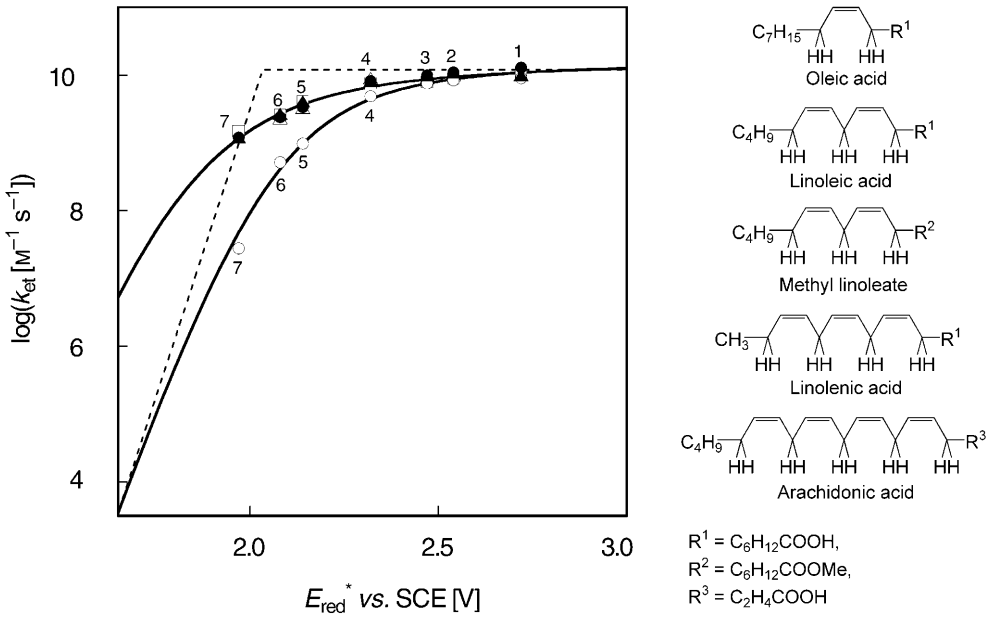


Fig. 5. *Plots of log* k_{et} *of photoinduced electron-transfer from unsaturated fatty acids* (oleic acid (○), linoleic acid (△), methyl linoleate (□), linoleic acid (●), and arachidonic acid (▲)) *to the singlet excited state of organic sensitizers* vs. *one-electron reduction potentials* (E^*_{red}) *of the singlet excited states of organic sensitizers* (1: $CNQuH^+$; 2: QuH^+; 3: NDC; 4: $AcrH^+$; 5: $AcrPh^+$; 6: Acr^iPr^+; 7: ADC).

Table 3. *One-Electron Oxidation Potentials* (E_{ox}) *of Unsaturated Fatty Acids and Intrinsic Barrier* ($\Delta G_0^{\neq}$) *of the Electron-Transfer Oxidation in MeCN*

Unsaturated fatty acid[a])	E_{ox} *vs.* SCE [V]	$\Delta G_0^{\neq}$ [kcal mol^{-1}]
Oleic acid	2.03 ± 0.02	15.8 ± 0.42
Linoleic acid	1.76 ± 0.04	18.5 ± 0.96
Methyl linoleate	1.76 ± 0.04	18.5 ± 0.96
Linolenic acid	1.76 ± 0.04	18.5 ± 0.96
Arachidonic acid	1.76 ± 0.04	18.5 ± 0.96

[a]) See *Fig. 5* for structures.

The E_{ox} values of linoleic acid, methyl linoleate, linolenic acid, and arachidonic acid are all the same, *i.e.*, 1.76 V ± 0.04 *vs.* SCE, whereas a higher E_{ox} value, *i.e.*, 2.03 V ± 0.02 *vs.* SCE, is obtained for oleic acid. The E_{ox} values of unsaturated fatty acids are well correlated with the first adiabatic ionization energies (I_{obs}) determined by the HeI photoelectron spectra: The I_{obs} value of oleic acid (8.63 eV) is significantly larger than that of linoleic acid (8.45 eV), which is the same as that of linolenic acid (8.45 eV) [35].

The occurrence of photoinduced electron-transfer reactions of unsaturated fatty acids was confirmed by laser-flash-photolysis experiments. Laser excitation of an MeCN solution of $AcrH^+$ ($1.0 \cdot 10^{-4}$ M) and linoleic acid ($5.0 \cdot 10^{-2}$ M) afforded a transient absorption spectrum at 10 μs with appearance of a new absorption band at 460 nm due to the radical cation of linoleic acid and a shoulder at 510 nm due to $AcrH^{\cdot}$, as shown in *Fig. 6* [30]. This indicates that photoinduced electron transfer from linoleic acid to $^1AcrH^{+*}$ occurs to produce $AcrH^{\bullet}$ and the radical cation of linoleic acid. The decay of absorbance at 460 nm is much faster than that at 510 nm due to $AcrH^{\bullet}$, coinciding with the rise of absorbance at 320 nm as shown in the inset of *Fig. 6*. This indicates that the radical cation of linoleic acid deprotonates to produce 'linoleyl' radical which has absorption at 320 nm. The deprotonation rate constant was determined as $8.1 \cdot 10^3$ s^{-1} from the first-order plots for the decay of absorbance at 460 nm as well as the rise of absorbance at 320 nm (inset of *Fig. 6*).

When linoleic acid was replaced by [11,11-$^2H_2$]linoleic acid under the same experimental conditions, the same transient absorption spectra were obtained as in the case of linoleic acid in *Fig. 6* [30]. However, the decay of absorbance at 460 nm became slower, and the deuterium kinetic isotope effect on the deprotonation of the radical cation of linoleic acid was determined as $k_H/k_D = 7.4 \pm 0.4$ [30].

The large difference in the E_{ox} values between linoleic acid (1.76 V ± 0.04 *vs.* SCE) and oleic acid (2.03 V ± 0.02 *vs.* SCE) has been confirmed in the photoinduced electron transfer to the singlet excited state of ADC ($^1ADC^*$), because the photoinduced electron transfer from linoleic acid to $^1ADC^*$ is exergonic ($\Delta G_{et} = -0.21$ eV), whereas the photoinduced electron-transfer from oleic acid is endergonic ($\Delta G_{et} = +0.06$ eV). In such a case, the products of the photoinduced electron transfer would only be observed in the case of linoleic acid. In fact, laser excitation (355 nm from Nd: YAG laser) of an MeCN solution of ADC ($2.7 \cdot 10^{-4}$ M) in the presence of linoleic acid (0.30M) afforded a transient absorption spectrum at 8 μs, which exhibits appearance of the absorption bands at 510, 640, and 710 nm due to $ADC^{\cdot -}$ [36] [37] as shown in *Fig. 7* [30]. In contrast to the case of linoleic acid, only triplet–triplet absorption due to the triplet excited state

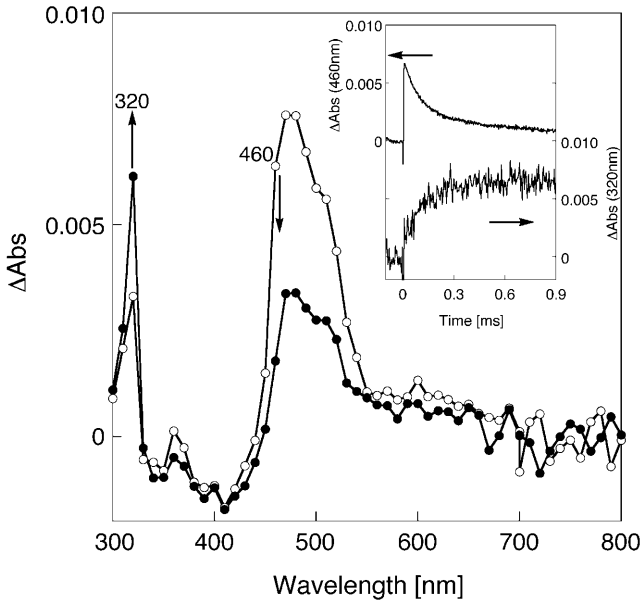


Fig. 6. *Transient absorption spectra observed in photoinduced electron transfer from linoleic acid* ($5.0 \cdot 10^{-2}$ M) *to the singlet excited state of AcrH*$^+$ ($1.0 \cdot 10^{-4}$ M) *in deaerated MeCN at 298 K observed at 10 μs* (○) *and 200 μs* (●) *after irradiation by a laser pulse at λ 355 nm with 64 mJ/pulse.* Inset: Plots of the time profiles of the absorbance at λ 320 and 460 nm.

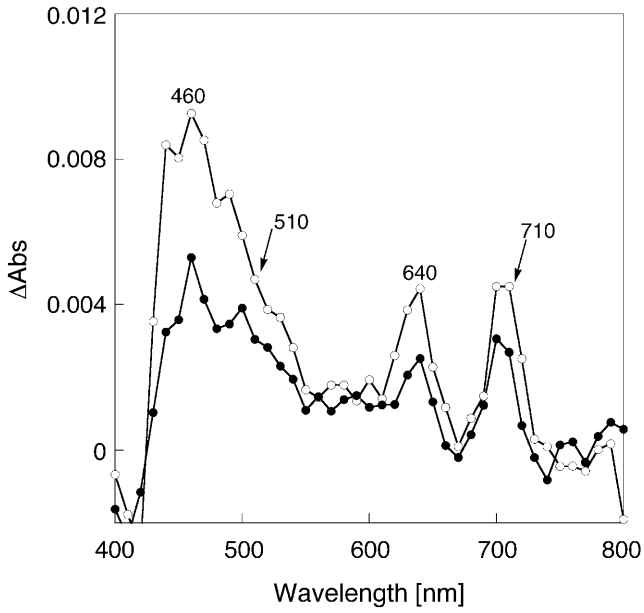


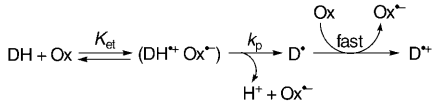
Fig. 7. *Transient absorption spectra observed in photoinduced electron transfer from linoleic acid* (0.30 M) *to the singlet excited state of ADC* ($2.7 \cdot 10^{-4}$ M) *in deaerated MeCN at 298 K observed at 8 μs* (○) *and 200 μs* (●) *after irradiation by a laser pulse at λ 355 nm with 64 mJ/pulse.*

of ADC (^{3}ADC*) appears at 460 nm [36] in the case of oleic acid under otherwise the same experimental conditions [30].

5. Proton-Coupled Electron Transfer (PCET) of Unsaturated Fatty Acids in Lipoxygenase. – Since deprotonated radicals are much stronger one-electron reductants than the parent electron-donor molecules [38], proton-coupled electron transfer (PCET) becomes thermodynamically feasible even if initial electron transfer is endergonic as shown in *Scheme 6*. In such a case, the observed rate constant of overall electron transfer (k_{obs}) to form 2 equiv. of $Ox^{\bullet-}$ is given by *Eqn. 2*, under the conditions that the deprotonation rate constant (k_p) is much smaller than the back-electron-transfer rate constant (k_{bet}) and the equilibrium constant of electron transfer ($K_{et}=k_{et}/k_{bet}$) « 1. In such a case, no intermediate could be detected in the PCET process because of the small equilibrium constant (K_{et} « 1).

$$k_{obs}=2k_pK_{et} \tag{2}$$

Scheme 6



Since the K_{et} value is determined from the E_{ox} value of an electron donor and the E_{red} value of an electron acceptor, and the k_p value has been determined as $8.1\cdot10^3$ s^{-1} (*vide supra*), the k_{obs} value of outer-sphere electron transfer followed by proton transfer can be estimated with *Eqn. 2*. For example, the k_{obs} value of PCET from linoleic acid to a strong one-electron oxidant, $[Ru(bpy)_3]^{3+}$ (bpy = 2,2′-bipyridine; E_{red} = 1.24 V *vs.* SCE)), is estimated as $2.6\cdot10^{-5}$ M^{-1} s^{-1}. In such a case, virtually no electron transfer from linoleic acid to $[Ru(bpy)_3]^{3+}$ occurs. This has been confirmed experimentally [30], when $[Ru(bpy)_3]^{3+}$ acts as an outer-sphere oxidant because $[Ru(bpy)_3]^{3+}$ is a coordinatively saturated complex.

The one-electron reduction potential of an iron(III) hydroxide cofactor ($Fe^{III}-OH$) of soybean lipoxygenase (0.6 V) [13] is much lower than those of $[Ru(bpy)_3]^{3+}$. The difference in the one-electron reduction potentials between an iron(III) hydroxide cofactor of soybean lipoxygenase (0.6 V) and $[Ru(bpy)_3]^{3+}$ (1.24 V) indicates that the electron-transfer rate with an iron(III) hydroxide cofactor is $6.5\cdot10^{10}$ times slower than that with $[Ru(bpy)_3]^{3+}$, because ΔE_{red} (0.64 V) corresponds to $F\Delta E_{red}/2.3RT$ (= 10.81, T = 298 K) in terms of logarithm of the rate constant. In such a case, an outer-sphere PCET pathway in the reaction of linoleic acid with $Fe^{III}-OH$ can be definitely ruled out.

As far as the relative reactivity between different substrates is concerned, however, the relative reactivity in lipoxygenases is similar to that in electron-transfer reactions of unsaturated fatty acids as compared with that in H-atom-transfer reactions. For example, a 10^5 slower rate of soybean lipoxygenase-1 (SLO)-catalyzed oleic acid oxygenation than of the oxygenation of linoleic acid [7] is consistent with a large rate difference ($3.7\cdot10^4$) in electron transfer between linoleic acid and oleic acid, which is expected

from the difference in the E_{ox} values (1.76 V *vs.* SCE of linoleic acid and 2.03 V *vs.* SCE of oleic acid). Such a large difference is not observed in the H-atom-transfer reactions of linoleic acid and oleic acid (*Fig. 3*). On the other hand, the SLO-catalyzed oxygenation rate of arachidonic acid has been reported to be similar to that of linoleic acid [10]. This is also consistent with the same E_{ox} values between arachidonic acid and linoleic acid in contrast with the observed difference in the HAT reactivity (*Fig. 3*).

The similar relative reactivity of LO despite the highly endergonic outer-sphere electron transfer suggests that a strong interaction between linoleic acid and the reactive center of the lipoxygenases (Fe^{III}–OH) is involved to make a PCET process to occur efficiently, when an inner-sphere electron transfer from linoleic acid to the Fe^{III} state is strongly coupled with the proton transfer to the OH group. The important distinction between the outer-sphere electron transfer and inner-sphere electron transfer has previously been discussed in detail [39]. The PCET process is clearly distinguished from an HAT process or an outer-sphere electron-transfer process, followed by proton transfer, because an electron and a proton are transferred at the same time but separately to the Fe^{III} site and the OH site in the PCET process, respectively.

REFERENCES

[1] R. I. Cukier, D. G. Nocera, *Annu. Rev. Phys. Chem.* **1998**, *49*, 337.
[2] J. M. Mayer, I. J. Rhile, *Biochim. Biophys. Acta* **2004**, *1655*, 51.
[3] T. Funabiki, in 'Oxygenases and Model Systems', Ed. T. Funabiki, Kluwer Academic Publishers, Dordrecht, 1997; M. J. Nelson, S. P. Seitz, in 'Active Oxygen in Biochemistry', Eds. J. S. Valentine, C. S. Foote, A. Greenberg, and J. F. Liebman, Chapman & Hall, London, 1995.
[4] E. I. Solomon, T. C. Brunold, M. I. Davis, J. N. Kemsley, S.-K. Lee, N. Lehnert, F. Neese, A. J. Skulan, Y.-S. Yang, J. Zhou, *Chem. Rev.* **2000**, *100*, 235; L. Que Jr., R. Y. N. Ho, *Chem. Rev.* **1996**, *96*, 2607.
[5] G. A. Veldink, M. P. Hilbers, W. F. Nieuwenhuizen, J. F. G. Vliegenthart, in 'Eicosanoids and Related Compounds in Plants and Animals', Eds. A. F. Rowley, H. Kuhn, and T. Schewe, Princeton University Press, Princeton, 1998.
[6] N. A. Porter, *Acc. Chem. Res.* **1986**, *19*, 262; N. A. Porter, D. G. Wujek, *J. Am. Chem. Soc.* **1984**, *106*, 2626.
[7] C. H. Clapp, S. E. Senchak, T. J. Stover, T. C. Potter, P. M. Findeis, M. J. Novak, *J. Am. Chem. Soc.* **2001**, *123*, 747.
[8] E. J. Corey, R. Nagata, *J. Am. Chem. Soc.* **1987**, *109*, 8107.
[9] T. Jonsson, M. H. Glickman, S. Sun, J. P. Klinman, *J. Am. Chem. Soc.* **1996**, *118*, 10319; M. J. Knapp, F. P. Seebeck, J. P. Klinman, *J. Am. Chem. Soc.* **2001**, *123*, 2931; M. J. Knapp, K. Rickert, J. P. Klinman, *J. Am. Chem. Soc.* **2002**, *124*, 3865.
[10] S. Peng, W. A. van der Donk, *J. Am. Chem. Soc.* **2003**, *125*, 8988.
[11] M. H. Glikman, J. S. Wiseman, J. P. Klinman, *J. Am. Chem. Soc.* **1994**, *116*, 793; M. H. Glikman, J. P. Klinman, *Biochemistry* **1995**, *34*, 14077.
[12] C.-C. Hwang, C. B. Grissom, *J. Am. Chem. Soc.* **1994**, *116*, 795; E. R. Lewis, E. Johansen, T. R. Holman, *J. Am. Chem. Soc.* **1999**, *121*, 1395.
[13] C. R. Goldsmith, R. T. Jonas, T. D. P. Stack, *J. Am. Chem. Soc.* **2002**, *124*, 83; C. R. Goldsmith, A. P. Cole, T. D. P. Stack, *J. Am. Chem. Soc.* **2005**, *127*, 9904.
[14] H. Fischer, L. Radom, *Angew. Chem., Int. Ed.* **2001**, *40*, 1340.
[15] B. Knühl, S. Marque, H. Fischer, *Helv. Chim. Acta* **2001**, *84*, 2290.
[16] P. J. Krusic, J. K. Kochi, *J. Am. Chem. Soc.* **1968**, *90*, 7155.
[17] S. Fukuzumi, K. Shimoosako, T. Suenobu, Y. Watanabe, *J. Am. Chem. Soc.* **2003**, *125*, 9074.
[18] H. Kitaguchi, K. Ohkubo, S. Ogo, S. Fukuzumi, *J. Am. Chem. Soc.* **2005**, *127*, 6605.
[19] E. Bascetta, F. D. Gunstone, J. C. Walton, *J. Chem. Soc., Perkin Trans. 2* **1983**, 603.

[20] J. E. Wertz, J. R. Bolton, in 'Electron Spin Resonance: Elementary Theory and Practical Applications', McGraw-Hill, New York, 1972.
[21] J. E. Bennett, R. Summers, *J. Chem. Soc., Faraday Trans. 2* **1973**, *69*, 1043.
[22] K. Suga, K. Ohkubo, S. Fukuzumi, *J. Phys. Chem. A* **2003**, *107*, 4339.
[23] D. L. Sloane, R. Leung, C. S. Craik, E. Sigal, *Nature (London)* **1991**, *354*, 149; Q.-F. Gan, M. F. Browner, D. L. Sloane, E. Sigal, *J. Biol. Chem.* **1996**, *271*, 25412.
[24] J. C. Boyington, B. J. Gaffney, L. M. Amzel, *Science (Washington, D.C.)* **1993**, *260*, 1482.
[25] K. Schwarz, S. Borngraber, M. Anton, H. Kuhn, *Biochemistry* **1998**, *37*, 15327; A. R. Brash, W. E. Boeglin, M. S. Chang, B.-H. Shieh, *J. Biol. Chem.* **1996**, *271*, 20949.
[26] W. Minor, J. Steczko, B. Stec, Z. Otwinowski, J. T. Bolin, R. Walter, B. Axelrod, *Biochemistry* **1996**, *35*, 10687.
[27] M. J. Knapp, J. P. Klinman, *Biochemistry* **2003**, *42*, 11466.
[28] H. Kitaguchi, K. Ohkubo, S. Ogo, S. Fukuzumi, *Chem. Commun.* **2006**, 979.
[29] E. Hatcher, A. V. Soudackov, S. Hammes-Schiffer, *J. Am. Chem. Soc*. **2004**, *126*, 5763.
[30] H. Kitaguchi, K. Ohkubo, S. Ogo, S. Fukuzumi, *J. Phys. Chem. A* **2006**, *110*, 1718.
[31] K. Ohkubo, K. Suga, K. Morikawa, S. Fukuzumi, *J. Am. Chem. Soc.* **2003**, *125*, 12850.
[32] S. Fukuzumi, N. Satoh, T. Okamoto, K. Yasui, T. Suenobu, Y. Seko, M. Fujitsuka, O. Ito, *J. Am. Chem. Soc.* **2001**, *123*, 7756.
[33] D. Rehm, A. Weller, *Ber. Bunsenges. Phys. Chem.* **1969**, *73*, 834; D. Rehm, A. Weller, *Isr. J. Chem.* **1970**, *8*, 259.
[34] S. Fukuzumi, M. Fujita, J. Otera, Y. Fujita, *J. Am. Chem. Soc.* **1992**, *114*, 10271; S. Fukuzumi, H. Miyao, K. Ohkubo, T. Suenobu, *J. Phys. Chem. A* **2005**, *109*, 3285; S. Fukuzumi, K. Hironaka, N. Nishizawa, T. Tanaka, *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2220.
[35] S. Katsumata, *J. Electron Spectrosc. Relat. Phenom*. **2005**, *142*, 265.
[36] M. Fujita, A. Ishida, T. Majima, S. Takamuku, *J. Phys. Chem.* **1996**, *100*, 5382.
[37] J. Eriksen, C. S. Foote, *J. Phys. Chem.* **1978**, *82*, 2659.
[38] D. D. M. Wayner, D. J. McPhee, D. Griller, *J. Am. Chem. Soc.* **1988**, *110*, 132.
[39] S. Fukuzumi, C. L. Wong, J. K. Kochi, *J. Am. Chem. Soc*. **1980**, *102*, 2928.

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