



**Mechanism and kinetics in reactions of caffeic acid with radicals  
by pulse radiolysis and calculation**

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**[Abstract]** The interaction of caffeic acid with  $e_{aq}^-$ ,  $(CH_3)_2(OH)CCH_2^{\cdot}$ ,  $CO_2^{\cdot-}$ ,  $H^{\cdot}$ ,  $\cdot OH$  and  $N_3^{\cdot}$  radicals were studied by  $\gamma$ -, pulse radiolysis and molecular orbital calculation. UV-visible spectra of electron/ $\cdot OH$  adducts, semi-quinone radicals of caffeic ions, and the stable products from the reactions were derived. The rate constants were determined. The attacked sites and the most favorable structures of the transient radicals were predicted. Reaction mechanisms were proposed.

**[Key words]:** Caffeic acid, Free radical, pulse radiolysis, calculation.

### Introduction

Caffeic acid(3,4-dihydroxy cinnamic acid) is one of the major phenolic components of dietary phytochemicals, widely exists in fruits, olive oil, wine, tea and coffee etc.<sup>[1]</sup> Many studies have demonstrated the antioxidant properties of caffeic acid, such as scavenging superoxide, peroxy and hydroxyl radicals<sup>[2-4]</sup>, etc. On the other hand, some research has shown its prooxidant activities, such as enhance of dopa oxidation<sup>[5]</sup>, auto-oxidization to form superoxide and direct reduction of Fe(III) to Fe(II)<sup>[6]</sup>. The redox properties also indicate the potentiality of caffeic acid as antioxidant, prooxidant and oxidant.<sup>[7]</sup> However, few studies have probed into its oxidant properties as scavenger of reducing radicals. Our previous studies on interaction of hydrated electron with dietary flavonoids and phenolic acids demonstrated their ability as electron scavengers.<sup>[8]</sup> This work aims to further understand the dual properties of caffeic acid toward both oxidizing and reducing species.

### Materials and methods

Caffeic acid (97%, predominantly trans-isomer) was obtained from Aldrich and all the other chemicals were guaranteed reagents. Solutions were prepared in 1mM phosphate buffer (pH 6.9) with appropriate additive such as t-BuOH or  $NaN_3$ , and bubbled with Ar or  $N_2O$  as needed. For pulse radiolysis experiment, a 28MeV-electron beam with pulse

duration of 10ns from a linear accelerator was utilized. The absorbed dose per pulse was 75-86Gy. The steady-state irradiation was carried out with a 0.22 PBq  $^{60}\text{Co}$   $\gamma$ -ray source at dose rate 0.15Gy/s.

The analyses of formate and oxalate were performed on a Hitachi IC System. Kinetic simulation was carried out with Fascimile V4.0. The electronic structure calculation was performed with PC Spartan Pro 1.0.

## Results

Fig. 1 showed the decay of  $e_{\text{aq}}^-$  at 720nm, which followed pseudo-first-order kinetics. The second order rate constant for the reaction of caffeic acid with  $e_{\text{aq}}^-$  was derived to be  $(8.3 \pm 0.5) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ , which was in good agreement with the reference data ( $1.4 \times 10^{10} \text{ dm}^3\text{mol}^{-1}\text{s}^{-1}$ ) at pH 7<sup>[9]</sup>. The electron adduct of caffeic acid was unstable and disappeared within 150 $\mu\text{s}$  with a second order decay rate constant estimated to be  $(1.1 \pm 0.2) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ .

From the build up of transients at 400nm, the rate constants of caffeic acid reacting with  $\cdot\text{OH}$  and  $\text{N}_3\cdot$  were determined as  $(5.5 \pm 0.8) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$  and  $(6.2 \pm 0.4) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ , respectively. By kinetic simulation, the rate constant of caffeic acid reacting with  $\text{CO}_2\cdot^-$  was estimated to be no less than  $1 \times 10^5 \text{ dm}^3\text{mol}^{-1}\text{s}^{-1}$ .

The absorption coefficients of the  $\cdot\text{OH}$  and  $\text{N}_3\cdot$  resulted stable products at 390nm were derived to 700 and 600  $\text{M}^{-1}\text{cm}^{-1}$ , respectively. The decay rate constant  $k$  were estimated to be  $(3.0 \pm 0.2) \times 10^7 \text{ M}^{-1}\text{s}^{-1}$  for caffeic ion -  $\cdot\text{OH}$  adduct radicals and  $(1.0 \pm 0.1) \times 10^7 \text{ M}^{-1}\text{s}^{-1}$  for  $\text{N}_3\cdot$  resulted semi-quinoid radicals.

A series of typical spectra for  $\gamma$ -ray irradiated  $\text{N}_2\text{O}$ -saturated 1.01mM caffeic acid + 10.1mM  $\text{HCOONa}$  (III) at pH 6.9 were shown in Fig. 2. As the absorbed dose increased, the

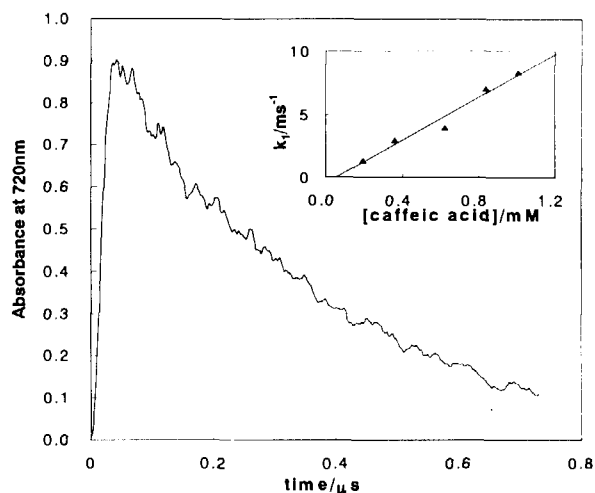


Fig. 1  $e_{\text{aq}}^-$  decay in 0.4mM caffeic acid + 0.1M t-BuOH, pH 6.9. Inserted: Dependence of pseudo first order decay rate constants on caffeic acid

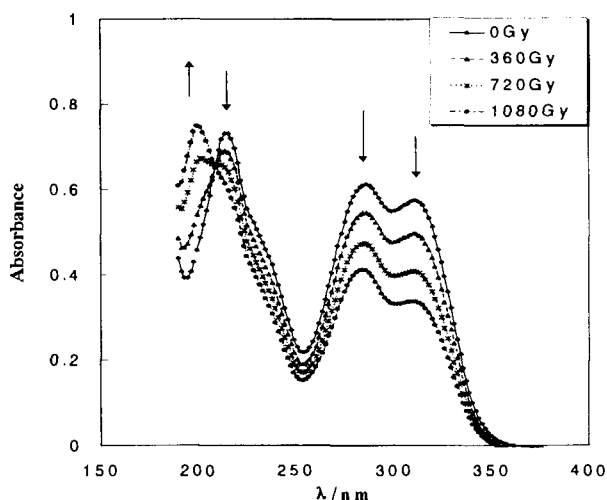


Fig. 2 UV spectra of  $\gamma$ -irradiated  $\text{N}_2\text{O}$ -saturated 1mM caffeic acid + 10mM  $\text{HCOONa}$  at pH 6.9

absorbance at 215, 286 and 310nm linearly decreased. From analysis of the changes in absorbance, it can be concluded that recombination was the main reaction route for the transient species of caffeic acid reacting with both reducing and oxidizing radicals.

Fig. 3 showed the electrostatic atomic charges of caffeic ions and the predicted radical attack sites. It was generally believed that  $\cdot\text{OH}$ , less selective oxidant than  $\text{N}_3\cdot$ , reacts with phenolic compounds via either addition or hydrogen-abstraction, while  $\text{N}_3\cdot$  selectively oxidized phenolic compounds via hydrogen-abstraction.

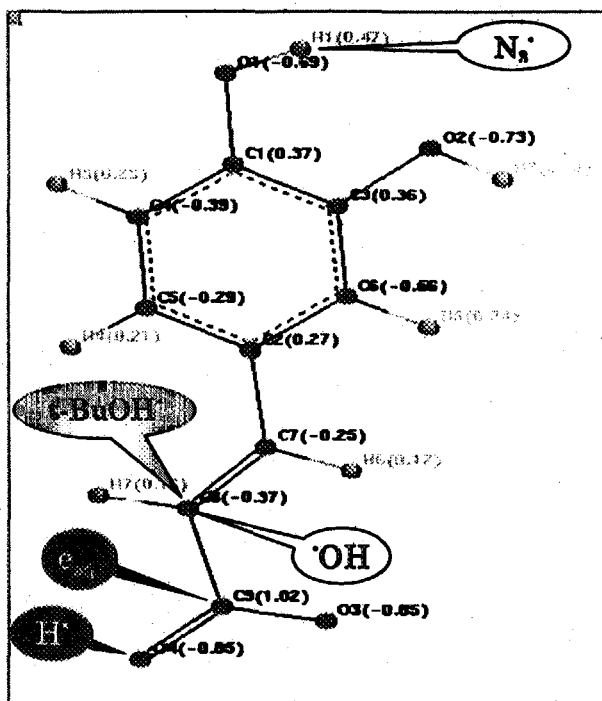


Fig.3 Atomic charges and radical attack sites on caffeic ions.

The lowest heat of formation for semiquinoid radicals ( $-150.9\text{kcal mol}^{-1}$ ) was lower than that of caffeic- $\text{N}_3$  adducts, but higher than that of caffeic-OH adducts, suggesting that for the reaction of caffeic ion with  $\cdot\text{OH}$  addition was more favorable than H-abstraction, but with  $\text{N}_3\cdot$ , the tendency was reversed. Comparison of the heat of formation for various radical conformers suggests that  $\text{H}\cdot$  tended to attack caffeic ion on O4,  $(\text{CH}_3)_2(\text{OH})\text{CCH}_2\cdot$  and  $\cdot\text{OH}$  on C8,  $e_{\text{aq}}^-$  on C9, and  $\text{N}_3\cdot$  tended to abstract H1, forming a semiquinoid radical. The lower value of heat of formation for radical of H adduct ( $-179.9\text{kcal mol}^{-1}$ ) than that of electron adduct ( $-120.9\text{kcal mol}^{-1}$ ) suggested that the electron adduct tended to undergo hydration to form radical of H adduct.

## Conclusion

Caffeic acid has been well-known as anti-oxidant, being able to scavenge various oxidizing radicals, however, little attention has been paid to the interaction of caffeic acid with reducing radicals. This work demonstrated that caffeic acid scavenged not only oxidizing radicals like  $\text{N}_3\cdot$  and  $\cdot\text{OH}$ , but also reducing radicals such as  $e_{\text{aq}}^-$ ,  $\text{CO}_2^{\cdot-}$ ,  $(\text{CH}_3)_2(\text{OH})\text{CCH}_2\cdot$  and  $\text{H}\cdot$ . Moreover, the reactivity toward caffeic acid was  $e_{\text{aq}}^- > \text{N}_3\cdot > \cdot\text{OH} > \text{CO}_2^{\cdot-}$ . However, The stability order of transient radicals was caffeic acid- $e_{\text{aq}}^-$  adducts  $<$  caffeic-OH adducts  $<$   $\text{N}_3\cdot$ -resulted o-semiquinone radicals.

The reducing radical scavenging ability of caffeic acid and other flavonoids has been neglected. The findings of this work further supported our previous proposal<sup>[8]</sup> that attentions should be also paid to the biological functions of their reducing radical

scavenging ability.

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