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Interaction of Hydroxycinnamic Acid Derivatives with the Cl_3COO Radical: A Pulse Radiolysis Study

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The electron transfer reactions between the trichloromethylperoxyl radical ($Cl_3COO^•$) and hydroxycinnamic acid derivatives, including chlorogenic acid, sinapic acid, caffeic acid, ferulic acid and 3,4-(methylenedioxy)cinnamic acid, have been studied by pulse radiolysis. The hydroxycinnamic acid derivatives, especially sinapic acid, are identified as good antioxidants for reduction of $Cl_3COO^•$ via electron transfer reactions. From buildup kinetic analysis of phenoxyl radical, the rate constant for reaction of $Cl_3COO^•$ with sinapic acid has been determined to be $8.2 \times 10^7\,dm^3\,mol^{-1}\,s^{-1}$, while the rate constants of electron transfer from other hydroxycinnamic acid derivatives to $Cl_3COO^•$ were obtained to be about $2 \times 10^7 dm^3 mol^{-1} s^{-1}$. The reaction of 3,4-(methylenedioxy) cinnamic acid with $Cl_3COO^•$ was investigated as an evidence for the electron transfer mechanism.

Keywords: Hydroxycinnamic acid derivatives, antioxidant, pulse radiolysis, electron transfer

INTRODUCTION

CCl_4 is well known as a selective toxin to liver, it can be metabolized into the free radical $CCl_3^•$ by cytochrome P450 through a reductive dehalogenation.[1–3] In the presence of oxygen, $CCl_3^•$ reacts rapidly with O_2 to yield $Cl_3COO^•$ ($k = 3.3 \times 10^9\,dm^3\,mol^{-1}s^{-1}$),[1,4] which reacts with various biochemical substances, including amino acids, DNA bases, lipids[5–8] and some organic reductants.[9,10] In most cases studied the radical was shown to bring about one-electron oxidation, and a few cases showing that $Cl_3COO^•$ radicals react by addition.[11,12] Temperature dependence and solvent effects in the reactions of the radical with organic reductant have been reported.[9,10] It is very convenient to use $Cl_3COO^•$ as a peroxyl radical model because it can be generated in

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water/alcohol solution, in which sufficient solubility of the substrate can be obtained.

The antioxidant activity of naturally occurring hydroxycinnamic acid derivatives have been observed in our laboratory,[13–15] it was shown that they can repair oxidizing OH adducts of DNA components effectively. The inhibition of lipid peroxidation by chlorogenic acid and caffeic acid was carried out by Joao *et al.*[16] using fluorescence probe method. To gain further insight into the antioxidant activity toward peroxyl radicals, we studied the reactions between $Cl_3COO^\bullet$ and hydroxycinnamic acid derivatives by pulse radiolysis.

EXPERIMENTAL

Chlorogenic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate), CHL, Sigma), sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid, SIN, Fluka), caffeic acid (3,4-dihydroxycinnamic acid, CAF, Aldrich), and ferulic acid (4-hydroxy-3-methoxycinnamic acid, FER, Aldrich) were used as received. 3,4-(methylenedioxy)cinnamic acid (MCI) was obtained from Shanghai Institute of Materia Medica with HPLC purity. Except where stated otherwise, the solvent used throughout this work is a 20/80 (v/v) mixture of 2-propanol and water containing 6 mM CCl_4 saturated with air. Henceforth we will simply refer to it as the solvent.

Pulse radiolysis experiments were carried out using the linear accelerator providing 8 MeV electron pulse with a duration of 8 ns. The dosimetry of the electron pulse was determined by a thiocyanate dosimeter containing 10 mM KCNS solution saturated with nitrous oxide, and by taking $\varepsilon_{(SCN)_2^-} = 7600\,dm^3\,mol\,s^{-1}$ at 480 nm. Detailed descriptions of the pulse radiolysis equipment have been given elsewhere.[17] In this experiment, the pulse dose was 15 Gy. All the experiments were performed at room temperature.

RESULTS AND DISCUSSION

1. Yield of Primary Radicals

$Cl_3COO^\bullet$ can be conveniently produced by pulse radiolysis of aqueous alcohol solutions containing carbon tetrachloride:

$$H_2O \rightsquigarrow OH + e_{aq}^- + H + \cdots \tag{1}$$

$$OH + (CH_3)_2CHOH \rightarrow (CH_3)_2COH + H_2O \tag{2}$$

$$H + (CH_3)_2CHOH \rightarrow (CH_3)_2COH + H_2 \tag{3}$$

$$(CH_3)_2COH + CCl_4 \rightarrow CCl_3^\bullet + (CH_3)_2CO + H^+ + Cl^- \tag{4}$$

$$e_{aq}^- + CCl_4 \rightarrow CCl_4^{-*[18]} \rightarrow CCl_3^\bullet + Cl^- \tag{5}$$

$$CCl_3^\bullet + O_2 \rightarrow Cl_3COO^\bullet \tag{6}$$

$k_2 = 1.9 \times 10^9\,dm^3\,mol^{-1}\,s^{-1}$, $k_3 = 7.4 \times 10^7\,dm^3\,mol^{-1}\,s^{-1}$,[19] $k_4 = 7.0 \times 10^8\,dm^3\,mol^{-1}\,s^{-1}$,[20] $k_5 = 3.0 \times 10^{10}\,dm^3\,mol^{-1}\,s^{-1}$, $k_6 = 3.3 \times 10^9\,dm^3\,mol^{-1}\,s^{-1}$.[4] Thus, the primary products generated by radiolysis of water were all converted into $Cl_3COO^\bullet$, all reactions could complete within 1 μs.

2. The Addition Reaction of $CCl_3^\bullet$ with Antioxidants

Figure 1 shows the transient absorption spectra at 2 μs and 50 μs after pulse radiolysis of N_2 saturated neutral solution containing 2.5 mM CHL respectively. At 50 μs after the pulse, a new absorption peak appears at 480 nm. It is very different from the absorption band of phenoxyl radical of CHL,[15] so the absorption peak at 480 nm should be assigned to the radical adduct formed by the addition of $CCl_3^\bullet$ to CHL. By varying the concentrations of CHL (0.5–2.5 mM), the rate constant of the addition reaction has been determined as $1.4 \times 10^7\,dm^3\,mol^{-1}\,s^{-1}$, the rate constants for SIN, CAF and FER have been determined as 1.5×10^7, 1.9×10^7 and $1.4 \times 10^7\,dm^3\,mol^{-1}\,s^{-1}$, respectively. In air-saturated solutions, the concentration of O_2 is $3 \times 10^{-4}\,\mathrm{mol\,dm^{-3}}$,[21] so when the concentration of antioxidant is < 2.5 mM, $> 95\%$ of $CCl_3^\bullet$ react with O_2 to yield $Cl_3COO^\bullet$ rather than form an adduct with the substrate.

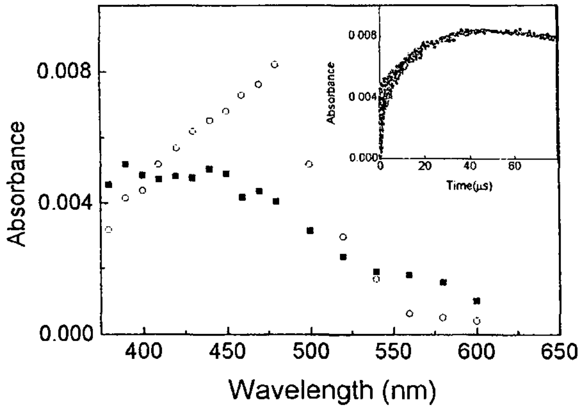

FIGURE 1 Transient absorption spectra from pulse radiolysis of 2.5 mM CHL neutral solution saturated with N_2: (■) 2 μs, (○) 50 μs. Inset: Growth trace of the transient absorption at 480 nm.

3. Electron Transfer Reaction of $Cl_3COO^\bullet$ with Hydroxycinnamic Acid Derivatives

Figure 2A shows the transient absorption spectrum after pulse radiolysis of the neutral solvent containing 2.5 mM CHL saturated with air. At 20 μs after the pulse, a new absorption band appears around 390 nm. Since the absorption peak of $Cl_3COO^\bullet$ is shorter than 320 nm,[22] the absorption band around 390 nm should be assigned to the phenoxyl radical of CHL[15] arising from electron transfer reaction between $Cl_3COO^\bullet$ and CHL. After capture of one electron, $Cl_3COO^\bullet$ should be reduced to Cl_3COO^-, which protonate to give Cl_3COOH.

$$\begin{aligned} \mathrm{Cl_3COO^\bullet + D} &\rightarrow \mathrm{Cl_3COO^- + D^{\bullet+}} \\ &\rightarrow \mathrm{Cl_3COOH + D(-H)^\bullet} \qquad (7) \end{aligned}$$

The absorption peak at 390, 380 and 380 nm in Figure 2B, C and D should be assigned to the phenoxyl radical of SIN, CAF and FER, respectively,[13] which were also formed via reaction (7).

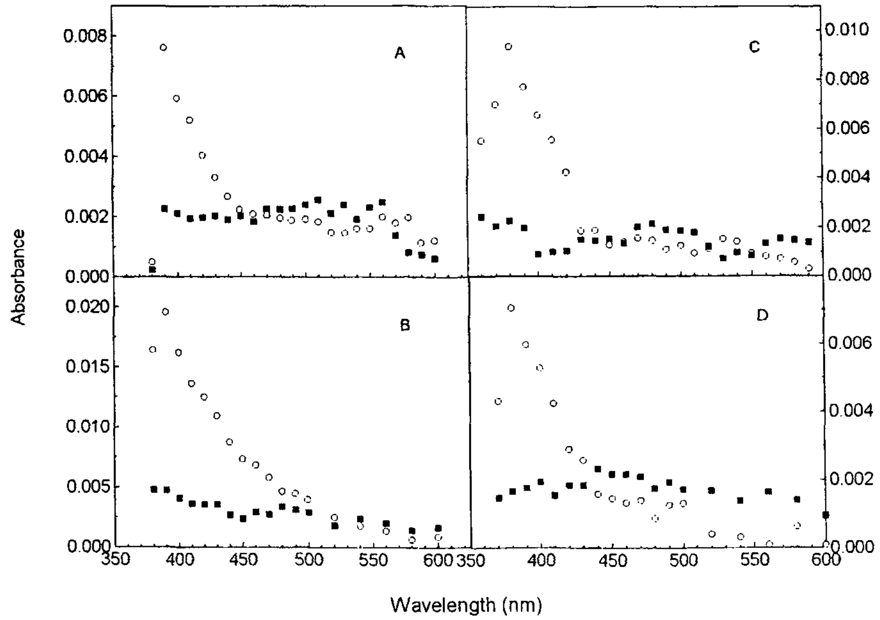

FIGURE 2 Transient absorption spectra from pulse radiolysis of the neutral solvent saturated with air. A: 2.5 mM CHL (■) 2 μs, (○) 25 μs; B: 2.5 mM SIN (■) 2 μs, (○) 12 μs. C: 2.5 mM CAF (■) 2 μs, (○) 45 μs; D: 2.5 mM FER (■) 2 μs, (○) 40 μs.

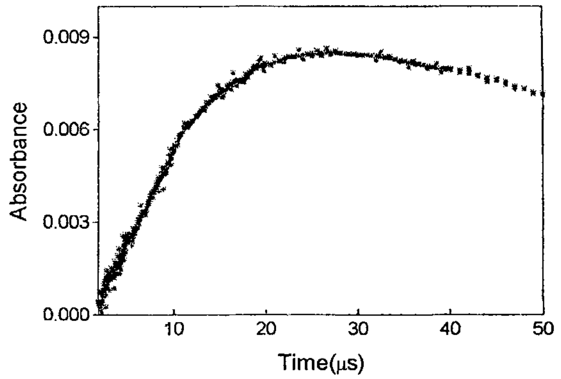

FIGURE 3 Growth trace of the transient absorption of the phenoxyl radical of CHL at 390 nm.

TABLE I The rate constants ($10^7\,dm^3\,mol^{-1}s^{-1}$) of electron transfer from hydroxycinnamic acid derivatives to $Cl_3COO^\bullet$

Substrate	CHL	SIN	CAY	FER
k	2.0	8.2	2.0	2.8

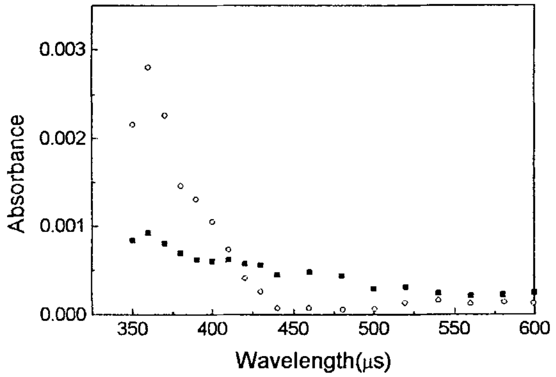

FIGURE 4 Transient absorption spectra after pulse radiolysis of 0.5 mM MCI neutral solution saturated with air. (■) 2 µs, (○) 45 µs.

Figure 3 shows the buildup trace of the phenoxyl radical of CHL at 390 nm. The kinetic of buildup follows pseudo-first-order rate law. By varying CHL concentration (0.5–2.5 mM), the rate constant of electron transfer from CHL to $Cl_3COO^\bullet$ has been determined as $2.0 \times 10^7\,dm^3\,mol^{-1}\,s^{-1}$, the rate constants of electron transfer to $Cl_3COO^\bullet$ from SIN, CAF and FER have also been determined (see Table I).

4. Evidence for Electron Transfer Mechanism

Because of the poor solubility of MCI in the solvent, the transient absorption spectra in Figure 4 were obtained from pulse radiolysis of 0.5 mM MCI neutral solution saturated with air. The spectra are very similar to those of other hydroxycinnamic acid derivatives, the absorption peak at 360 nm should be attributed to $MCI^{\bullet +}$ formed from MCI via one-electron transfer to $Cl_3COO^\bullet$. Having no phenolic hydroxyl in its molecular structure, MCI cannot give H-atom to $Cl_3COO^\bullet$ to yield phenoxyl radical, but form $MCI^{\bullet +}$ via electron transfer reaction with $Cl_3COO^\bullet$. The rate constant of electron transfer was determined to be $2.5 \times 10^7\,dm^3\,mol^{-1}\,s^{-1}$. From discussion above, we conclude that the reaction between $Cl_3COO^\bullet$ and hydroxycinnamic acid derivatives is electron transfer, not H-atom transfer reaction.

CONCLUSIONS

Our experimental results have demonstrated that the hydroxycinnamic acid derivatives, especially sinapic acid, are good antioxidants in reducing $Cl_3COO^\bullet$ via electron transfer reaction. It is consistent with the prediction of Lin that sinapic acid may be more potent antioxidant than other members of the hydroxycinnamic acid derivatives.[23]

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