



AP-08

Interaction of hydrated electron with dietary flavonoids and phenolic acids

— Rate constants and transient spectra studied by pulse radiolysis

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Abstract: The reaction rate constants and transient spectra of 11 flavonoids and 4 phenolic acids reacting with e_{aq}^- at neutral pH were measured. The results suggest that C_4 keto group is the active site for e_{aq}^- to attack on flavonoids and phenolic acids, while the *o*-dihydroxy structure in B-ring, the $C_{2,3}$ double bond, the C_3 -OH group and glucosylation have little effects on the e_{aq}^- scavenging activities.

Keywords: hydrated electron, flavonoids, rate constants, pulse radiolysis.

Introduction

The antioxidant activities of flavonoids and simple phenolic acids have been extensively studied^[1-7] and their beneficial activities as antioxidants are highly recognized. No matter whether flavonoids and phenolic acids act as antioxidants or pro-oxidants, both activities originate from their reducing activities. On the other hand, Cai *et. al.*^[8,9] reported that baicalin, a compound of flavone, scavenged reducing radicals such as $\cdot H$ and α -hydroxyethyl radicals. The results demonstrate the oxidizing abilities of flavonoids and phenolic acids, which should also be involved in their physiological activities.

This work studied the interaction of e_{aq}^- with a series of flavonoids and phenolic acids, by pulse radiolysis and aimed to derive the structure-oxidizing activity relationship.

Materials and Methods

A 28MeV electron beam with a pulse duration of 10ns was utilized for pulse radiolysis experiment. The absorbed dose per pulse was 20-75Gy, measured with N_2O -saturated 10mM KSCN solutions. Samples were dissolved in water containing 0.1M *t*-BuOH, 1mM Na_2HPO_4 + 1mM KH_2PO_4 (pH 6.9). For measurement of the transient spectra, a flow cell system was used with the cell length of 2.0cm, and the solutions were bubbled with Ar for 20min before and during the measurement. The decay of e_{aq}^- was followed to derive the pseudo-first-order rate constants and further derive the second order rate constants. Samples were all sealed for irradiation after being bubbled with Ar for 30 min.

Results

Fig. 1 showed the decay of e_{aq}^- in O_2 -free baicalin solution after electron. The decay of e_{aq}^- was assumed to obey pseudo-first-order kinetics. From the slopes the pseudo-first-order rate

constants were obtained at various concentrations of baicalin. The second order rate constant for the reaction of baicalin with e_{aq}^- was derived to be $(1.3 \pm 0.1) \times 10^{10} \text{M}^{-1} \text{s}^{-1}$ by the slope of plotting the pseudo-first-order rate constants versus concentrations of baicalin.

With the same method described above, the rate constants for the reactions of e_{aq}^- with other flavonoids and phenolic acids at pH 6.9 were also determined and listed in Table 1. The compounds, with either a benzoyl or styryl keto group, but without a bulky group neighbor to the keto group, were the most reactive toward e_{aq}^- . These results suggests that a benzoyl or a styryl keto group was important for flavonoids and phenolic acids to scavenge e_{aq}^- and the keto group might be the site on which e_{aq}^- attacked, as supported by Simic and Hoffman^[10].

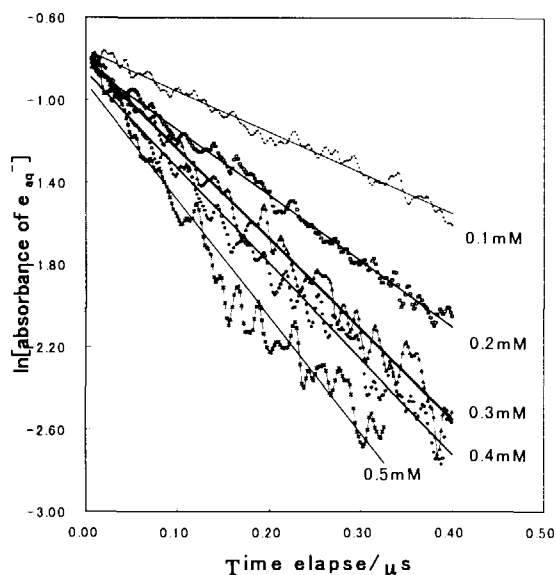


Fig.1 Decay of e_{aq}^- in baicalin solution containing 0.1M t-BuOH, pH 6.9.

Table 1 Rate constants and spectra of the transients for the reactions of e_{aq}^- with flavonoids and phenolic acids at pH 6.9 and room temperature

compound	rate constants* / $\text{M}^{-1} \text{s}^{-1}$	λ_{max} / nm	$\epsilon / \text{M}^{-1} \text{cm}^{-1}$
(+)-catechin	$(1.2 \pm 0.1) \times 10^8$	<320	$> 7 \times 10^3$
4-chromanol	$(4.4 \pm 0.4) \times 10^8$	<320	$> 5 \times 10^3$
genistein	$(6.2 \pm 0.4) \times 10^9$	<350, 430	$> 2 \times 10^3, 5 \times 10^2$
genistin	$(8 \pm 1) \times 10^9$	<350, 460	$> 2 \times 10^3, 8 \times 10^2$
rutin	$(7.6 \pm 0.4) \times 10^9$	<400	$> 2 \times 10^3$
caffeic acid	$(8.3 \pm 0.5) \times 10^9$	360	1.4×10^4
trans-cinnamic acid	$(1.1 \pm 0.1) \times 10^{10}$	370, 490	$1.8 \times 10^4, 2.5 \times 10^3$
p-coumaric acid	$(1.1 \pm 0.1) \times 10^{10}$	365, 470	$1.7 \times 10^4, 2 \times 10^3$
2,4,6-trihydroxyl-benzoic acid	$(1.1 \pm 0.1) \times 10^{10}$	<350, 500	$> 5 \times 10^3, 1 \times 10^3$
baicalein	$(1.1 \pm 0.5) \times 10^{10}$	<400, 460	$> 2 \times 10^3, 1 \times 10^3$
baicalin	$(1.3 \pm 0.1) \times 10^{10}$	365	1.7×10^4
naringenin	$(1.2 \pm 0.1) \times 10^{10}$	<370, 480	$> 2 \times 10^3, 1.5 \times 10^3$
naringin	$(1.0 \pm 0.1) \times 10^{10}$	<370, 480	$> 2 \times 10^3, 1.5 \times 10^3$
quercetin	$(1.3 \pm 0.5) \times 10^{10}$	<400, 540	$> 2 \times 10^3, 1 \times 10^3$
gossypin	$(1.2 \pm 0.1) \times 10^{10}$	<400, 560	$> 6 \times 10^3, 1.5 \times 10^3$

* \pm SD, by 5 experiments.

The transient spectra of flavonoids and phenolic acids reacting with e_{aq}^- and $\cdot H$ were also recorded, as summarized in Table 1. Competitive reaction calculation clearly shows that both reducing species, $\cdot H$ and e_{aq}^- , contributed to the obtained spectrum. All of the transients showed tendency of sharp rise of absorbance below 400nm and a minor peak at wavelength of 460-560nm. A sample transient spectra is shown in Fig.2. These

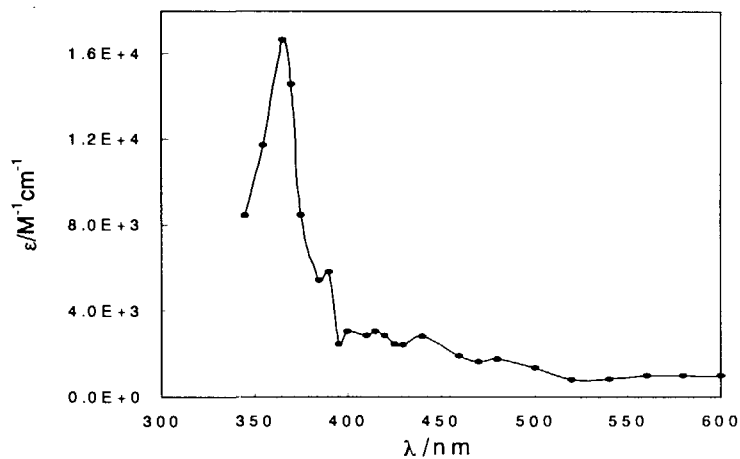
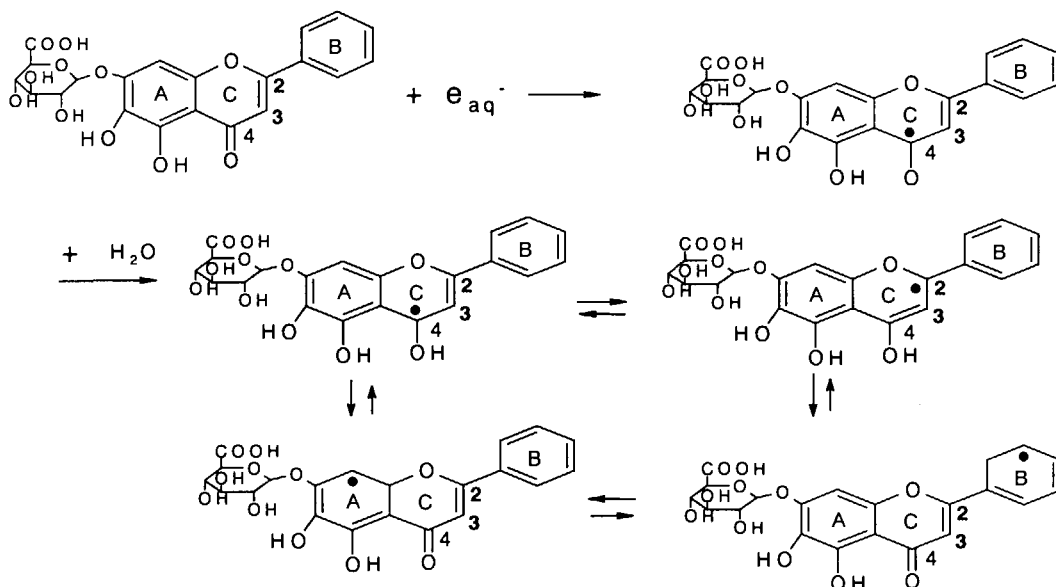


Fig. 2 Transient spectra of e_{aq}^- reacting with baicalin, obtained in pulse radiolysis of O_2 -free 0.1mM baicalin+0.1M t-BuOH +1mM phosphate buffer(pH 6.9).

characteristics of transient spectra were in accord with that of ketyl radical of flavone, which had a main absorption peak at 350nm and a small peak at 500nm.^[11]

We assumed that e_{aq}^- first attacks the keto group of flavonoids and phenolic acids and forms a ketyl radical ion. The ketyl radical ion is unstable and quickly protonize into the same transient as that of H-adduct, which might exist in several resonance states. For example, the reaction of baicalin e_{aq}^- may follow the scheme below:



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