Electron-Transfer Oxidation Properties of Unsaturated Fatty Acids and Mechanistic Insight into Lipoxygenases

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Rate constants of photoinduced electron-transfer oxidation of unsaturated fatty acids with a series of singlet excited states of oxidants in acetonitrile at 298 K were examined and the resulting electron-transfer rate constants (k_{et}) were evaluated in light of the free energy relationship of electron transfer to determine the one-electron oxidation potentials (E_{ox}) of unsaturated fatty acids and the intrinsic barrier of electron transfer. The k_{et} values of linoleic acid with a series of oxidants are the same as the corresponding k_{et} values of methyl linoleate, linolenic acid, and arachidonic acid, leading to the same E_{ox} value of linoleic acid, methyl linoleate, linolenic acid, and arachidonic acid (1.76 V vs SCE), which is significantly lower than that of oleic acid (2.03 V vs SCE) as indicated by the smaller k_{et} values of oleic acid than those of other unsaturated fatty acids. The radical cation of linoleic acid produced in photoinduced electron transfer from linoleic acid to the singlet excited state of 10-methylacridinium ion as well as that of 9,10-dicyanoanthracene was detected by laser flash photolysis experiments. The apparent rate constant of deprotonation of the radical cation of linoleic acid was determined as 8.1×10^3 s^{-1}. In the presence of oxygen, the addition of oxygen to the deprotonated radical produces the peroxyl radical, which has successfully been detected by ESR. No thermal electron transfer or proton-coupled electron transfer has occurred from linoleic acid to a strong one-electron oxidant, $Ru(bpy)_3^{3+}$ (bpy = 2,2′-bipyridine) or $Fe(bpy)_3^{3+}$. The present results on the electron-transfer and proton-transfer properties of unsaturated fatty acids provide valuable mechanistic insight into lipoxygenases to clarify the proton-coupled electron-transfer process in the catalytic function.

Introduction

Lipoxygenases are non-heme iron proteins that catalyze the oxidation of unsaturated fatty acids via hydrogen atom abstraction from a bisallylic position of unsaturated fatty acids, followed by addition of oxygen to generate a hydroperoxide product.[1−3] In mammals, lipoxygenases oxygenate arachidonic acid, resulting in production of leukotrienes and lipoxins, which regulate responses in inflammation and immunity.[4] Thus, lipoxygenase inhibitors have been used as drug agents to treat inflammatory diseases such as asthma, atherosclerosis, and psoriasis.[4,5] In addition, lipoxygenase inhibitors have been proposed as promising cancer chemopreventive agents.[6,7] Extensive kinetic studies of lipoxygenases using soybean lipoxygenase-1 (SLO)[8] and human lipoxygenase[9] as well as the model studies[10] have revealed that the abstraction of the pro-*S* hydrogen atom from carbon atom C11 of linoleic acid by a ferric hydroxide cofactor (Fe(III)−OH) with an unusually high redox potential (0.6 V)[11] is rate-limiting to form Fe(II)−OH_2 and a radical intermediate substrate. The five-coordinate ferric center consisting of three His, the C-terminal carboxylate, and a hydroxide, with an Asn further removed from the iron, is believed to be responsible for the high reactivity of the hydrogen-abstraction process.[12] Subsequent reaction with molecular oxygen eventually leads to 13-(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid (13-HPOD), accompanied by regeneration of Fe(III)−OH.[8,9] The hydrogen-abstraction step has received considerable interest, since the unusually large deuterium kinetic isotope effects up to 81 were reported in the SLO-catalyzed oxidation of linoleic acid and arachidonic acid.[8,13,14] Quantum mechanical calculations have indicated that the hydrogen-transfer step involves an electron transfer from the π-system of the linoleic acid to an orbital localized on the Fe(III) center, and a proton transfer from the donor carbon to the oxygen acceptor.[15,16] Such a proton-coupled electron transfer (PCET) has been regarded an important mechanism for biological redox reactions.[17−22] In a PCET reaction, the electron and proton may transfer consecutively (ET/PT) or concertedly (ETPT).[18] The concerted pathway without an intermediate is merged into hydrogen atom transfer.[18] The unusually high deuterium kinetic isotope effect of 81 has been interpreted as evidence for a rate-determining hydrogen-tunneling step, which has been well simulated theoretically.[16,23−25] Understanding such reactions certainly requires knowledge of the thermodynamics and kinetics of the possible ET, PT, and PCET steps. The consecutive (ET/PT) pathway has been ruled out for lipoxygenases based on the unfavorable thermodynamics.[16,18] However, the reported estimation of the one-electron oxidation potential (E_{ox}) of an unsaturated fatty acid is quite inaccurate.[26] Thus, the previous discussion on the thermodynamics associated with an electron transfer for lipoxygenases has yet to be verified. The deprotonation step of radical cations of unsaturated fatty acids has never been examined, either.

We report herein the detailed kinetic investigations on both photoinduced and thermal electron-transfer oxidation of unsaturated fatty acids to determine the standard one-electron oxidation potentials (E_{ox}) and the intrinsic barriers of electron transfer ($\Delta G^{\neq}_0$), both of which would otherwise be difficult to obtain.

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Once the E_{ox} values of unsaturated fatty acids are determined, the free energy changes (ΔG_{et}) of photoinduced electron-transfer reactions of unsaturated fatty acids with various one-electron oxidants can be readily estimated. When the ΔG_{et} values are negative, the electron-transfer products would be detected as the transient absorption spectra. In contrast, no electron-transfer products would be detected when the ΔG_{et} values are positive. This is confirmed by laser flash photolysis measurements of photoinduced electron-transfer reactions of unsaturated fatty acids to demonstrate the validity of the E_{ox} values of unsaturated fatty acids determined in this study. The rate constant of the deprotonation of the radical cation of linoleic acid has been determined for the first time from the decay and rise profiles of the radical cation and the deprotonated radical, respectively. The formation of peroxyl radicals derived from the electron-transfer oxidation of linoleic acid, followed by deprotonation and addition of oxygen is directly detected by ESR. The possibility of proton-coupled electron-transfer oxidation of linoleic acid has also been examined using strong one-electron oxidants such as $Ru(bpy)_3^{3+}$ and $Fe(bpy)_3^{3+}$ (bpy = 2,2′-bipyridine). These data provide the solid energetic basis for the postulated PCET process in lipoxygenases.

Experimental Section

Materials. Oleic acid, linoleic acid, linolenic acid, arachidonic acid, and tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate [$Ru(bpy)_3Cl_2$] were purchased from Aldrich Co., Ltd. Methyl linoleate was purchased from Wako Pure Chemical Industries, Ltd. [11,11-$^2H_2$]linoleic acid was synthesized according to the literature,[27] but an alternate procedure was used to convert 2-octyn-1-ol to the corresponding bromide.[28] 9-Decynoic acid used in this procedure was synthesized according to the literature.[29] Tris(2,2′-bipyridyl)ruthenium(III) hexafluorophosphate [$Ru(bpy)_3(PF_6)_3$] was prepared by oxidizing $Ru(bpy)_3^{2+}$ with lead dioxide in aqueous H_2SO_4 followed by the addition of KPF_6.[30,31] Tris(2,2′-bipyridyl)iron(III) hexafluorophosphate [$Fe(bpy)_3(PF_6)_3$] was prepared by adding three equivalents of 2,2′-bipyridine to an aqueous solution of ferrous sulfate and then by oxidizing the iron (II) complex with lead dioxide in an aqueous H_2SO_4 solution, followed by the addition of KPF_6.[31] 10-Methylacridinium iodide ($AcrH^+$), 1-methylquinolinium iodide (QuH^+), and 1-methyl-3-cyanoquinolinium iodide ($CNQuH^+$) were prepared by the reactions of acridine, quinoline, and 3-cyanoquinoline with methyl iodide in acetone;[32] the resulting iodide salts were converted to the perchlorate salts ($AcrH^+ClO_4^-$, $QuH^+ClO_4^-$, and $CNQuH^+ClO_4^-$) by addition of $Mg(ClO_4)_2$ to the iodide salt and purified by recrystallization from methanol.[33] 9-Substituted 10-methylacridinium perchlorate ($AcrR^+ClO_4^-$: R = Pr^i and Ph) was prepared by the reaction of 10-methylacridone in dichloromethane with the corresponding Grignard reagent (RMgX), followed by addition of sodium hydroxide for the hydrolysis and perchloric acid for the neutralization, and purified by recrystallization from ethanol−diethyl ether.[34] Organic photosensitizers (1,4-dicyanonaphthalene (DCN) and 9,10-dicyanoanthracene (DCA)) were obtained commercially from Tokyo Kasei Kogyo Co., Ltd and purified by the standard method.[35] Di-*tert*-butyl peroxide (Bu^tOOBu^t) was purchased from Nacalai Tesque Co., Ltd. and purified by chromatography through alumina, which removes traces of the hydroperoxide.[35] Acetonitrile was purified and dried with calcium hydride by the standard procedure, and stored under nitrogen atmosphere.[35]

Fluorescence Quenching. Quenching experiments of the fluorescence of organic photosensitizers were carried out on a Shimadzu RF-5300 spectrofluorophotometer. The excitation wavelengths were 355, 340, 345, 450, 465, 460, and 430 nm for $CNQuH^+$ (1.0×10^{-4} M), QuH^+ (1.0×10^{-4} M), DCN (1.0×10^{-4} M), $AcrH^+$ (1.0×10^{-4} M), $AcrPh^+$ (1.0×10^{-4} M), $AcrPr^{i+}$ (1.0×10^{-4} M), and DCA (1.0×10^{-5} M).[36,37] The monitoring wavelengths were those corresponding to the maxima of the respective emission bands at 435, 395, 380, 490, 505, 500, and 460 nm, respectively.[36,37] The solutions were deoxygenated by argon purging for 10 min prior to the measurements. Relative emission intensities were measured for a solution of each photosensitizer with an unsaturated fatty acid quencher at various concentrations. There was no change in the shape but there was a change in the intensity of the fluorescence spectrum by the addition of a quencher. The Stern−Volmer relationship (eq 1) was obtained for the ratio of the emission

$$I_0/I = 1 + K_{SV}[D] \quad (1)$$

intensities in the absence and presence of an electron donor (I_0/I) and the concentrations of unsaturated fatty acid donors used as quenchers [D]. In the case of fluorescence quenching of $AcrH^+$ by some quenchers, the Stern−Volmer plot showed a deviation from a linear correlation between I_0/I and [D] in the high concentrations of donors which absorb light at the excitation wavelength. In such a case, the longer excitation wavelength (e.g., $\lambda = 486$ nm) was selected and the quenching constant was determined from the initial slope of the Stern−Volmer plot.

Time-resolved fluorescence spectra were measured by a Photon Technology International GL-3300 with a Photon Technology International GL-302 and a nitrogen laser/pumped dye laser system equipped with a four-channel digital delay/pulse generator (Standard Research System Inc. DG535) and a motor driver (Photon Technology International MD-5020). The laser excitation with a wavelength of 337 nm was obtained from a nitrogen laser and that with a wavelength of 431 nm was generated using dimethyl-POPOP (Dojindo Laboratory, Japan) as a dye. The fluorescence lifetimes τ were determined by a single-exponential curve fit using a microcomputer. Fluorescence lifetimes of photosensitizers in deaerated MeCN were determined as 45, 31, 12, 37, 1.5, 34, and 18 ns for $CNQuH^+$ QuH^+, DCN, $AcrH^+$, $AcrPh^+$, $AcrPr^{i+}$, and DCA, respectively. The observed rate constants k_{et} ($= K_{SV}\tau^{-1}$) of photoinduced electron transfer were determined from the Stern−Volmer constants K_{SV} and the emission lifetimes τ.

Laser Flash Photolysis Measurements. Measurements of transient absorption spectra in photoinduced electron transfer from linoleic acid to $AcrH^+$ and DCA were performed according to the following procedures. Degassed MeCN solutions containing $AcrH^+$ (1.0×10^{-4} M) with linoleic acid (5.0×10^{-2} M), $AcrH^+$ (1.0×10^{-4} M) with oleic acid (5.0×10^{-2} M), DCA (2.7×10^{-4} M) with linoleic acid (0.30 M), and DCA (2.7×10^{-4} M) with oleic acid (0.10 M) were excited by Nd:YAG laser (Continuum, SLII-10, 4−6 ns fwhm) at 355 nm. Time courses of the transient absorption spectra were measured by using a continuous Xe-lamp (150 W) and an In GaAs−PIN photodiode (Hamamatsu 2949) as a probe light and a detector, respectively. The output from the photodiodes and a photomultiplier tube was recorded with a digitizing oscilloscope (Tektronix, TDS3032, 300 MHz). The transient spectra were recorded using fresh solutions in each laser excitation. All experiments were performed at 298 K.

Kinetic Measurements. Changes in the UV−vis spectra in electron-transfer reactions were monitored using a Hewlett-Packard 8453 diode array spectrophotometer. The reaction of

linoleic acid with $Fe(bpy)_3^{3+}$ was examined by measuring the change in the UV−vis spectra of $Fe(bpy)_3^{2+}$ ($\epsilon = 8400\ M^{-1}\ cm^{-1}$ at 520 nm)[31] in the presence of various concentrations of linoleic acid ($(0-4.0) \times 10^{-4}$ M). The reaction of linoleic acid with $Ru(bpy)_3^{3+}$ was examined by measuring the change in the UV−vis spectra of $Ru(bpy)_3^{2+}$ ($\epsilon = 14\,600\ M^{-1}\ cm^{-1}$ at 452 nm)[31] in the presence of various concentrations of linoleic acid ($(0-2.0) \times 10^{-4}$ M).

ESR Measurements. The ESR spectra were performed on a JEOL X-band ESR spectrometer (JES-ME-LX) at 233 or 253 K. A quartz ESR tube (internal diameter: 1.5 mm) containing an oxygen-saturated CH_2Cl_2 solution of $AcrPr^{i+}$ (1.0×10^{-2} M) and linoleic acid (0.10 M) at 233 K or an O_2-saturated Bu^t-$OOBu^t$ (neat) solution containing linoleic acid (1.3 M) at 253 K was irradiated in the cavity of the ESR spectrometer with the focused light of a 1000-W high-pressure Hg lamp (Ushio-USH1005D) through an aqueous filter. The ESR spectra were measured under nonsaturating microwave power conditions. The amplitude of modulation was chosen to optimize the resolution and the signal-to-noise (S/N) ratio of the observed spectra: typically 1.0 G for peroxyl radicals. The g values were calibrated with a Mn^{2+} marker.

Results and Discussion

Photoinduced Electron-Transfer Oxidation of Unsaturated Fatty Acids. The irreversible behavior upon the electron-transfer oxidation of unsaturated fatty acid due to facile deprotonation together with the strong interaction of unsaturated fatty acid with electrodes has precluded the accurate determination of the one-electron oxidation potentials (E_{ox}) by the direct electrochemical measurements. Thus, 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 can be deduced (vide infra).

The dynamics of intermolecular photoinduced electron-transfer reactions from an electron donor (D) to the excited-state acceptor (A*) has been formulated as shown in eq 2, where k_{12} and k_{21} are the diffusion and dissociation rate constants in the encounter complex (DA*), and k_{23} and k_{32} are the rate

$$D + A^* \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} (DA^*) \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} (D^{\bullet+}A^{\bullet-}) \xrightarrow{k_{30}} D + A \qquad (2)$$

constants of forward electron transfer from D to A* and the back electron transfer to the excited state, respectively, and k_{30} is the rate constant of back electron transfer to the ground state.[38,39] The over-all rate constant (k_{et}) of the emission quenching by electron transfer is given by eq 3 under the conditions that the back electron transfer to the ground state is

$$k_{et} = k_{12}k_{23}/(k_{32} + k_{21}) \qquad (3)$$

much faster than that to the excited state, i.e., $k_{30} \gg k_{32}$. From eq 3 is derived eq 4, where $\Delta G^{\neq}$ is the activation Gibbs energy

$$\Delta G^{\neq} = 2.3RT\log[Z(k_{et}^{-1} - k_{12}^{-1})] \qquad (4)$$

of the electron-transfer process in (DA*), Z [$= (k_BT/h)(k_{12}/k_{21})$; k_B is the Boltzmann constant) is the collision frequency that is taken as $1 \times 10^{11}\ M^{-1}\ s^{-1}$, F is the Faraday constant, the k_{12} value in MeCN is $2.0 \times 10^{10}\ M^{-1}\ s^{-1}$,[38] and the other notations are conventional. The dependence of $\Delta G^{\neq}$ on the Gibbs energy change of adiabatic photoinduced electron transfer (ΔG_{et}) has well been established as given by the Gibbs energy relationship (eq 5), where $\Delta G^{\neq}_0$ is the intrinsic barrier that represents the

$$\Delta G^{\neq} = (\Delta G_{et}/2) + [(\Delta G_{et}/2)^2 + (\Delta G^{\neq}_0)^2]^{1/2} \qquad (5)$$

activation Gibbs energy when the driving force of electron transfer is zero, i.e., $\Delta G^{\neq} = \Delta G^{\neq}_0$ at $\Delta G_{et} = 0$.[38-41] On the other hand, the ΔG_{et} values of photoinduced electron transfer are obtained from the one-electron oxidation potential of the donor (E_{ox}) and the one-electron reduction potential of the excited state of the acceptor (E_{red}*) by using eq 6.[42,43] The E_{red}* values are determined from the E_{red} values of the ground state by subtracting the excitation energies (ΔE), which are obtained

$$\Delta G_{et} = F(E_{ox} - E_{red}^*) \qquad (6)$$

as the average of the absorption and emission energies.[38]

From eqs 5 and 6 is derived a linear relation between $\Delta G^{\neq}/F + E_{red}$* and $(\Delta G^{\neq}/F)^{-1}$ (eq 7).[39] The $\Delta G^{\neq}/F$ values are obtained from the rate constants of photoinduced electron transfer (k_{et})

$$(\Delta G^{\neq}/F) + E_{red}^* = E_{ox} + (\Delta G^{\neq}_0/F)^2/(\Delta G^{\neq}/F) \qquad (7)$$

by using eq 4. We can choose appropriate acceptors whose E_{red}* values are known or readily determined. Thus, the unknown values of E_{ox} and $\Delta G^{\neq}_0/F$ of unsaturated fatty acids (electron donors) can be determined from the intercept and slope of plots of $\Delta G^{\neq}/F + E_{red}$* vs $(\Delta G^{\neq}/F)^{-1}$ by using eq 7, respectively.[37,39]

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 ($CNQuH^+$, QuH^+, DCN, $AcrH^+$, $AcrPh^+$, $AcrPr^{i+}$, and DCA) were determined by the fluorescence quenching in MeCN at 298 K (see Experimental Section). Typical Stern−Volmer plots (eq 1) are shown in Figure 1. The k_{et} values thus determined from the slopes of the Stern−Volmer plots and the fluorescence lifetimes (eq 1) are listed in Table 1 together with the E_{red}* values of the singlet excited states of electron acceptors.[36,37] The k_{et} values of oleic acid are significantly smaller than those of linoleic acid, methyl linoleate, linolenic acid, and arachidonic acid, which are virtually the same irrespective of the difference in the number of double bonds. Thus, the electron-transfer properties are solely determined by the bisallylic structure of unsaturated fatty acids. It was also confirmed that the k_{et} value

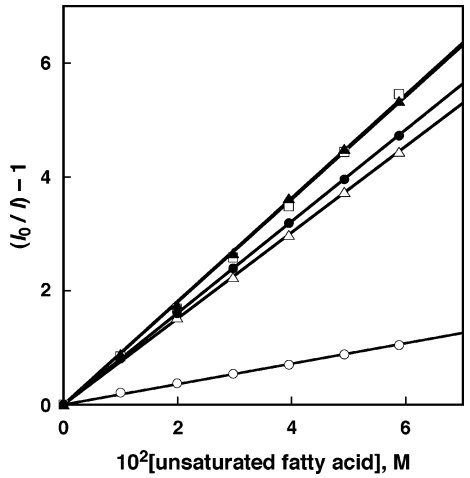


Figure 1. Stern−Volmer plots for fluorescence quenching of $^1AcrPr^{i+}$* (1.0×10^{-4} M) by unsaturated fatty acids [oleic acid (○), linoleic acid (△), methyl linoleate (□), linoleic acid (●), and arachidonic acid (▲)] in deaerated MeCN at 298 K.

TABLE 1: Rate Constants (k_{et}) for the Photoinduced Electron Transfer from Unsaturated Fatty Acids to Organic Sensitizers in Deaerated MeCN at 298 K and One-Electron Reduction Potentials (E_{red}*) of Singlet Excited States of Organic Sensitizers in MeCN at 298 K

			k_{et}, $M^{-1}s^{-1}$				
no.	organic sensitizer	E_{red}* (V vs SCE)	oleic acid	linoleic acid	methyl linoleate	linolenic acid	arachidonic acid
1	$CNQuH^+$	2.72	9.1×10^9	1.3×10^{10}	1.1×10^{10}	1.3×10^{10}	1.0×10^{10}
2	QuH^+	2.54	8.4×10^9	1.0×10^{10}	9.0×10^9	1.1×10^{10}	1.1×10^{10}
3	DCN	2.47	7.6×10^9	9.0×10^9	8.0×10^9	1.0×10^{10}	1.0×10^{10}
4	$AcrH^+$	2.32	4.9×10^9	9.0×10^9	7.6×10^9	8.2×10^9	8.1×10^9
5	$AcrPh^+$	2.14	9.8×10^8	3.3×10^9	4.1×10^9	3.4×10^9	4.1×10^9
6	$AcrPr^{i+}$	2.08	5.2×10^8	2.3×10^9	2.6×10^9	2.4×10^9	2.7×10^9
7	DCA	1.97	2.8×10^7	1.2×10^9 (1.3×10^9)[a]	1.5×10^9	1.2×10^9	1.2×10^9

[a] The k_{et} value is determined from the fluorescence lifetime measurements. Others are from the fluorescence quenching measurements.

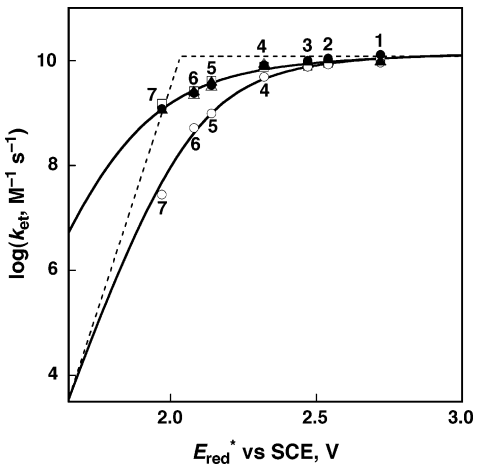


Figure 2. Plots of log k_{et} of photoinduced electron-transfer from unsaturated fatty acids [oleic acid (○), linoleic acid (△), methyl linoleate (□), linoleic acid (●), and arachidonic acid (▲)] to the singlet excited state of organic sensitizers vs one-electron reduction potentials (E_{red}*) of the singlet excited states of organic sensitizers. Numbers refer to organic sensitizers in Table 1. The solid lines are drawn based on eqs 4−6. The two straight broken lines are drawn based on eq 7 and the observed diffusion limit, respectively.[44]

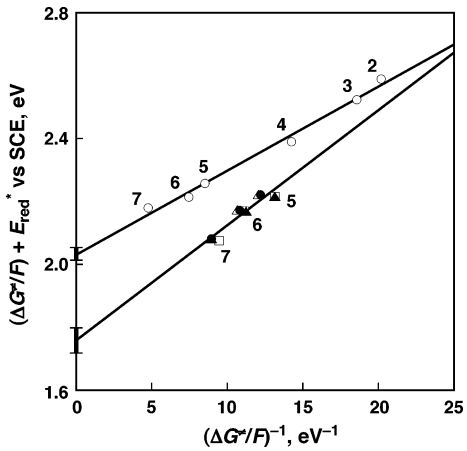


Figure 3. Plots of $(\Delta G^{\neq}/F) + E_{red}$* vs $(\Delta G^{\neq}/F)^{-1}$ for photoinduced electron-transfer reactions of unsaturated fatty acids [oleic acid (○), linoleic acid (△), methyl linoleate (□), linolenic acid (●), and arachidonic acid (▲)]. Numbers refer to organic sensitizers in Table 1.

determined from the steady-state fluorescence quenching of DCA by linoleic acid was the same as that determined from the dynamic quenching (the fluorescence lifetime measurements). To examine the deuterium isotope effect on the rate constant of photoinduced electron transfer, fluorescence quenching rate constant of DCA by [11,11-$^2H_2$]linoleic acid is also determined as 1.0×10^9 M^{-1} s^{-1}, which agrees with that of DCA by undeuterated linoleic acid (1.2×10^9 M^{-1} s^{-1}). Thus, there is no deuterium kinetic isotope effect on the photoinduced electron-transfer step.

The plots of log k_{et} vs E_{red}* are shown in Figure 2, where the k_{et} values increase with increasing in the E_{red}* value to reach a diffusion-limited value. This is a typical driving force dependence of photoinduced electron-transfer reactions, which agrees with eqs 4 and 5.[38,39] When $\Delta G_{et}/2 \gg \Delta G^{\neq}_0$ in eq 5, $\Delta G^{\neq} \cong \Delta G_{et}$. In such a case, log k_{et} value is linearly correlated with E_{red}* with the slope of $F/2.3RT$ (=16.9 at 298 K) as shown in eq 8, which is derived from eqs 4−6. This is drawn as the broken straight line for oleic acid in Figure 2. When $-\Delta G_{et}/2$

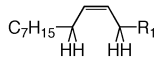
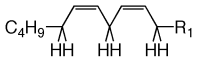
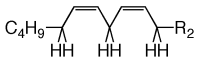
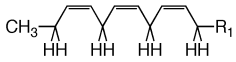
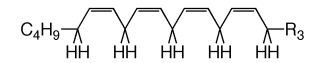
$$\log k_{et} \cong \log Z - (F/2.3RT)(E_{ox} - E_{red}^*) \qquad (8)$$

$\gg \Delta G^{\neq}_0$ in eq 5, on the other hand, $\Delta G^{\neq} \cong 0$. In such a case, k_{et} becomes virtually the same as k_{12} (the diffusion rate constant) in eq 4 as indicated by the broken line at the plateau region in Figure 2. The intersect E_{red}* value of two broken lines corresponds to the E_{ox} value, because $\Delta G_{et} = 0$ in eq 6, when $k_{et} = k_{12}$ in eq 4. Thus, the E_{ox} value of oleic acid is determined as 2.03 V from the intersection value. The graphically determined E_{ox} value is not sensitive to the diffusion-limited rate constant, because the difference in factor of 2 in the diffusion rate constant results in an error of only 0.02 V in E_{ox} value. In the case of linoleic acid (△), methyl linoleate (□), linoleic acid (●), and arachidonic acid (▲) in Figure 2, the lack of points in the endergonic region ($\Delta G_{et} > 0$) has precluded such graphic determination of the E_{ox} value.

The E_{ox} and $\Delta G^{\neq}_0$ of these unsaturated fatty acids can be determined from the intercepts and the slopes of linear plots of $\Delta G^{\neq} + E_{red}$* vs $(\Delta G^{\neq}/F)^{-1}$ (Figure 3) by using eq 7, respectively. Good linear correlations in Figure 3 in accordance with eq 7 despite different molecular sizes of sensitizers confirm the validity of the present analysis. The E_{ox} values of linoleic acid, methyl linoleate, linolenic acid, and arachidonic acid are obtained as all the same as 1.76 ± 0.04 V vs SCE by the least-squares analysis. On the other hand, a higher E_{ox} value is obtained as 2.03 ± 0.02 V vs SCE for oleic acid. This value agrees with the value determined graphically as the intersection of the two broken lines in Figure 2 (vide supra). The experimental errors in these E_{ox} values are obtained as the standard deviation (see the error bar in Figure 3). The standard deviation in the case of oleic acid (±0.02 V) is also consistent with the error estimate of the graphically determined value in Figure 2 (vide supra). The experimental errors in the E_{red}* values are also included well within the standard deviation in the E_{ox} values.

The E_{ox} and $\Delta G^{\neq}_0$ values of unsaturated fatty acids are listed in Table 2. The E_{ox} values of unsaturated fatty acids are well correlated with the first adiabatic ionization energies (I_{obs})

TABLE 2: One-Electron Oxidation Potentials (E_{ox}) of Unsaturated Fatty Acids and Intrinsic Barrier ($\Delta G^{\neq}_0$) of the Electron-Transfer Oxidation in MeCN

unsaturated fatty acid	E_{ox} vs SCE, V	$\Delta G^{\neq}_0$, kcal mol^{-1}	structure of unsaturated fatty acid
oleic acid	2.03 ± 0.02	3.77 ± 0.10	
linoleic acid	1.76 ± 0.04	4.41 ± 0.23	
methyl linoleate	1.76 ± 0.04	4.41 ± 0.23	
linolenic acid	1.76 ± 0.04	4.41 ± 0.23	
arachidonic acid	1.76 ± 0.04	4.41 ± 0.23	

$R_1 = C_6H_{12}COOH$, $R_2 = C_6H_{12}COOMe$, $R_3 = C_2H_4COOH$

determined by the HeI photoelectron spectra: The I_{obs} value of oleic acid (8.63 eV) is significantly larger than of linoleic acid (8.45 eV), which is the same as that of linolenic acid (8.45 eV).[45] The validity of the E_{ox} values of unsaturated fatty acids determined herein is further confirmed by the laser flash photolysis experiments (vide infra).

Detection of Radical Intermediates in Photoinduced Electron Transfer. The occurrence of photoinduced electron-transfer reactions of unsaturated fatty acids is confirmed by the laser flash photolysis experiments (see Experimental Section). In the case of photoinduced electron transfer from unsaturated fatty acids to the singlet excited state of $AcrH^+$ ($^1AcrH^{+}$*), the electron transfer from all unsaturated fatty acids examined herein is highly exergonic ($\Delta G_{et} < 0$), since the E_{red}* value of $^1AcrH^{+}$* (2.32 V vs SCE)[36,46] is much more positive than the E_{ox} values of unsaturated fatty acids (Table 2). Laser excitation of an MeCN solution of $AcrH^+$ (1.0×10^{-4} M) and linoleic acid (5.0×10^{-2} M) affords a transient absorption spectrum at 10 μs with appearance of new absorption band 510 nm due to $AcrH^{\bullet}$,[46] as shown in Figure 4. This indicates that photoinduced electron transfer from linoleic acid to $^1AcrH^{+}$* occurs to produce $AcrH^{\bullet}$ and the radical cation of linoleic acid.[47] The difference spectrum obtained by subtraction of the spectrum at 200 μs from the spectrum at 8 μs exhibits an absorption maximum at 460 nm, which is clearly different from the absorption band due to

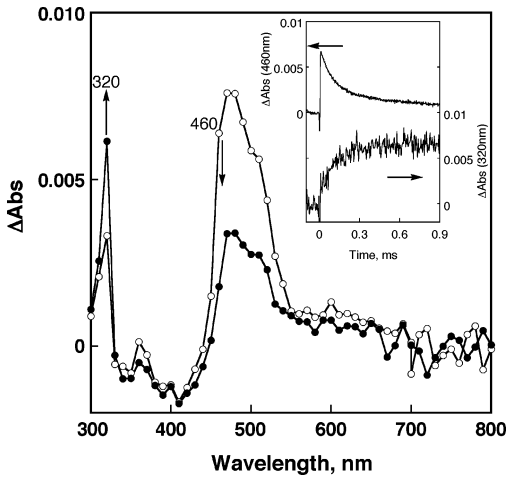


Figure 4. Transient absorption spectra observed in photoinduced electron transfer from linoleic acid (5.0×10^{-2} M) to the singlet excited state of $AcrH^+$ (1.0×10^{-4} M) in deaerated MeCN at 298 K observed at 10 (○) and 200 μs (●) after irradiation of laser pulse at $\lambda = 355$ nm with 64 mJ/pulse.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

$AcrH^{\bullet}$ (see Supporting Information, Figure S1). Thus, this absorption band at 460 nm is assigned to 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 Figure 4. This indicates that the radical cation of linoleic acid deprotonates to produce linoleyl radical which has absorption at 320 nm. The apparent deprotonation rate constant is determined as 8.1×10^3 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 (see Supporting Information, Figure S2).[48]

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 the case of linoleic acid in Figure 4. However, the decay of absorbance at 460 nm becomes slower and the rise in absorbance at 320 nm due to linoleyl radical was hardly observed. This indicates a large deuterium kinetic isotope effect on the deprotonation process of the radical cation of linoleic acid. Although the decay of absorbance at 460 nm involved both the bimolecular back electron transfer from $AcrH^{\bullet}$ to the radical cation of linoleic acid and the deprotonation process, the deuterium kinetic isotope effect is roughly estimated as $k_H/k_D = 7 \pm 1$. The more accurate k_H/k_D value was determined as $k_H/k_D = 7.4 \pm 0.4$ from the comparison of decay rate of the absorption band due to $AcrH^+$ under photoirradiation of an MeCN solution of $AcrH^+$ containing linoleic acid and [11,11-$^2H_2$]linoleic acid.

Laser excitation (355 nm from Nd: YAG laser) of an MeCN solution of $AcrH^+$ (1.0×10^{-4} M) and oleic acid (5.0×10^{-2} M) also affords a transient absorption band at 510 nm due to $AcrH^{\bullet}$,[47] as shown in Figure 5. In this case, however, a transient absorption band at 650 nm appears at 10 μs after the laser excitation. The broad absorption band at 650 nm is characteristic of that of $AcrH_2^{\bullet+}$.[49] The rapid rise of the absorption band is shown the inset of Figure 5. This indicates that the fast proton transfer takes place from the radical cation of oleic acid to $AcrH^{\bullet}$ in the cage.[49] The proton-transfer rate constant is determined as 4.9×10^5 s^{-1} from the first-order plot of the rise in absorbance around at 650 nm (see Supporting Information, Figure S3), which is much larger than the apparent deprotonation rate constant of the radical cation of linoleic acid.[50]

Judging from the E_{ox} value of linoleic acid (1.76 V vs SCE), photoinduced electron transfer from linoleic acid to the singlet excited state of 9,10-dicyanoanthracene (^1DCA*) is exergonic ($\Delta G_{et} = -0.21$ eV), whereas the photoinduced electron transfer from oleic acid (2.03 V vs SCE) is endergonic ($\Delta G_{et} = +0.06$ eV). Thus, the products of the photoinduced electron transfer

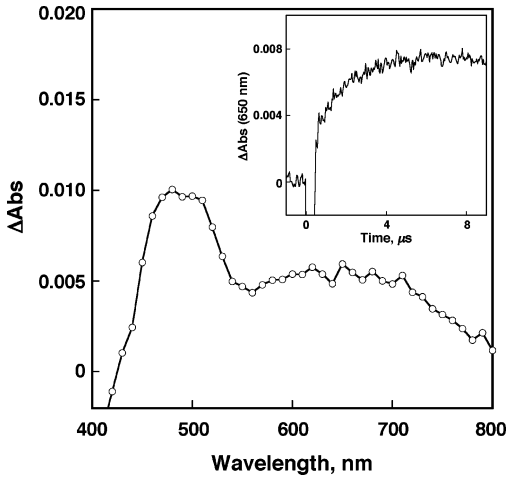


Figure 5. Transient absorption spectra observed in photoinduced electron transfer from oleic acid (5.0×10^{-2} M) to the singlet excited state of $AcrH^+$ (1.0×10^{-4} M) in deaerated MeCN at 298 K observed at 10 μs after irradiation of laser pulse at $\lambda = 355$ nm with 64 mJ/pulse. Inset: Plot of the time profile of the absorbance at $\lambda = 650$ nm.

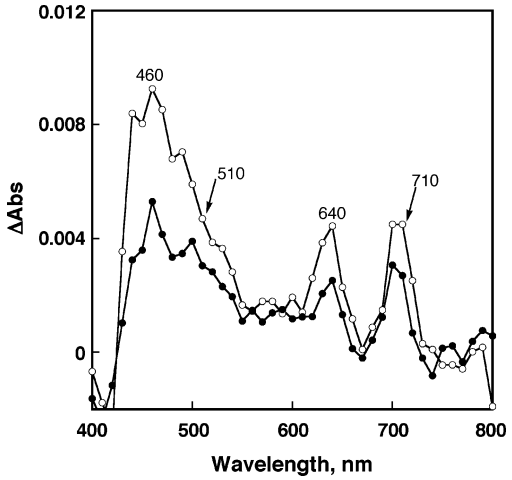


Figure 6. Transient absorption spectra observed in photoinduced electron transfer from linoleic acid (0.30 M) to the singlet excited state of DCA (2.7×10^{-4} M) in deaerated MeCN at 298 K observed at 8 (○) and 200 μs (●) after irradiation of laser pulse at $\lambda = 355$ nm with 64 mJ/pulse.

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 DCA (2.7×10^{-4} M) in the presence of linoleic acid (0.30 M) affords a transient absorption spectrum at 8 μs, which exhibits appearance of the absorption bands at 510, 640, and 710 nm due to $DCA^{\bullet-}$,[51,52] as shown in Figure 6. Since there is little absorbance at 460 nm for $DCA^{\bullet-}$,[51,52] the transient absorption at 460 nm is ascribed to that due to the radical cation of linoleic acid as the case of photoinduced electron transfer from linoleic acid to $^1AcrH^{+}$* (Figure 4).[53] In contrast to the case of linoleic acid, only triplet−triplet absorption due to the triplet excited state of DCA ($^3DCA^*$)[54] appears at 460 nm in the case of oleic acid under otherwise the same experimental conditions (see Supporting Information S4). Thus, the E_{ox} values of unsaturated fatty acids determined from the plots of log k_{et} of photoinduced electron transfer from unsaturated fatty acids to the singlet excited state of organic sensitizers vs one-electron reduction potentials (E_{red}*) of the singlet excited states of organic sensitizers in Figure 2 (Table 2) are quite consistent with the results of the laser flash photolysis experiments.

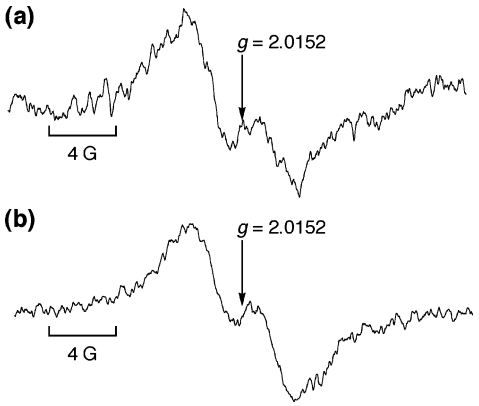
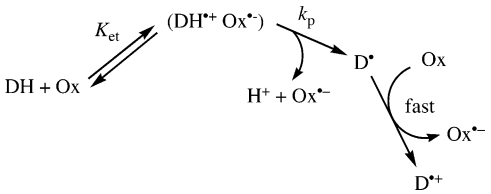


Figure 7. ESR spectra of peroxyl radicals observed under photoirradiation of (a) an O_2-saturated CH_2Cl_2 solution containing $AcrPr^{i+}$ (1.0×10^{-2} M) and linoleic acid (0.10 M) at 233 K and (b) an O_2-saturated Bu^tOOBu^t (neat) solution containing linoleic acid (1.3 M) at 253 K with a high-pressure Hg lamp.

SCHEME 1



The ESR spectra were measured under photoirradiation of a CH_2Cl_2 solution containing $AcrPr^{i+}$ (1.0×10^{-2} M) and linoleic acid (0.10 M) at 233 K (see Experimental Section). The linoleyl radical, which is supposed to be formed by deprotonation of the radical cation of linoleic acid, could not be detected under the present experimental conditions probably due to the instability of the radical.[55,56] When the photoirradiation is performed in an oxygen-saturated CH_2Cl_2 solution, the doublet ESR signal with a g value (2.0152) is observed as shown in Figure 7a. The g value and the hyperfine coupling constant (a(H) = 4.06 G) are diagnostic of secondary alkylperoxyl radicals.[57,58] The same ESR signal is observed when the photoirradiation was carried out using an oxygen-saturated solution containing linoleic acid and Bu^tOOBu^t (Figure 7b).[54] Thus, the linoleyl radical produced by deprotonation of the radical cation of linoleic acid is trapped by oxygen to produce the peroxyl radical.

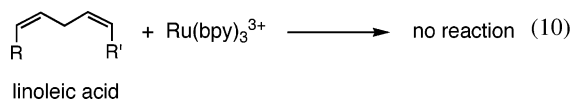
Possibility of PCET of Linoleic Acid. Since the deprotonated radicals are much stronger one-electron reductants than the parent electron donor molecules,[59] proton-coupled electron transfer (PCET) becomes thermodynamically feasible even if initial electron transfer is endergonic as shown in Scheme 1.[60] In such a case, the observed rate constant of overall electron transfer (k_{obs}) to form 2 equiv of $Ox^{\bullet-}$ is given by eq 9, under the conditions that the deprotonation rate constant (k_p) is much smaller than the back electron-transfer rate constant (k_{bet}) and

$$k_{obs} = 2k_pK_{et} \quad (9)$$

the equilibrium constant of electron transfer ($K_{et} = k_{et}/k_{bet}$) $\ll$ 1. In this case, no intermediate could be detected in the PCET process because of the small equilibrium constant ($K_{et} \ll 1$).

Such an outer-sphere PCET process is tested for the reaction of linoleic acid with a strong one-electron oxidant, $Ru(bpy)_3^{3+}$ ($E_{red} = 1.24$ V vs SCE) in MeCN at 298 K. $Ru(bpy)_3^{3+}$ acts as an outer-sphere oxidant because $Ru(bpy)_3^{3+}$ is a coordinatively saturated complex. No electron-transfer reaction occurred, since no increase in absorbance at 450 nm due to $Ru(bpy)_3^{2+}$ was

observed in the reaction of linoleic acid (1.0×10^{-2} M) and $Ru(bpy)_3^{3+}$ (1.0×10^{-4} M) after 3 h (eq 10). Now that the E_{ox}



value of linoleic acid (1.76 V vs SCE) and the deprotonation rate constant (8.1×10^3 s^{-1}) have been determined (vide supra), the k_{obs} value is estimated as 2.6×10^{-5} M^{-1} s^{-1} by using eq 11. In such a case, virtually no electron transfer from linoleic

$$\log k_{obs} = \log 2k_p - (F/2.3RT)(E_{ox} - E_{red}) \qquad (11)$$

acid to $Ru(bpy)_3^{3+}$ occurs, in agreement with the experimental observation (eq 10). It was also confirmed that no electron transfer occurred from linoleic acid to $Fe(bpy_3)^{3+}$ ($E_{red} = 1.06$ V vs SCE), which is a weaker one-electron oxidant than $Ru(bpy)_3^{3+}$.

Mechanistic Insight into Lipoxygenase. As described above, we have successfully determined the fundamental electron-transfer oxidation properties of unsaturated fatty acids (the one-electron oxidation potentials and the intrinsic barrier of electron transfer) for the first time. The deprotonation rate constant of the radical cation of linoleic acid has also been determined as 8.1×10^3 s^{-1}. No outer-sphere PCET from linoleic acid to strong one-electron oxidants ($Ru(bpy)_3^{3+}$ and $Fe(bpy)_3^{3+}$) would occur judging from the equilibrium constant of the electron transfer, which can be estimated from the redox potentials, and the deprotonation rate constant. This was confirmed experimentally by the spectroscopic method. Since the one-electron reduction potential of a ferric hydroxide cofactor (Fe(III)−OH) of soybean lipoxygenase (0.6 V)[10] is much lower than those of $Ru(bpy)_3^{3+}$ and $Fe(bpy)_3^{3+}$, an outer-sphere PCET pathway in the reaction of linoleic acid with Fe(III)−OH can be definitely ruled out.[26,61] As far as the difference in the relative reactivity between linoleic acid and oleic acid as substrates of soybean lipoxygenase-1 (SLO) is concerned,[62] however, the 10^5 slower rate of SLO-catalyzed oleic acid oxygenation than the oxygenation of linoleic acid is rather consistent with a large rate difference (3.7×10^4) of electron transfer between linoleic acid and oleic acid, 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).[63] On the other hand, the SLO-catalyzed oxygenation rate of arachidonic acid has been reported to be similar to that of linoleic acid.[14] This is also consistent with the same E_{ox} values between arachidonic acid and linoleic acid. Thus, a strong interaction between linoleic acid and Fe(III)−OH should be involved to make a PCET process to occur efficiently, when an inner-sphere electron transfer from linoleic acid to the Fe(III) state may be strongly coupled with the proton transfer to the OH group.[64] In such a case, a hydrogen atom is virtually transferred from linoleic acid to Fe(III)−OH, when an electron and a proton may be transferred at the same time but separately to the Fe(III) site and the OH site, respectively.

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Supporting Information Available: Figures showing a difference transient absorption spectrum (Figure S1), first-order plots (Figures S2 and S3), and a triplet−triplet absorption spectrum of ^{3}DCA* (Figure S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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(47) If the previously reported E_{ox} value of arachidonic acid (2.5 V vs SCE)[26] were correct, there would be no observation of the products of photoinduced electron transfer from linoleic acid and oleic acid to $^1AcrH^{+*}$ because the electron-transfer processes would be endergonic. This shows sharp contrast to the results in Figure 4 and Figure 5.

(48) The first-order decay indicates that the deprotonation of linoleic acid radical cation involves the solvent. However, the residual water may also be involved in the deprotonation step.

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(50) The first-order decay of oleic acid radical cation with the concomitant rise of $AcrH_2^{\bullet+}$ indicates that the proton transfer occurs in the cage in competition with the escape from the cage. The rather slow escape rate from the cage indicates that there is $\pi-\pi$ interaction between oleic radical cation and $AcrH^{\bullet}$.

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(53) If the previously reported E_{ox} value of arachidonic acid (2.5 V vs SCE)[26] were correct, it would be impossible to observe the products of photoinduced electron transfer from linoleic acid to $^1DCA^*$ in contrast to the results in Figure 6.

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(60) The deprotonation step in Scheme 1 shows only the stoichiometry. It should be noted that there is no free H^+ in solution; see ref 49.

(61) The difference in the one-electron reduction potentials between a ferric hydroxide cofactor (Fe(III)−OH) of soybean lipoxygenase (0.6 V)[10] and $Ru(bpy)_3^{3+}$ (1.24 V) indicates that the electron-transfer rate with a ferric hydroxide cofactor is 6.5×10^{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.

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(63) This value is obtained assuming that the difference in the free energy change in electron transfer is directly reflected in the rate difference, which corresponds to $\exp(F\Delta E_{ox}/RT)$; F is Faraday constant and T = 298 K.

(64) For the distinction between outer-sphere and inner-sphere electron-transfer processes, see: Fukuzumi, S.; Wong. C. L.; Kochi, J. K. *J. Am. Chem. Soc.* **1980**, *102*, 2928.