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Reactions of reducing and oxidizing radicals with caffeic acid: a pulse radiolysis and theoretical study

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Abstract

Molecular calculations coupled with pulse radiolysis studies are performed to understand the reactions of radicals with caffeic acid. From molecular calculation, we find that e_{aq}^- and $\cdot OH$ tend to form adducts with caffeic acid, while $N_3\cdot$ tends to abstract H from 4-hydroxyl group in benzene ring, generating a semi-quinoid radical. Based on comparison of the heat of formation, the most favorable radical attack sites and the most stable radical structures are predicted. The calculation results suggest that the stability of the electron adducts < semi-quinoid radicals < $\cdot OH$ adducts of caffeic ions, in good agreement with their experimental second-order decay rate constants ($2k = (1.1 \pm 0.2) \times 10^9$, $(6.0 \pm 0.4) \times 10^7$ and $(2.0 \pm 0.2) \times 10^7 M^{-1} s^{-1}$, respectively), determined by pulse radiolysis. Molecular calculations seem to be a powerful tool to predict the stability and structures of transient radicals. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Caffeic acid; Free radical; Pulse radiolysis; Molecular orbital calculation

1. Introduction

Radical reactions, especially with participation of oxidative radical, have been known to be involved in many biological processes that cause damage to lipids, proteins, membranes and DNA or result in carcinogenesis (Pryor, 1987). Compounds such as phenolic acid and flavonoids can scavenge oxidative radicals, thus reduce the possible damages. Such antioxidant properties have caused a great interest on their research. Caffeic acid (3,4-dihydroxy cinnamic acid) is one of the phenolic acids that widely exists in fruits, wine, tea, coffee and olive oil, etc. (Ho, 1992). It has been demonstrated to be able to scavenge superoxide, peroxy and hydroxyl radicals (Kono et al., 1997; Wang et al., 1993; Facino et al., 1995), lipid alkoxyl radical (Milic et al., 1998) and nitronium ions (Pannala

et al., 1998; Kerry and Rice-Evans, 1998). Some researches also demonstrated its prooxidant activities, such as enhancement of dopa oxidation (Takahama, 1997), auto-oxidization to form superoxide and direct reduction of Fe(III) to Fe(II) (Odnick et al., 1988). All the antioxidant or prooxidant activities involve radical reactions. A major concern in medicinal application of antioxidants is the possible side effects caused by products resulted from radical reactions. Thus, it is important to know the details of radical reaction as well as possible structures of final products.

Recently, Lien et al. (1999) has reported a quantitative analysis of phenolic antioxidants using calculation methods. They used AM1 semi-empirical method to obtain heat of formation (H_f) and energy (E_{homo} , E_{lumo}) of antioxidant radicals, and found some correlation between antioxidant activities and calculated parameters. They derived a model to calculate the redox potentials of phenolic compounds based on the parameters such as H_f , E_{lumo} and number of the OH group.

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Lots of the free radical reactions with caffeic acid have been reported (Wang et al., 1993; Zuo et al., 1995; Hapiot et al., 1996; Kono et al., 1997), but no attempt with molecular calculation has been done on the prediction of the stability and structure of most favorable transient radicals. This work employs caffeic acid as a model compound, and systematically investigates its reaction with several common radicals with molecular orbital calculation coupled with pulse radiolysis. Molecular orbital calculation is carried out and shows a powerful tool to understand the radical attack mode and position on its backbone, and predict the stability and possible structures of most favorable transient radicals.

2. Materials and methods

2.1. Molecular orbital calculations

The molecular orbital calculations were performed with Gaussian 98 (Gaussian Inc., USA) on an alpha workstation (OS/Digital Unix) or PC Spartan Pro 1.0 (Wavefunction Inc., USA).

There are two types of basic radical reactions: addition and hydrogen abstraction. On the other hand, there are multiple positions on caffeic acid backbone that can be subjected to radical attack. Thus a series of different transient structures may be generated. In this work, the heat of formation of each possible transient radical was calculated from equilibrium geometry with semi-empirical PM3 model. The electrostatic charge of caffeic ions were obtained from the calculation on equilibrium geometry at B3LYP/6-31+G level.

2.2. Pulse radiolysis

Caffeic acid (97%) was from Aldrich (Milwaukee, WI) and all the other chemicals were guaranteed reagents from Wako (Osaka, Japan). All solutions were prepared with 1 mM phosphate buffer (pH 6.9) in Milli-Q water.

Pulse radiolysis experiments were carried out with a 28 MeV-electron beam with pulse duration of 10 ns from a linear accelerator at the Nuclear Engineering Research Laboratory, University of Tokyo. Details of the pulse radiolysis system have been given elsewhere (Kobayashi and Tabata, 1989). The absorbed dose was measured with N₂O-saturated 10 mM KSCN aqueous solutions, taking $G_{\text{e}} = 5.3 \times 10^{-4} \text{ m}^2 \text{ J}^{-1}$ for SCN₂^{•−} at 472 nm (Buxton and Stuart, 1995). The absorbed dose per pulse was 75–86 Gy. High dose was chosen to increase absorption signal at low wavelength.

A flow cell system with the cell length 2.0 cm was used for measurement of the transient spectra. The solutions

were bubbled with Ar or N₂O for 20 min before and during the measurement. The transient spectrum was recorded after electron pulse at wavelength from 350 to 700 nm. The absorption coefficients were calculated from the maximum absorbance around 5 μs after electron pulse, and taking $G_{\text{e}^{\text{aq-}}}$ 0.29 μmol J^{−1}, G_{H} 0.053 μmol J^{−1} and G_{OH} 0.29 μmol J^{−1} for O₂-free solutions (Bjergbakke et al., 1989) and $G_{\text{e}^{\text{aq-}}}$ 0.33 μmol J^{−1}, G_{H} 0.053 μmol J^{−1} and G_{OH} 0.30 μmol J^{−1} for N₂O-saturated solutions (Draganic and Draganic, 1973), respectively.

O₂-free aqueous solutions of 0.1–1 mM caffeic acid + 0.2 M t-BuOH were used to observe the reaction between caffeic acid and e_{aq}[−], in which all ·OH radicals were scavenged by t-BuOH (Motohashi and Saito, 1993), thus e_{aq}[−] was the only active species at the time scale of pulse radiolysis with $G_{\text{e}^{\text{aq-}}}$ 0.29 μmol J^{−1}. N₂O-saturated aqueous solutions of 0.1–1 mM caffeic acid were used to observe the reaction between caffeic acid and ·OH, in which all e_{aq}[−] was converted into ·OH by N₂O (Elliot, 1989), thus ·OH was the only active species at that time scale with $G(\cdot\text{OH})$ 0.63 μmol J^{−1}. N₂O-saturated aqueous solutions of 0.1–1 mM caffeic acid + 10 mM NaN₃ were used to observe the reaction between caffeic acid and N₃[•], in which all ·OH was converted into N₃[•] (Motohashi and Saito, 1993), thus N₃[•] was the only active species at the time scale, with $G(\text{N}_3^{\bullet})$ 0.63 μmol J^{−1}.

For measurement of rate constants, samples were all sealed after being bubbled with Ar or N₂O for 20 min. The decay of e_{aq}[−] at 720 nm (Jou and Freeman, 1977) or the buildup of the transients of caffeic acid with ·OH and N₃[•] at 400 nm were followed in order to determine the rate constants of caffeic acid reacting with e_{aq}[−], ·OH and N₃[•]. To determine the decay rate constants of the transients, the absorbance at 365 nm for caffeic acid – e_{aq}[−] adducts, at 390 nm for caffeic acid – ·OH adducts and – N₃[•] resulted semi-quinoid radicals were followed.

3. Results

3.1. Molecular orbital calculation

The calculation started from caffeic ion, since caffeic acid exists in dissociated form in neutral aqueous solution, with a pKa less than 4. However, solvent effects are neglected during calculation.

The atomic numbering and electrostatic charges of caffeic ions were shown in Fig. 1. On the carbon backbone of caffeic ions, atomic charges fell in the order of C₉(1.02) > C₁(0.37) > C₃(0.36) > C₂(0.27) > C₇(−0.25) > C₅(−0.29) > C₈(−0.37) > C₄(−0.39) > C₆(−0.56), showing the decreasing accessibility of nucleophilic attack of e_{aq}[−].

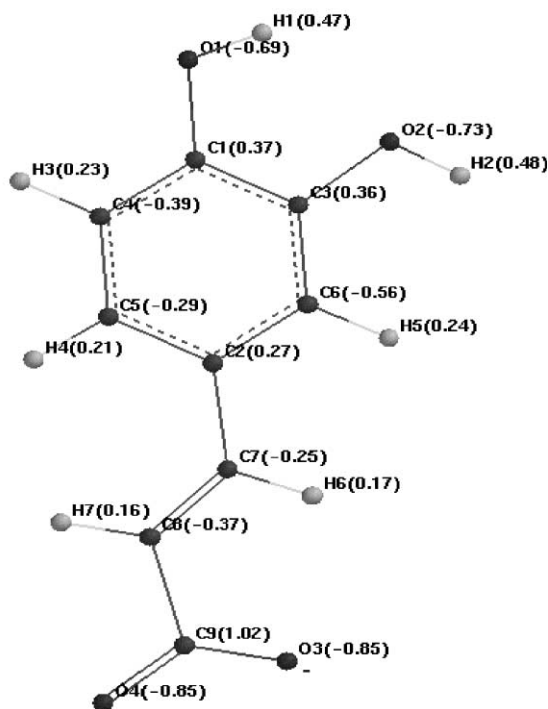


Fig. 1. Electrostatic atomic charges of caffeic ions calculated on the equilibrium geometry with ab initio B3LYP/6-31+G model.

In order to derive the most favorable structure of initial transient radicals for the reactions of e_{aq}^- , $\cdot OH$ and N_3^\bullet with caffeic ions, the heat of formation of all the possible transient radicals was derived from the calculation on their equilibrium geometry with semi-empirical PM3 model and compared in Table 1. The possible initial transient radicals included radicals 1–9, formed when $\cdot OH$ or N_3^\bullet attacked the C1–9 of caffeic ions, caffeic–electron adducts 1–3, resulted from the attack of e_{aq}^- on benzene ring, C7=C8 double bond or keto group, and semi-quinoid radicals 1–3, the radicals

located at O1, O2 or O3. The lowest heat of formation for semi-quinoid radicals ($-150.9 \text{ kcal mol}^{-1}$) was lower than that of caffeic- N_3 adducts, but higher than that of caffeic-OH adducts, suggesting that for the reaction of caffeic ion with $\cdot OH$ addition was more favorable than the H-abstraction, but with N_3^\bullet , the tendency was reversed. Comparison of the heat of formation for various radical conformers suggested that mostly $\cdot OH$ tended to attack caffeic ion on C8 to form radical 8, e_{aq}^- on C9 to form electron adduct 3, and N_3^\bullet tended to abstract H1 to form semi-quinoid radical 1. The equilibrium geometry of the most favorable initial transient radicals of caffeic ions derived by the calculation with semi-empirical PM3 model was shown in Fig. 2. Moreover, the stability of the most favorable initial transients of caffeic ions attacked by e_{aq}^- , N_3^\bullet and $\cdot OH$ could be predicted as electron adduct 3 ($-120.9 \text{ kcal mol}^{-1}$) < semi-quinoid radical 1 ($-150.9 \text{ kcal mol}^{-1}$) < caffeic-OH adduct radical 8 ($-218.8 \text{ kcal mol}^{-1}$).

3.2. Pulse radiolysis

3.2.1. Kinetics of caffeic acid reacting with e_{aq}^- , $\cdot OH$ and N_3^\bullet

The decaying absorbance after electron pulse of e_{aq}^- at 720 nm and the buildup of $\cdot OH$ or N_3^\bullet resulted transients at 400 nm were followed with the concentration of caffeic acid varying from 0.1 to 1 mM. The solution compositions were described in experimental section. They followed pseudo-first-order kinetics. From the slope of plotting, the pseudo-first-order rate constants versus concentrations of caffeic acid, the second order rate constants were derived to be $(8.3 \pm 0.5) \times 10^9$, $(5.5 \pm 0.8) \times 10^9$ and $(6.2 \pm 0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, for the reaction with e_{aq}^- , $\cdot OH$ and N_3^\bullet , respectively. The rate constant for e_{aq}^- determined here was in good agreement with the reference data ($1.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$) at pH 7 (Zuo et al., 1995), and that for $\cdot OH$ was in accordance with literature data: $7.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7 (Wang et al., 1993). Up to

Table 1

The heat of formation of initial transient radicals, calculated by equilibrium geometry with semi-empirical PM3 model (unit: kcal mol^{-1})

Radicals ^a	1	2	3	4	5	6	7	8	9
Caffeic-OH	-206.0	-188.5	-204.1	-199.2	-202.1	-202.7	-38.3	-218.8	-147.2
Caffeic- N_3	-81.1	-87.1	-79.8	-76.6	-79.7	-78.8	-59.2	-103.3	29.0
Caffeic- e^-	-108.2	-52.6	-120.9						
Semi-quinoid	-150.9	-148.2	-70.0						

^a Radicals 1–9 represent the radical formed when $\cdot OH$ or N_3^\bullet attacked the C1–9 of caffeic ions. Caffeic–electron adduct 1–3 are resulted from the attack of e_{aq}^- on benzene ring, C7=C8 double bond and keto group, respectively. Semi-quinoid radicals 1–3 represent the radicals located at O1, O2 and O3, respectively. The italic number showed the lowest value of the heat of formation for a set of radical conformers.

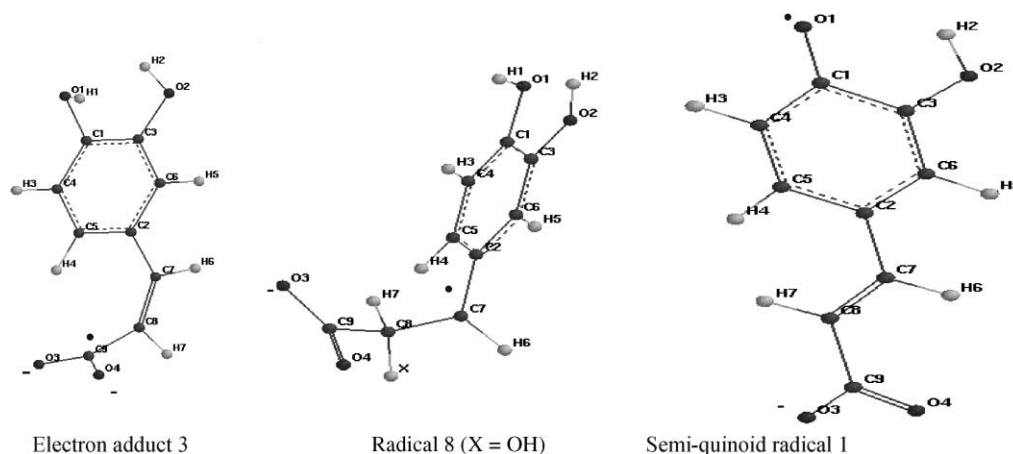


Fig. 2. Equilibrium geometries of the most favorable initial transient radicals for the reaction of caffeic ions with e_{aq}^- , $\cdot OH$ and N_3^\cdot , derived by the calculation with semi-empirical PM3 model.

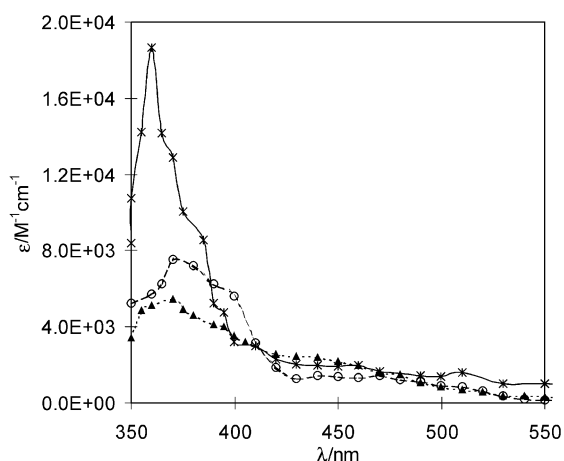


Fig. 3. Transient spectra of caffeic acid reacting with e_{aq}^- , $\cdot OH$ and N_3^\cdot at pH 6.9, obtained by pulse radiolysis of solutions: O_2 -free 0.1 mM caffeic acid + 0.2 M t-BuOH (star); N_2O -saturated 0.1 mM caffeic acid (triangle); N_2O -saturated 0.1 mM caffeic acid + 10 mM NaN_3 (circle).

our knowledge, the rate constant of caffeic acid reacting with N_3^\cdot had not been reported.

3.2.2. Spectra and stability for the transients of caffeic acid reacting with e_{aq}^- , $\cdot OH$ and N_3^\cdot

The spectra of transients resulting from caffeic acid reacting with e_{aq}^- , $\cdot OH$ and N_3^\cdot were shown in Fig. 3. All the three transients had an absorption maximum around 370 nm with a shoulder at 450 nm, but their molar absorption coefficients were different, implying that the transients still all keep the backbone structure of caffeic acid. N_3^\cdot was reported to react with phenolic compounds

forming semi-quinoid radicals selectively, while $\cdot OH$ was less selective, led to either OH adducts or semi-quinoid radicals. Our calculation results suggested that N_3^\cdot reacted with caffeic ions mainly to form semi-quinoid radicals, while $\cdot OH$ favorably resulted in OH adducts.

Both $\cdot OH$ and N_3^\cdot resulted transients had lifetime around 4 ms, and led to stable products which had weak absorption at 400 nm. Assuming the transients followed second-order decay kinetics to form one stable product, the absorption coefficients of the $\cdot OH$ and N_3^\cdot resulted stable products at 390 nm were derived to be 600 and 700 $M^{-1}cm^{-1}$, respectively, based on the initial and final steady absorbance of the decay curves. And the decay rate constant $2k$ was estimated to be $(2.0 \pm 0.2) \times 10^7 M^{-1}s^{-1}$ for $\cdot OH$ resulted transients (mainly caffeic ion $\cdot OH$ adduct radicals) and $(6.0 \pm 0.4) \times 10^7 M^{-1}s^{-1}$ for N_3^\cdot resulted transients (mainly semi-quinoid radicals), respectively, by parameter fitting for the time profiles of absorbance at 390 nm with a program, Fascimile V4.0 (AEA Technology). Hapiot et al. (1996) reported the semiquinone formed by the reaction of caffeic acid with N_3^\cdot decayed via second-order kinetics with $2k = 8.4 \times 10^7 M^{-1}s^{-1}$ at pH 5.7. This value is in good agreement with the data derived in this work.

The electron adduct of caffeic acid was unstable and disappeared within 150 μs , at a second-order rate constant estimated to be $2k = (1.1 \pm 0.2) \times 10^9 M^{-1}s^{-1}$ from the slope of $1/\text{absorbance}$ versus time.

4. Discussion

From pulse radiolysis, we can obtain kinetic data and transient spectra of the reactions of caffeic acid with

radicals such as e_{aq}^- , $\cdot OH$ and $N_3\cdot$. This work demonstrates that caffeic acid scavenges not only oxidizing radicals like $N_3\cdot$ and $\cdot OH$, but also reducing radicals such as e_{aq}^- . Moreover, the reactivity toward caffeic acid is $e_{aq}^- ((8.3 \pm 0.5) \times 10^9 M^{-1} s^{-1}) > N_3\cdot ((6.2 \pm 0.4) \times 10^9 M^{-1} s^{-1}) > \cdot OH ((5.5 \pm 0.8) \times 10^9 M^{-1} s^{-1})$. The stability of initial transient radicals is caffeic acid $-e_{aq}^-$ adducts $< N_3\cdot$ -resulted radicals $< \cdot OH$ -resulted radicals, with rate constants $2k$ of recombination estimated as $(1.1 \pm 0.2) \times 10^9$, $(6.0 \pm 0.4) \times 10^7$ and $(2.0 \pm 0.2) \times 10^7 M^{-1} s^{-1}$, respectively. This order well agrees with our prediction based on the heat of formation of the most favorable initial transient radicals: electron adduct 3 ($-120.9 kcal mol^{-1}$) $<$ semi-quinoid radical 1 ($-150.9 kcal mol^{-1}$) $<$ caffeic- $\cdot OH$ adduct radical 8 ($-218.8 kcal mol^{-1}$).

Simply from pulse radiolysis, it is difficult to know exactly on which position the radicals tend to attack caffeic acid, and whether the radical reaction is hydrogen abstraction or addition. One way to derive the radical reaction mechanism is by product analysis, however, it is quite time consuming. Based on the heat of formation for all the possible initial transients, we successfully predict the structure of the most favorable transient. The application of theoretical calculation method on the study of radical reactions is demonstrated to be a powerful tool to further understand the reaction mechanism.

The calculation helps to predict the structure of the most favorable transient for a radical reaction, however, a mixture of transients are possible to be formed and the calculation done here is not enough to estimate how many kinds of transients are formed and how many percentage that the most favorable transient accounts for.

At this trial study, only the heat of formation of possible initial transient radicals is calculated. Thus, the prediction is limited to the structure of the most favorable initial transients. The foretelling for the further reactions of the initial transients need additional experiments and calculation.

In this work, the prediction of radical attack site is based solely on comparison of the heat of transient formation. These predictions show the thermodynamic tendency of the reactions, and imply the predominant final products. However, the real reactions are affected not only by thermodynamic tendency, but also many other factors such as temperature, solvent and pH. And when the overall effects of other factors surpass the thermodynamic tendency, the reactions may proceed in other way. Several models such as Onsager model (Wong et al., 1992), polarized continuum model (PCM) (Cossi et al., 1998; Barone and Cossi, 1998), IPCM and SCI-PCM model (Foresman et al., 1996) are available to deal with the environmental factors. Usually, such model can produce satisfying results with

small molecule, but with the increase of molecule size, the accuracy decreases, while the time cost increases rapidly. Theoretically, CPU time required scale as the fourth power of the number of heavy atoms.

The choice of level of theoretical calculation and an appropriate base set size is important. The key points to consider are accuracy of calculation and time cost. The ab initio methods such as Hartree-Fock (HF), MP2, B3LYP usually are time consuming, especially the latter two methods. In comparison, semi-empirical methods such as AM1, PM3 can produce satisfactory accuracy but cost very little time. Thus, PM3 was used in this trial work in prediction of heat of formation.

Activation energy is a good index for the reactivity of the radical reaction. If a transition structure can be located, from the difference of energy between the transition structure and reactants, the activation energy can be derived. This work is under progress in our lab.

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