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Phenol Acidity and Ease of Oxidation in Isoflavonoid/β-Carotene Antioxidant Synergism

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ABSTRACT: Regeneration of β-carotene from the β-carotene radical cation by the 4′-propylpuerarin anion (second-order rate constant = 1.5×10^9 L mol^{-1} s^{-1} in methanol/chloroform = 1:9 (v/v) solution at 25 °C as determined by laser flash photolysis) was found to be marginally slower than regeneration by the 7-propylpuerarin anion (2.3×10^9 L mol^{-1} s^{-1}), in agreement with the 7-propylpuerarin anion being more reducing (E' = 0.56 V vs NHE) than the 4′-propylpuerarin anion (E' = 1.01 V vs NHE). The potentials were calculated from $E°$ = 1.12 and 1.44 V (vs NHE) as determined by cyclic voltametry in aqueous solution and pK_a = 9.51 and 7.23 obtained previously for 7-propylpuerarin and 4′-propylpuerarin, respectively. The less reducing but more acidic 4′-propylpuerarin showed less antioxidant activity in liposome of pH 7.4, but more significant antioxidant synergism with β-carotene than the more reducing but less acidic 7-propylpuerarin for oxidation initiated in the liposome lipid phase. Electrostatic effects are concluded to be important in the regeneration of β-carotene from the radical cation in the water/lipid interface because approximately 50% of 4′-propylpuerarin is present as the anion, whereas only 0.5% of 7-propylpuerarin is present as the anion. In contrast, penetration of the undissociated phenolic group into the lipid phase, more significant for 7-propylpuerarin than for 4′-propylpuerarin according to the calculated water/lipid partition coefficients, becomes important for the chain-breaking action in lipid oxidation of the puerarin derivatives as models for (iso)flavonoids and their glycosides.

KEYWORDS: β-carotene, puerarin, isoflavonoid, antioxidant, laser flash photolysis

INTRODUCTION

Puerarin, a bioactive isoflavonoid *C*-glycoside isolated from the root of *Pueraria lobata* traditionally used in Chinese herbal medicine, is getting increasing attention for a variety of pharmacological effects.[1−4] Puerarin is an antioxidant like other (iso)-flavonoids, and synergistic effects have been demonstrated for puerarin and carotenoids, when present together in liposomes at physiological pH, as models for biological membranes.[5,6] Such synergism between hydrophilic (iso)flavonoids and carotenoids active as antioxidant in the lipid phase depends on regeneration by the (iso)flavonoid at the water/lipid interface of the lipophilic carotenoid from the carotenoid radical cation resulting from carotenoid scavenging of lipid radicals. A proper orientation of the reducing and regenerating (iso)flavonoid at the interface depends on the polarity and charge of the (iso)flavonoid and has been confirmed to be important for such synergistic effects.[7] Regeneration of lipophilic antioxidants such as tocophenol and carotenoids by aqueous phase antioxidants such as ascorbate and plant phenols in general seems to be of importance for antioxidant synergism.[8−11]

Assignment of experimentally determined pK_a values to individual phenolic groups in plant polyphenols is often difficult and without consensus.[12,13] Radical scavenging capacities determined for individual plant phenols in homogeneous solution are moreover often not very informative for an understanding of the synergistic effects due to the importance of the actual locations of the antioxidants at the lipid/water interface. Puerarin is an example of a plant phenol for which assignment of both acidity and ease of oxidation to the individual phenolic groups seems unambiguous as based on specific chemical derivatization as the 7- and 4′-propyl ethers combined with theoretical calculations.[5] Notably, the more acidic of the two phenol groups of puerarin is the least reducing, whereas the less acidic is the more reducing.[5,14] A comparison between 7-propylpuerarin and 4′-propylpuerarin (Scheme 1) as antioxidants in the absence and presence of β-carotene in liposomes is accordingly expected to provide mechanistic information relevant to an understanding of the regeneration of β-carotene in the water/lipid interface by puerarin and other (iso)flavonoids under physiological conditions. Theoretical calculations have recently revived interest in carotenoids as electron donors and acceptors of importance for radical communications,[15−17] making assignment of individual phenolic groups and/or phenolates as electron donors highly relevant. The acidity of phenolic groups has further been found to be important for the rate of electron transfer reactions to radicals.[12]

MATERIALS AND METHODS

Chemicals. *all-trans*-β-Carotene (β-carotene) and natural soybean phospholipid mixture L-α-phosphatidylcholine (PC, content ∼ 23%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

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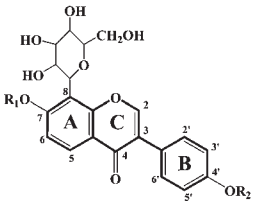
Scheme 1. Molecular Structures of Puerarin and Derivatives 7-Propylpuerarin, 4′-Propylpuerarin, and 7,4′-Dipropylpuerarin



compound	R_1	R_2
puerarin	H	H
7-propylpuerarin	C_3H_7	H
4′-propylpuerarin	H	C_3H_7
7,4′-dipropylpuerarin	C_3H_7	C_3H_7

Chloroform (>99.0%, Beijing Chemical Works) was purified by distillation and passage through an alumina column before use. Methanol of high-performance liquid chromatography (HPLC) grade (Caledon Laboratories, Georgetown, ON, Canada) was used as received, and 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) was from Wako Pure Chemicals Inc. (Osaka, Japan). Other chemicals were of analytical grade. Puerarin was obtained from Huike Plant Exploitation Inc. (Shaanxi, China) and used as received. 7-Propylpuerarin, 4′-propylpuerarin, and 7,4′-dipropylpuerarin were prepared and characterized as previously described.[18] The anionic 7- and 4′-propylpuerarin and monoanionic puerarin in methanol/chloroform = 1:9 (v/v) solution were prepared by the addition of 1 equiv of tetramethylammonium hydroxide, $(CH_3)_4N^+OH^-$ (97%, Sigma, St. Louis, MO), to neutral solutions.

Determination of Oxidation Potentials. Cyclic voltammetry (CV) was performed on a CHI 660B electrochemical analyzer (CH Instruments Inc., Austin, TX) with a three-electrode configuration, for which the solutions of puerarin at a concentration of 2.0×10^{-5} M in Britton−Robinson (B-R) buffer were used (2 < pH < 12). The working electrode was a glassy carbon piece (diameter = 4 mm), the reference electrode was of the Ag/AgCl type (KCl-saturated), and the counter electrode was a platinum wire. Ionic strength was adjusted to 0.10 with NaCl.

Laser Flash Photolysis. Submicrosecond time-resolved absorption spectra were obtained at room temperature (25 ± 1 °C). The excitation laser pulses at 500 nm (2 mJ/pulse, 7 ns, 10 Hz) were obtained by an optical parametric oscillator (OPO, Quanta-Ray MOPO-SL, Spectra Physics, Mountain View, CA) driven by an Nd^{3+}:YAG pumped laser (Quanta-Ray PRO-230; Spectra Physics). The optical path length of the flow cuvette used for laser flash photolysis was 1.0 cm. The anaerobic condition was achieved by bubbling the solution with high-purity argon for ∼30 min. NIR kinetics at 950 nm was recorded with a xenon lamp probe (CW, 350 W) and a photodiode detector (S8890-02, Hamamatsu, Hamamatsu City, Japan).

Antioxidant Evaluation in Liposomes. Liposome was prepared following ref 7 with certain modifications. Briefly, soybean L-α-phosphatidylcholine (0.75 mM) together with AMVN (1.60 mM) and/or β-carotene (7.5 μM) in chloroform was mixed in methanol, and the volume ratio of chloroform and methanol was 2:1. Solvents were removed under reduced pressure (0.01 MPa) by using a rotary evaporator at the bath temperature of 22 °C. Afterward, high-purity nitrogen was introduced to re-establish the atmospheric pressure. The flask was covered with aluminum foil and kept under vacuum for 1.5 h.

The lipid residues with or without β-carotene were rehydrated with 0.01 M phosphate buffer solution (pH 7.40) under sonication, and then 25.0 μL of ethanol solution of 7- or 4′-propylpuerarin (3.0×10^{-4} M, 7,4′-propylpuerarin was also investigated for comparison) was added to 5.0 mL of liposome suspension. The preparation was passed through hydrophilic polyethersulfone membranes (200 nm pore size) 20 times using Acrodisc syringe filters (Pall Corp., East Hills, NY) and then incubated for 90 min under room temperature conditions with reduced light and atmospheric oxygen partial pressure. The final concentrations of L-α-phosphatidylcholine and AMVN were 0.15 and 0.32 mM, respectively. The final β-carotene and/or isoflavonoid concentrations corresponded to a 1% molar fraction of L-α-phosphatidylcholine.

Three milliliters of the suspension in a sealed 1.0 cm quartz cuvette was thermostated, and peroxidation was initiated at 43 °C. Absorbance at 234 nm corresponding to the formation of conjugated dienes of L-α-phosphatidylcholine was continuously recorded for 100 min at 43 °C on a Cary50 spectrophotometer (Agilent Technologies Inc., Santa Clara, CA). Results from the conjugated diene measurements are expressed as ΔAUC, the difference of the area under curve (AUC) of absorbance versus time between control liposomes and liposomes with antioxidants.[19,20] Any synergistic effect was confirmed for $AUC_{\text{puerarin derivative+}\beta\text{-carotene}} > \Delta AUC_{\text{puerarin derivative}} + \Delta AUC_{\beta\text{-carotene}}$.

Solution Stability of 7- and 4′-Propylpuerarin Derivative Anions. Steady state absorption spectra of 2.5×10^{-4} M 7 or 4′-propylpuerarin derivatives in alkaline aqueous solution (pH 12.0) were recorded on a spectrophotometer at a serial delay time in darkness under aerobic conditions at 25 ± 1 °C.

Calculated Partition Coefficients of Puerarin and Its Derivatives. Calculated partition coefficients (ClogP) for neutral and anionic 7- or 4′-propylpuerarin as seen in Table 1 together with values for neutral puerarin, 7,4′-dipropylpuerarin, and the puerarin dianion for comparison, were obtained using Marvin 5.4 with Calculator Plugins, 2010 (ChemAxon, http://www.chemaxon.com).

Table 1. Calculated Partition Coefficients (ClogP), Standard Redox Potentials $E°$, and Redox Potential E' for Neutal and Anionic Puerarin and Derivatives and Second-Order Rate Constants (k) for Electron Transfer from 1.2×10^{-5} M 4′- and 7-Propylpuerarin Anions and Puerarin Dianion to β-Carotene in Methanol/Chloroform (1:9, v/v) at 25 °C

compound	ClogP	$E°/E'$ (V)	k (L mol^{-1} s^{-1})
4′-propylpuerarin	0.99	1.44	
4′-propylpuerarin$^-$	−0.47	1.01	1.5×10^9
7-propylpuerarin	1.00	1.12	
7-propylpuerarin$^-$	0.87	0.56	2.3×10^9
puerarin	−0.03	1.13[a]	
7,4′-dipropylpuerarin	2.02		
puerarin^{2-}	−3.72	0.69	5.0×10^9 [b]

[a] From ref 6. [b] From ref 5.

RESULTS

Standard Reduction Potentials. A cyclic voltammogram (CV) scan of 7-propylpuerarin and 4′-propylpuerarin aqueous solution with constant pH (2 < pH < 12) as shown in Figure 1 indicates no reversible reduction peak after the initial oxidation peak. The oxidation peaks seen are ascribed to the oxidation of the B-ring phenolic 4′-OH group for 7-propylpuerarin and that of the A-ring 7-OH group for 4′-propylpuerarin, respectively. The B-ring phenolic hydroxyl is found to be more reducing than the A-ring hydroxyl group, which agrees with our previous experimental and theoretical results.[5,11,21] The electrode oxidation processes for the neutral and monoanionic forms of puerarin derivatives are suggested to be

$$\text{P-7/4′-OH} \rightarrow \text{P-7/4′-O}^{\bullet} + \text{H}^+ + \text{e}^- \quad (1)$$

$$\text{P-7/4′-O}^- \rightarrow \text{P-7/4′-O}^{\bullet} + \text{e}^- \quad (2)$$

in which P represents the 7- or 4′-propylpuerarin skeleton.

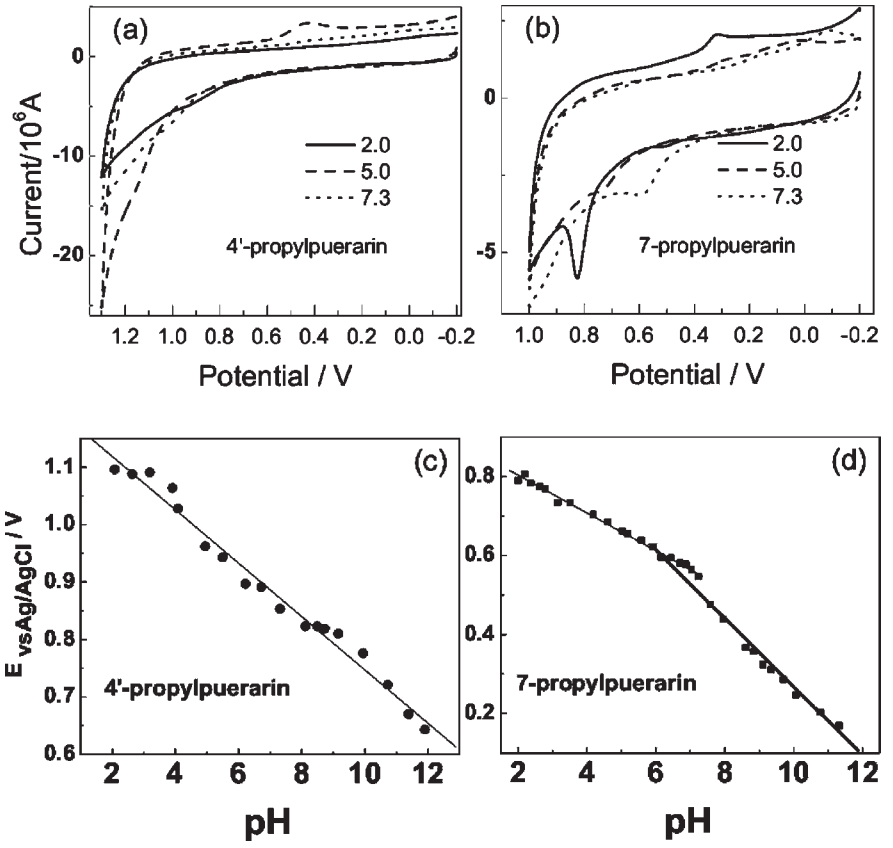


Figure 1. Cylic voltammogram and pH dependence of half-wave potential (oxidation potential) of 2.0×10^{-5} M (a, c) 4′-propylpuerarin and (b, d) 7-propylpuerarin at various pH values in aqueous solution of ionic strength 0.1 adjusted with NaCl at a scan rate of 100 mV s^{-1} at room temperature (~25 °C).

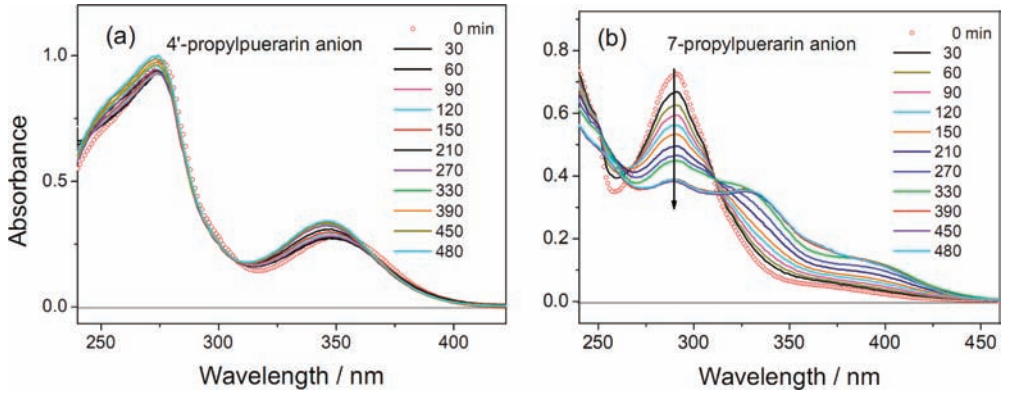


Figure 2. Absorption spectra of (a) 4′-propylpuerarin and (b) 7-propylpuerarin anions in aqueous solution at pH 12 in darkness at indicated times under aerobic conditions at 25 °C showing the difference in degradation rate for the two isomers.

The dependence of the oxidation of 4′-propylpuerarin on pH is found to be linear with a slope $\alpha = 0.047 \pm 0.002$ in the whole pH range, similar to the slope found for puerarin.[6] These deviations from the expected slope of 0.059 are most likely due to irreversibility of the electrode process. The dependence on pH for 7-propylpuerarin oxidation potential was linear up to pH 7.5 with a similar slope of 0.047 ± 0.001, whereas the slope was 0.082 ± 0.001 for pH >7.5. The linear dependence of the oxidation potential of 4′-propylpuerarin on pH above the pK_a value can be ascribed to a slow degradation of the 4′-propylpuerarin anion formed from deprotonation of parent molecules under alkaline conditions as seen in Figure 2a. The nonlinearity of the oxidation potential for 7-propylpuerarin in the extended pH region as seen in Figure 2b may be due to a higher reducing ability of the 4′-hydroxyl group, which also makes it more suspectible to degradation under alkaline conditions. However, neutral conditions were used for the kinetics studies for which the degradation reactions are very slow.

The standard reduction potential $E°$ of 7-propylpuerarin or 4′-propylpuerarin was determined by linear extrapolation of the pH dependence ($2 < \text{pH} < 7.5$) for both 7-propylpuerarin and 4′-propylpuerarin to pH 0, as shown in Figure 1c,d and Table 1.

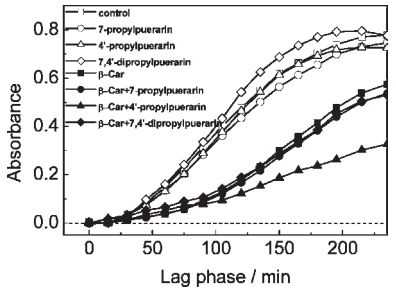


Figure 3. Time evolution profiles of conjugated diene formation measured spectrophotometrically at 234 nm arising from the lipid oxidation initiated by AMVN in L-α-phosphatidylcholine (PC) liposomes at 43 °C in the presence/absence of puerarin derivatives without and with β-carotene. Final concentrations: [PC] = 0.15 mM, [AMVN] = 0.32 mM, [antioxidant] = 1% molar fraction of PC.

The standard reduction potentials of the protonated 7-propylpuerarin and 4′-propylpuerarin are estimated to be $E°$ = 1.12 and 1.44 V vs NHE (0.90 ± 0.01 V and 1.21 ± 0.01 vs Ag/AgCl) as shown in Table 1, respectively. Notably, the standard reduction potential of 7-propylpurarin is almost equal to that of puerarin ($E°$ = 1.13 V), in agreement with the specific derivatization of the less reducing 4′-hydroxyl group in 7-propylpuerarin.[5]

In view of the possible deprotonation of puerarin phenoxyls for physiological pH 7.4 conditions, redox potentials of 7- or 4′-propylpuerarin anion should be considered. Redox potentials of 7- or 4′-propylpuerarin anions, corrected for acid dissociation of the phenolic groups, were calculated to be E' = 0.56 and 1.07 V, respectively, and are listed together in Table 1 as based on the Gibbs free energy $\Delta G°$ for the reactions of eq 1 for their oxidation and of eq 3 for their deprotonation.

$$\text{P-7/4′-OH} \rightarrow \text{P-7/4′-O}^- + \text{H}^+ \quad (3)$$

Antioxidant Evaluation in Liposomes. The antioxidant efficiency for the two puerarin derivates in the absence and presence of β-carotene was determined in L-α-phosphatidylcholine liposomes with pH 7.4 at 43 °C, and the corresponding time evolution curves for formation of the conjugated dienes as the primary oxidation products of L-α-phosphatidylcholine are shown in Figure 3. Oxidation was initiated thermally in the lipid phase by the lipophilic free radical initiator AMVN and was monitored at 234 nm, at which wavelength the dienes as the primary lipid oxidation product absorb. ΔAUC, the difference between the area under curve (AUC) of aborbance versus time of the conjugated diene formation between control and the sample, was used to quantify the antioxidant activity of total antioxidants added. AUC and ΔAUC values for puerarin derivatives without and with β-carotene are summarized in Table 2.

It can be seen directly from Figure 3 and further from Table 2 that 4′-propylpuerarin has almost no effect on the rate of lipid oxidation. 7-Propylpuerarin is found to inhibit lipid oxidation slightly better than 4′-propylpuerarin, whereas 7,4′-dipropylpuerarin is found to be somewhat prooxidative, an effect that possibly results from a looser structure of the liposome in the presence of the surface active 7,4′-dipropylpuerarin. β-Carotene has a clear antioxidative effect, confirming previous findings for lipid oxidation in liposomes with initiation of oxidation in the lipid phase.[5] It is worth noting that 4′-propylpuerarin, being a poorer antioxidant than β-carotene, remarkably enhanced the antioxidant efficiency of β-carotene. A clear synergistic antioxidant effect was accordingly observed in the case of the 4′-propylpuerarin and β-carotene mixture. Antioxidant synergism efficiency (SE) between β-carotene (β-Car) and puerarin derivatives (PD) expressed as

$$\text{SE}\ (\%) = \frac{\Delta\text{AUC}_{\beta\text{-Car}} + \Delta\text{AUC}_{\text{PD}} - \Delta\text{AUC}_{\beta\text{-Car+PD}}}{\Delta\text{AUC}_{\beta\text{-Car}} + \Delta\text{AUC}_{\text{PD}}} \times 100\% \quad (4)$$

is listed in Table 2. The β-carotene/4′-propylpuerarin synergism efficiency is seen to be 31%, which is somewhat lower than the 47% previously observed for the puerarin and β-carotene synergy efficiency.[5] In contrast, the addition of 7-propylpuerarin or 7,4′-dipropylpuerarin to β-carotene had almost no effect or even a slightly antagonistic effect in inhibiting lipid peroxidation in liposomes of pH 7.4.

Table 2. Area in Arbitrary Units under the Absorbance/Time Curve (AUC) and Difference between the Control and Antioxidants (ΔAUC[a]), As Determined by Spectrophotometric Measurements of Conjugated Diene Formation in Soybean L-α-Phosphatidylcholine Liposomes with or without β-Carotene Added with Free Radical Initiation by AMVN in the Lipid Phase, Together with Antioxidant Synergism Efficiency (SE) between Puerarin Derivatives and β-Carotene (See Figure 3)

compound	without β-carotene		with β-carotene		SE (%)
	AUC	ΔAUC	AUC	ΔAUC	
blank	99.5	0	52.4	47.0	
7-propylpuerarin	92.1	7.3	48.7	50.8	−6
4′-propylpuerarin	95.8	3.7	32.8	66.6	31
7,4′-dipropylpuerarin	107.3	−7.8	52.3	47.1	−8

[a] ΔAUC = AUC(blank) − AUC(antioxidant).

Laser Flash Photolysis. Upon acting as scavengers of lipid radicals β-carotene and most other carotenoids form the carotenoid radical cation through electron transfer. This radical cation is also found photochemically in electron-accepting solvents such as chloroform upon direct photoexcitation of carotenoids with 500 nm laser pulses.[22] A mixture of chloroform and methanol (9:1) was used in the present study to dissolve both β-carotene (5×10^{-6} M) and puerarin or puerarin derivative (1.2×10^{-5} M). The real time kinetics of the carotenoid radical cations formed by nanosecond laser photolysis was recorded at 950 nm as previously described[5] as shown in Figure 4.

For neutral puerarin and neutral 7- or 4′-propylpuerarin hardly any effect was seen for the decay of β-carotene radical cation, confirming previous findings (data not shown). In contrast, the decay of the β-carotene radical cation was accelerated by the presence of both the puerarin monoanion and the 7- or 4′-propylpuerarin anion as seen in Figure 4. The anions were obtained by adding 1 equiv of tetramethylammonium hydroxide to the solution. The increased rate is attributed to electron transfer from the resulting phenolate ion of puerarin or puerarin derivative to β-carotene according to

$$\beta\text{-Car}^{\bullet+} + \text{P-O}^- \rightarrow \beta\text{-Car} + \text{P-O}^{\bullet} \quad (5)$$

where P-O$^-$ is the puerarin or the puerarin derivative anion. The 7-propylpuerarin anion more significantly accelerates the decay

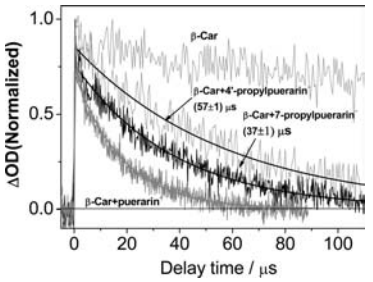


Figure 4. Time traces of β-carotene radical cations (5×10^{-6} M) at 900 nm measured by transient absorption spectroscopy following laser flash photolysis of carotenoid in methanol/chloroform (1:9, v/v) solutions at 25 °C in the absence and presence of 1.2×10^{-5} M 4′-propylpuerarin, 7-propylpuerarin anions, or puerarin monoanion. Full lines are exponential fits to yield the lifetimes indicated.

of carotenoid radical cations with a rate constant $k = 2.3 \times 10^9$ L mol^{-1} s^{-1} than the 4′-propylpuerarin anion with $k = 1.5 \times 10^9$ L mol^{-1} s^{-1}, which agrees with the lower oxidation potential of 7-propylpuerarin anion (1.12 V) compared to that of 4′-propylpuerarin (1.01 V). The even higher rate constant for the dianion of puerarin previously reported (5.0×10^9 L mol^{-1} s^{-1}) may indicate an important electrostatic contribution.[5] The puerarin anion obtained by the addition of 1 equiv of base to puerarin results in a faster decay of β-carotene radical cation than for 7- or 4′-propylpuerarin, which may be assigned to some resonance effects between the two possible phenolates in the monoanion of puerarin and their resulting radicals.[14]

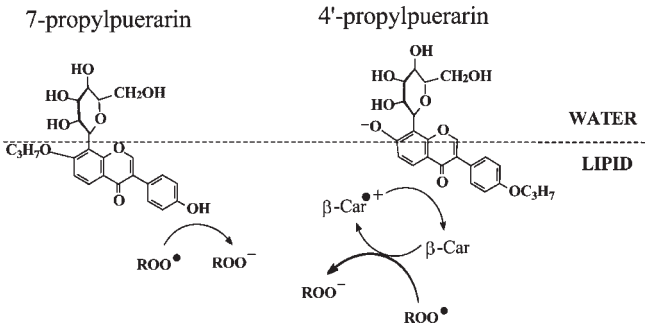
DISCUSSION

The phenolate anion of the more reducing of the two puerarin derivatives, 7-propylpuerarin ($E°$ = 1.12 V vs NHE in aqueous solution), was found to react more quickly with β-carotene radical cation in methanol/chloroform homogeneous solution than the less reducing 4′-propylpuerarin ($E°$ = 1.44 V) in regenerating β-carotene. The more reducing 7-propylpuerarin was also found to be the best antioxidant, hampering lipid oxidation in liposomes at physiological pH 7.4, although neither of the two derivatives had but marginal effects on the rate of formation of primary lipid oxidation products for oxidation initiated in the lipid phase. In contrast, the less reducing 4′-propylpuerarin showed a clearly synergistic effect with β-carotene as a lipid-soluble antioxidant in the liposome. β-Carotene alone was rather efficient as an antioxidant located in the lipid phase in contrast to any of the puerarin derivatives alone as present mainly in the aqueous phase.

The difference in radical scavenging rate for the reaction in eq 5 as shown in Table 1 in homogeneous solution accordingly depends on the driving force, that is, $-\Delta G°$, in agreement with the linear free relationship previously demonstrated for regeneration of four carotenoids including β-carotene by the puerarin dianion and the daidzein dianion.[6] However, factors other than the driving force for radical scavenging are important in a heterogeneous system such as membranes. In the present investigation, liposomes of physiological pH were used as models for such systems. β-Carotene was clearly a good antioxidant when oxidation was initiated in the lipid phase. Lipid-derived radicals are scavenged by β-carotene

$$\beta\text{-Car} + \text{ROO}^{\bullet} \rightarrow \beta\text{-Car}^{\bullet+} + \text{ROO}^{-} \quad (6)$$

Scheme 2. 7-Propylpuerarin and 4′-Propylpuerarin at Water/Lipid Interface at pH 7.4 with β-Carotene Radical Cation As Formed by Oxidation of β-Carotene by Free Radial Initiator



and β-carotene becomes more efficient as antioxidants when continuously regenerated by aqueous phase antioxidants in lipid/water interface at which β-Car$^{\bullet+}$ will concentrate due to the negative charge of the L-α-phosphatidylcholine at physiological pH.

The more reducing 4′-hydroxy group of puerarin is the less acidic, and at physiological pH, 7-propylpuerarin is only marginally dissociated, corresponding to 0.5% (pK_a = 9.51 for 7-propylpuerarin in water at 25 °C), whereas the less reducing but more acidic 4′-propylpuerarin is 48% dissociated (pK_a = 7.23 for 4′-propylpuerarin). Redox potentials for pH 7.4 were calculated to be E' = 1.01 V for the 4′-propylpuerarin anion and E' = 0.56 V for the 7-propylpuerarin anion, respectively. Clearly, the less reducing 4′-propylpuerarin is the more efficient in the regeneration of β-carotene as the effective lipophilic antioxidant, and factors other than the ease of oxidation clearly have to be considered for the puerarin derivatives. Partition between the lipid phase and the aqueous phase for the regenerating hydrophilic antioxidant has previously been found to be important.[7] The partition coefficients for 4′-propylpuerarin and 7-propylpuerarin in Table 1 show that the less reducing 4′-propylpuerarin is penetrating less into the lipid phase as shown in Scheme 2. Because the more acidic phenolic group in 4′-propylpuerarin is located in the more polar part of the molecule, the β-carotene radical cation being more polar and hydrophilic than β-carotene seems to be located close to the phenolate ion. The electrostatic interaction of β-carotene is accordingly concluded to be important for the regeneration of β-carotene by the puerarin derivative, whereas for the direct effect of the puerarin derivative as a chain-breaking antioxidant the penetration into the lipid phase seems to be more important.

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