Pulse radiolysis study on the mechanisms of reactions of CCl₃OO• radical with quercetin, rutin and epigallocatechin gallate

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Abstract The mechanisms of reactions between CCl₃OO• radical and quercetin, rutin and epigallocatechin gallate (EGCG) have been studied using pulse radiolytic technique. It is suggested that the electron transfer reaction is the main reaction between CCl₃OO• radical and rutin, EGCG, but there are two main pathways for the reaction of CCl₃OO• radical with quercetin, one is the electron transfer reaction, the other is addition reaction. The reaction rate constants were determined. It is proved that quercetin and rutin are better CCl₃OO• radical scavengers than EGCG.

Keywords: pulse radiolysis, antioxidant, radical, electron transfer.

CCl₄ is a well-known selective toxin to the liver. Its toxicity has been ascribed to the generation of radical species during its metabolism. It is generally believed that CCl₄ could be metabolized into the free radical CCl₃• by cytochrome P450 through a reductive dehalogenation. In the presence of oxygen, CCl₃• reacts rapidly with O₂ to produce CCl₃OO• radical (the reaction rate constant k = 3.3×10⁹ L·mol⁻¹·s⁻¹). CCl₃OO• radical is a stronger oxidizing radical, whose one-electron redox potential in neutral aqueous solution is between 1.1 and 1.3 V vs. SCE. As most of the oxygen radicals, CCl₃OO• radical can attack various biochemical substances, such as amino acids, DNA bases, and lipids. Therefore, a wide variety of studies have been carried out in order to find efficient scavengers of CCl₃OO• radical, especially the natural antioxidants. In addition, CCl₃OO• radical is usually used as a peroxyl radical model due to its convenient generating in homogeneous aqueous-organic solution and its stronger oxidizing property in the peroxyl radicals. The reaction of CCl₃OO• radical with antioxidants is more complex. The polarity of the solvent and the temperature can affect the reaction rate constants greatly. In most cases, the radical was shown to bring out one-electron oxidation, and in a few cases, it was shown to be addition reaction.

Quercetin, rutin and EGCG are all natural flavonoids. Their structures are shown in fig.1. They have been studied as scavengers of radicals extensively. The structures of quercetin and rutin are similar. Besides the active 2,3-double bond on the C ring like indole, they have a carbonyl
group conjugated with the double bond. The only difference between them is that rutin has a larger substituent at the 3-position than quercetin. The structure of EGCG is greatly different from them. There is no 2,3-double bond and carbonyl on its C ring. So it could be anticipated that the reactions of $\cdot\text{OOCCl}_3$ radical with quercetin and rutin might be the same as that with indole$^6$, both one-electron oxidation and addition are existent. But there would not exist an addition reaction between $\cdot\text{CCl}_3\text{OO}$ radical and EGCG. To gain further insight into the antioxidant activity of them, we carried out the experiments of quercetin, rutin and EGCG scavenging $\cdot\text{CCl}_3\text{OO}$ radical by pulse radiolysis technique.

![Fig. 1. The structures of quercetin, rutin and EGCG.](image)

1 Experimental

Quercetin and rutin were purchased from Shanghai Institute of Materia Medica. EGCG was obtained from Zhejiang Agriculture University, China. CCl$_4$ and 2-propanol were triply distilled. The solvent used throughout this work is a mixture of 2-propanol (25% in v/v) and triple-distilled water containing 1×10$^{-2}$ mol • L$^{-1}$ CCl$_4$ saturated with air. We simply referred to it as solvent in the work. The reactions were studied in the solvent at their natural pH (about 6—7) without the addition of buffers. All the solutions were prepared freshly prior to use. All experiments were carried out at room temperature.
The pulse radiolysis experiments were carried out using a linear accelerator providing 8 MeV electron pulse with a duration of 8 ns. The dosimetry of electron pulse was performed with a nitrous oxide saturated $1 \times 10^{-2}$ mol $\cdot$ L$^{-1}$ KSCN aqueous solution for which $\varepsilon_{(SCN)^-} = 7600$ L $\cdot$ mol$^{-1}$ $\cdot$ cm$^{-1}$ at 480 nm. The dose of a single pulse was around 15 Gy in this work. The detailed descriptions of the set-up of the radiolysis equipment and experimental conditions have been given elsewhere[7].

2 Results and discussion

2.1 Generation of $\text{CCl}_3\text{OO}^+$ radical

$\text{CCl}_3\text{OO}^+$ radical can be conveniently produced by pulse radiolysis of 2-propanol aqueous solution containing $\text{CCl}_4$ saturated with air. The main reactions are as follows:

\[
\begin{align*}
\text{H}_2\text{O} & \xrightarrow{\text{Ionizing Radiation}} \cdot\text{OH} + \cdot\text{H} + e_{\text{aq}}^- + \cdots \quad (1) \\
\cdot\text{OH} + (\text{CH}_3)_2\text{CHOH} & \rightarrow \text{H}_2\text{O} + (\text{CH}_3)_2\text{COH} \quad (2) \\
\cdot\text{H} + (\text{CH}_3)_2\text{CHOH} & \rightarrow \text{H}_2 + (\text{CH}_3)_2\text{COH} \quad (3) \\
(\text{CH}_3)_2\text{COH} + \text{CCl}_4 & \rightarrow \text{CCl}_3^- + (\text{CH}_3)_2\text{CO} + H^+ + \text{Cl}^- \quad (4) \\
e_{\text{aq}}^- + \text{CCl}_4 & \rightarrow \text{CCl}_4^- \rightarrow \text{CCl}_3^- + \text{Cl}^- \quad (5) \\
\text{CCl}_3^- + \text{O}_2 & \rightarrow \text{CCl}_3\text{OO}^+ \quad (6)
\end{align*}
\]

where $\cdot\text{OH}$, $\cdot\text{H}$ and $e_{\text{aq}}^-$ are the primary radicals of pulse radiolysis of water. The $G$ value of $\cdot\text{OH}$, $\cdot\text{H}$ and $e_{\text{aq}}^-$ are 2.9, 0.6 and 2.9 in this condition, respectively. Reactions (2) and (3) eliminated $\cdot\text{OH}$ and $\cdot\text{H}$ that may react with antioxidant directly. The reaction rate constants of (4) and (5) are $7.0 \times 10^8$ and $3.0 \times 10^{10}$ L $\cdot$ mol$^{-1}$ $\cdot$ s$^{-1}$[2,8], respectively. So at this condition, the $\text{CCl}_3\text{OO}^+$ radical was mainly formed through the reactions (1), (5) and (6).

2.2 Characteristic of transient absorption spectra of quercetin, rutin and EGCG scavenging $\text{CCl}_3\text{OO}^+$ radical

2.2.1 Transient absorption of quercetin scavenging $\text{CCl}_3\text{OO}^+$ radical. Fig. 2 shows the transient absorption spectra at different times after pulse radiolysis of air-saturated solution containing $8 \times 10^{-4}$ mol $\cdot$ L$^{-1}$ quercetin. The transient species with maximum absorption peaks at 480 and 540 nm were observed. The absorption peak at 480 nm is stronger and produced fast, the other one at 540 nm is broad and produced more slowly than that at 480 nm. The growth traces (fig. 3) at 480 and 540 nm also proved that the growth at 480 nm is much faster than that at 540 nm. Considering that the absorbance of $\text{CCl}_3\text{OO}^+$ radical is between 280 and 320 nm[9] and its produce is almost
completed within 1 µs, the transient species must be two different species excluding CCl₃OO⁻ radical. The absorption peak at 540 nm should be assigned to the phenoxy radical of quercetin[10]. It is more likely that the species with a maximum absorption peak at 480 nm is the CCl₃OO⁻ adduct of quercetin. And the addition position may be at the 2,3-double bond of quercetin as that of indole[6] (reaction (7a)). As most of such reactions, the quercetin may lose an electron firstly, then eliminate a proton rapidly to form the phenoxy radical (Q - O⁻) (reaction 7(b)). The mechanism could be illustrated as in the following

\[
\text{CCl}_3\text{OO}^- + \text{Q} - \text{OH} \rightarrow \text{Q} - \text{OH(CCl}_3\text{OO)} \tag{7a}
\]

\[
\text{CCl}_3\text{OO}^- + \text{Q} - \text{OH} \rightarrow \text{CCl}_3\text{OO}^- + \text{Q} - \text{OH}'' \rightarrow \text{CCl}_3\text{OOH} + \text{Q} - \text{O}^- \tag{7b}
\]

where Q-OH, Q'-OH(CCl₃OO), Q-OH'' and Q-O⁻ represent quercetin, adduct of quercetin, cation radical of quercetin and phenoxy radical of quercetin, respectively.

From kinetic analysis of the growth traces at 480 and 540 nm, the apparent rate constant of the reaction of CCl₃OO⁻ radical with quercetin was obtained. It was found that the kinetic of buildup follows pseudo first order rate law. By varying the concentration of quercetin from 3×10⁻⁴ to 8×10⁻⁴ mol • L⁻¹, a series of apparent rate constants were determined (fig. 3(b), (d)). According to the different apparent rate constants, the growth rate constant of quercetin phenoxy is calculated to be 2.43×10⁷ L • mol⁻¹ • s⁻¹, and that of CCl₃OO⁻ radical adduct of quercetin is 1.53×10⁸ L • mol⁻¹ • s⁻¹.

2.2.2 Transient absorption of rutin and EGCG scavenging CCl₃OO⁻ radical. As shown in fig. 4(a), (b), the transient absorption spectra were obtained from pulse radiolysis of 6×10⁻⁴ mol • L⁻¹ rutin or 2×10⁻³ mol • L⁻¹ EGCG solution, respectively. The only transient absorption peak with maximum at 460 nm for rutin system and at 350 nm for EGCG system was found in the determined wavelength. The growth trace was smooth (fig. 4(c), (d)). So it could be deduced that there was only one main transient species formed from the reaction of CCl₃OO⁻ radical with rutin and EGCG, respectively. The transient absorption peak at 460 and 350 nm should be assigned to the corresponding phenoxy of rutin and EGCG[11,12] formed through one-

![Fig. 2. Transient absorption spectra from pulse radiolysis of 8×10⁻⁴ mol • L⁻¹ quercetin solution recorded at ■, 1; ○, 5; ◆, 20 and ▼, 60 µs.](image)
Fig. 3. Growth trace observed at 480 nm (a) and 540 nm (c) after pulse radiolysis of $8 \times 10^{-4}$ mol L$^{-1}$ quercetin solution. (b) and (d) Dependence of $k_{\text{obs}}$ vs. concentration of quercetin at the corresponding wavelength of (a) and (c).

Fig. 4. Transient absorption spectra from pulse radiolysis of $6 \times 10^{-4}$ mol L$^{-1}$ rutin solution (a) and $2 \times 10^{-3}$ mol L$^{-1}$ EGCG solution (b) recorded at 1 $\mu$s (■) and 40 $\mu$s (○). (c) and (d) The growth trace at the corresponding wavelength of (a) and (b).
electron transfer reaction. The reaction can be described as follows:

$$\text{CCl}_3\text{OO}^+ + \text{R-OH} \rightarrow \text{CCl}_3\text{O}^\cdot + \text{R-OOH} + \text{R-O}^\cdot$$

(8)

where R-OH represents rutin or EGCG, and R-OOH$^+$ and R-O$^\cdot$ represent the corresponding cation radical and phenoxyl radical.

In the same way as that dealing with quercetin, the reaction rate constant of rutin with CCl$_3$OO$^+$ radical was determined to be $1.0 \times 10^8$ L $\cdot$ mol$^{-1}$ $\cdot$ s$^{-1}$ by varying the concentration of rutin from $5 \times 10^{-4}$ to $1 \times 10^{-3}$ mol $\cdot$ L$^{-1}$. And that of EGCG was determined to be $5.0 \times 10^7$ L $\cdot$ mol$^{-1}$ $\cdot$ s$^{-1}$ by varying the concentration of EGCG from $1 \times 10^{-3}$ to $5 \times 10^{-3}$ mol $\cdot$ L$^{-1}$.

As expected, the reaction between CCl$_3$OO$^+$ radical and EGCG is one-electron transfer. However, no addition transient absorption was found for the reaction of CCl$_3$OO$^+$ radical with rutin. The reason may be that the larger substituents at the 3-position prevent the occurrence of such a reaction.

3 Conclusion

The experimental results demonstrate that both one-electron transfer and addition exist in the reaction of CCl$_3$OO$^+$ radical with quercetin, but only the one-electron oxidation is the main mechanism for the reaction of CCl$_3$OO$^+$ radical with rutin and EGCG. So CCl$_3$OO$^+$ radical is not just a one-electron oxidant, and the mechanism depends on the environment of the reaction. In addition, the experimental results prove quercetin and rutin to be better CCl$_3$OO$^+$ radical scavengers than EGCG. It also demonstrates again that the antioxidant activity could be strengthened with the double bond on the C ring conjugated with B ring for the flavonoids$^{[13]}$.

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References