Research article

One-electron oxidation of antioxidants: A kinetic-thermodynamic correlation

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The values of the bimolecular rate constants for the reactions of 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) radical cation with epicatechin (((2.4 ± 0.2)) s^{-1} M^{-1}), and epigallocatechingallate ((29 ± 5) s^{-1} M^{-1}) were obtained by spectrophotometric measurements. We propose a correlation between the Gibbs energy ΔG^{o} for the one-electron charge-transfer reactions from several antioxidants to radical species and the rate constants of the corresponding bimolecular reactions. This correlation can be used to predict rate constants of reactions of known ΔG^{o} values.

Keywords: ABTS, Antioxidants, Epicatechin, Epigallocatechingallate, Kinetic-thermodynamic correlation

Introduction

Antioxidants, such as polyphenolic compounds (vitamins E and C and carotenoids) present in fruits, vegetables, tea, and wines are of great interest because of their involvement in mechanisms of defense against oxidizing species generated during aerobic respiration, known as reactive oxygen species.[1,2] Several publications have shown the existence of a potential relationship between the intake of antioxidants and the prevention of cardiovascular diseases, cancer, and chronic inflammation.[3]

In order to evaluate the antioxidant activity of different substances and natural products several methods have been developed, such as those employing Trolox Equivalent Antioxidant Capacity,[4,5] Total Radical-Trapping Antioxidant Parameter,[6] Ferric Reducing Ability of Plasma,[7] and Oxygen Radical Absorbance Capacity.[8] These methods are based on charge-transfer processes or on reactions involving an H-atom transfer between the analyzed substrate and a radical.

Most of the methods employed to measure the antioxidant capacity of samples of natural products are based on correlations of changes. For instance, the changes of absorbance in the UV–vis region of different oxidizing species after a certain time of incubation with the antioxidants are compared with those produced by incubation during the same period of time with a reference compound.[4–8] However, kinetic information is lost in these comparisons because the kinetics of the reactions between the radicals and the antioxidants is not always comparable. For instance, the decay of the 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical promoted by quercetin presents two phases, and can be approximately fitted to a biexponential decay of widely different lifetimes (ca. 3 and 200 seconds).[9] This behavior closely resembles that described by Pannala *et al.*[10] for flavonoids with a catechol-containing B ring, and can be explained in terms of the presence of two reactive sites of widely different reactivity.

We here extend the study of reactions of the $ABTS^{\cdot+}$ radical cation with the flavonoids epicatechin (EC) and epigallocatechingallate (EGCG), which also contain catechol groups in ring B, to prove whether these reactions also proceed in two phases.

As mentioned above, the radical species employed for measuring the antioxidant capacity of a sample react with the substrates by different mechanisms. Lien *et al.*[11] found for a large number of phenols a correlation between experimentally determined redox potential at pH 7, a direct measure of the antioxidant property,[12,13] and ΔH_f, which is the calculated difference of the heats of formation between phenoxyl radicals and the corresponding parent phenols. The ΔH_f value is determined by the bond dissociation energy of the O–H bond, which indicates that the tendency to donate an electron and an H-atom are not independent in the case of phenols. This finding and the disadvantage of employing the conventional methods for evaluating antioxidant capacities motivated us to

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search for a correlation between the rate constants of bimolecular reactions between phenolic substrates with species able to react with them (either by one-electron oxidation or by H-atom abstraction) and the standard Gibbs energy of the corresponding reaction. The use of the standard Gibbs energy of the reaction instead of the redox potential of the substrate was necessary because of the extension of our correlation to different oxidizing species.

Experimental

ABTS, EC, and EGCG (Sigma-Aldrich p.a., Saint Louis, MO, USA), hydrogen peroxide (Merck p.a.), sodium acetate (Merck p.a.), and glacial acetic acid (Merck p.a., Hohenbrunn, Germany) were used as received. Distilled water (>18 MΩ cm, <20 ppb of organic carbon) was obtained from a Millipore system.

The $ABTS^{\cdot+}$ radical cation was prepared by addition of H_2O_2 to a buffered (pH = 3.6) solution of sodium acetate. After incubating the solution at room temperature for an hour, the characteristic color of the radical was observed. This reactive species is stable at 4°C for 6 months.[14] The same concentration of the colored radical (10 μM) and variable amounts of the polyphenolic compounds in the range from 40 μM to 1 mM were employed.

All the kinetic studies were carried out in triplicate and at room temperature. The experiments performed to study the slow phase of the reaction were performed with a Shimadzu UV-1800 spectrophotometer.

The fast reaction was studied by mixing solutions of $ABTS^{\cdot+}$ with those of the flavonoids in a Hi-Tech Scientific SFA-20 Rapid Kinetics stopped-flow accessory. The absorbance at 700 nm was measured with a sensitive detection system conventionally used for flash-photolysis experiments.[15]

Results and discussion

Kinetics of the reaction of $ABTS^{\cdot+}$ with EC and EGCG

The reaction of $ABTS^{\cdot+}$ with EC or EGCG was studied by employing different concentrations of the flavonoids at room temperature. Solutions were prepared by mixing the reactants. The absorption spectra of the solutions showed the characteristic bands of $ABTS^{\cdot+}$ with maxima at 420 and 740 nm.[16] A decrease of these bands was observed after mixing. Fig. 1 shows the spectral changes observed for the mixture of $ABTS^{\cdot+}$ and EGCG.

In the presence of both flavonoids the absorbance changes analyzed at 700 nm, close to the second maximum of $ABTS^{\cdot+}$ show two different processes: a fast decrease of absorbance within a few seconds followed by a slower decrease in a time scale of several minutes. Fig. 2 shows both the faster and slower absorbance changes observed for two different concentrations of EC.

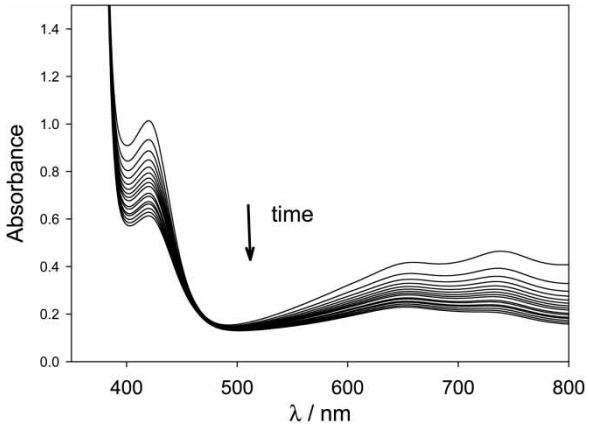

Figure 1 Spectral changes observed for the mixture of $ABTS^{\cdot+}$ 10 μM and EGCG 0.18 mM. The absorption spectra were taken every 3 minutes from $t = 1$ minute to $t = 64$ minutes.

The absorbance of $ABTS^{\cdot+}$ follows a biexponential decay. Due to the relatively high concentration of $ABTS^{\cdot+}$ necessary for the absorbance measurements, it was not possible to strictly maintain the pseudo first-order conditions during the complete decay of the radical. For that reason kinetic computer simulations were performed with the CKS stochastic program.[17] The rate constants of the faster and slower processes, k_1 and k_2, respectively, were systematically varied until the best coincidence between the experimental and simulated signals was obtained. The simulated decays obtained for two concentrations of EC are also shown in Fig. 2. The values of k_1 and k_2 are shown in Table 1.

The biexponential decay of $ABTS^{\cdot+}$ radical in the presence of EC or EGCG observed here is in line with the data reported by Pannala *et al.*,[10] although these authors do not report the bimolecular rate constants of both processes. The mechanism, which accounts for the biexponential behavior, is a

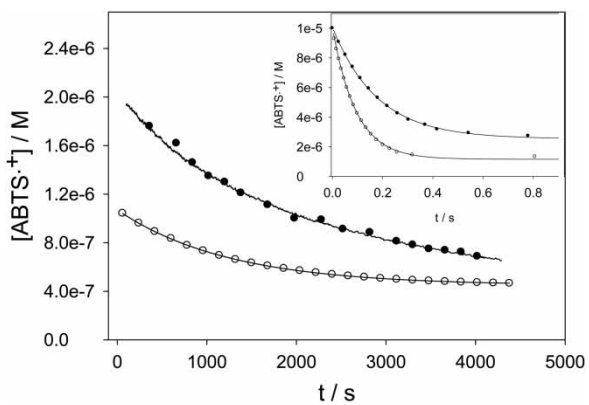

Figure 2 Average slow $ABTS^{\cdot+}$ concentration changes observed in the presence of different amounts of EC 0.33 mM (black circle), 0.77 mM (white circle), and the simulated decays (full line). Inset: Average fast concentration changes for the data shown in the main figure.

Table 1 Bimolecular rate constants of oxidation of $ABTS^{\cdot+}$ with EC and EGCG

	$k_1/M^{-1}\,s^{-1}$	$k_2/M^{-1}\,s^{-1}$
EC	12 000 ± 50	150 ± 10
ECG	13 000 ± 60	160 ± 10

The error bars represent standard deviations.

consecutive one with a first step involving the donation of a single electron from the flavonoid to the radical cation leading to the formation of a semiquinone. This species can donate a further electron to yield a quinone, as proposed by these authors.

Correlation between the rate constants for the reactions between phenolic compounds and one-electron oxidants with the reaction Gibbs energy

For the purpose of finding a relationship between kinetic parameters and antioxidant properties of phenolic compounds, the bimolecular rate constants of the reactions between phenolic compounds and one-electron oxidants were correlated with the corresponding Gibbs energy. Most rate constants were obtained from the literature[18,19] and the values measured in this paper were also included. Table 2 shows the rate

Table 2 Room-temperature bimolecular rate constants of oxidation of the flavonoids with oxidants, the difference in redox ($\Delta\varepsilon$) potentials, and Gibbs energy (ΔG)

		$k\times10^{-9}\,M^{-1}\,s^{-1}$	$\Delta\varepsilon^{o}$/V§	$\Delta G^{o}\times10^{-23}$/eV§
EC $\varepsilon^{o}/V=0.5700$	$NO_2^{\cdot}$	0.090*	0.4300	−2.5893
	$CO_3^{\cdot-}$	0.560*	0.7300	−4.3958
	$N_3^{\cdot}$	4.00*	1.0400	−6.2625
	$OH^{\cdot}$	6.4*	1.3300	−8.0087
	$H_2PO_4^{\cdot}$	0.780†	1.4800	−8.9120
	$SO_4^{\cdot-}$	1.460†	1.8600	−1.1200
	$Br_2^{\cdot-}$	0.090*	1.0500	−6.3227
	$O_2^{\cdot-}$	4.7926×10^{-4}*	0.2950	−1.7764
	$ABTS^{\cdot+}$	1.50×10^{-7}‡	0.0900	−5.4194
ECG $\varepsilon^{o}/V=0.5500$	$N_3^{\cdot}$	4.70*	1.0600	−6.3829
	$OH^{\cdot}$	5.80*	1.3500	−8.1292
	$H_2PO_4^{\cdot}$	0.850†	1.5000	−9.0324
	$SO_4^{\cdot-}$	1.20†	1.8800	−1.1321
	$O_2^{\cdot-}$	4.29×10^{-4}*	0.3150	−1.8968
EGCG $\varepsilon^{o}/V=0.4300$	$OH^{\cdot}$	7.10*	1.4700	−8.8518
	$N_3^{\cdot}$	4.80*	1.1800	−7.1055
	$SO_4^{\cdot-}$	1.040†	2.0000	−1.2043
	$NO_2^{\cdot}$	0.120*	0.5700	−3.4323
	$CO_3^{\cdot-}$	0.660*	0.8700	−5.2388
	$(SCN)_2^{\cdot-}$	0.420*	0.6700	−4.0345
	$H_2PO_4^{\cdot}$	0.680†	1.6200	−9.7550
	$O_2^{\cdot-}$	6.60×10^{-4}*	0.4350	−2.6194
	$ABTS^{\cdot+}$	1.60×10^{-7}‡	0.2300	−1.3850
	$(SCN)_2^{\cdot-}$	0.218*	0.6200	−3.7334
trolox C $\varepsilon^{o}/V=0.4800$	$Br_2^{\cdot-}$	0.600*	1.1400	−6.8646
	$N_3^{\cdot}$	0.500*	1.1300	−6.8044
	$HO_2^{\cdot}$	1.9879×10^{-4}*	0.3100	−1.8667
1,4-Dihydroquinone $\varepsilon^{o}/V=0.4590$	$ClO_2^{\cdot}$	3.90×10^{-5}*	0.9500	−5.7205
	$SCN)_2^{\cdot-}$	0.0600*	1.1000	−6.6238
	$SO_5^{\cdot-}$	2.70×10^{-3}*	1.1000	−6.6238
	I	6.40*	1.2700	−7.6474
	$N_3^{\cdot}$	4.00*	1.6100	−9.6948
	$BrO_2^{\cdot-}$	0.270*	1.3300	−8.0087
	$Br_2^{\cdot-}$	0.100*	1.6200	−9.7550
	$OH^{\cdot}$	20.0*	1.9000	−1.1441
	$Cl_2^{\cdot-}$	1.00*	2.3000	−1.3850
	$HO_2^{\cdot}$	4.70×10^{-5}*	0.7900	−4.7571
1,2-Dihydroquinone $\varepsilon^{o}/V=0.5310$	$SO_5^{\cdot-}$	2.70×10^{-3}*	1.1000	−6.6238
	$N_3^{\cdot}$	4.00*	1.6100	−9.6948
	$OH^{\cdot}$	11.0*	1.9000	−1.1441
	$HO_2^{\cdot}$	5.00×10^{-6}*	0.7900	−4.7571
1,3-Dihydroquinone $\varepsilon^{o}/V=0.8100$	$SO_5^{\cdot-}$	1.00×10^{-3}*	0.9500	−5.7205
	I	0.130*	1.1000	−6.6238
	$N_3^{\cdot}$	1.00*	1.2700	−7.6474
	$Br_2^{\cdot-}$	0.170*	1.6100	−9.6948
	$OH^{\cdot}$	12.0*	1.6200	−9.7550

All data correspond to undissociated phenols.
*Data from NDRL-NIST solution kinetics database.[18]
†Data from Villata *et al.*[19]
‡This work.
§Data from Wardman[20].

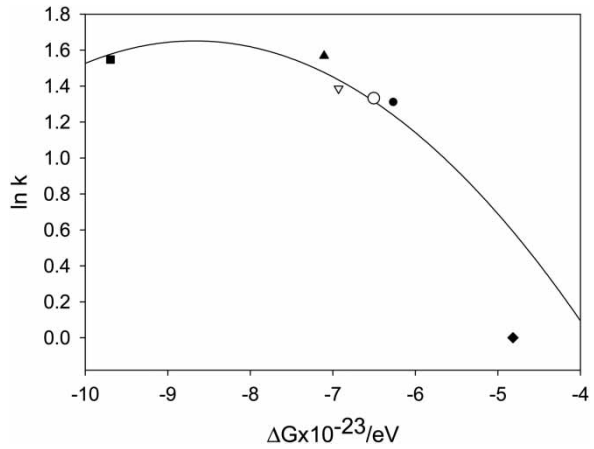

Figure 3 Marcus correlation for the reaction of the phenols EC (black circle); ECG (black square); EGCG (black triangle); 1,4-dihydroquinone (inverted triangle); 1,3-dihydroquinone (black rhombus); and 1,2-dihydroquinone (white circle) with azide radical. The rate constants were taken from NDRL-NIST Solution Kinetics Database[18] and the redox potentials from Wardman[20].

constants, as well as the difference in redox potentials ($\Delta\varepsilon$)[20] and Gibbs energy (ΔG^{o}).

As already mentioned, these reactions can proceed either by electron transfer or H-abstractions. However, Table 2 also includes data for azide radical reactions, which are known to take place by electron transfer.[21] For the reactions of the phenols with this radical, the expected Marcus[22] type correlation is shown in Fig. 3.

For all the oxidants and phenols shown in Table 2, it was possible to obtain an empirical correlation between the rate constant k and $\Delta G°$ (Fig. 4).

The experimental data were fitted to equation 1:

$$\ln k = a + b \times c^{(\Delta G^{o})^{2}} \qquad (1)$$

Equation 1 with a similar dependence of k with ΔG^{o} to that shown by Marcus equation,[22] is also valid for the azide radical reactions, which proceed by electron transfer.

From this correlation, by measuring the rate constant of the reaction of a phenolic compound or an antioxidant natural extract, it is possible to predict the antioxidant ability of the sample. As can be seen in Fig. 4, the correlation can be applied in the $\Delta G°$ range from -6 to -0.5×10^{-23} eV. All the values of k for $\Delta G°$ ranging between -12 and -6×10^{-23} eV are close to a diffusion-controlled rate constant.

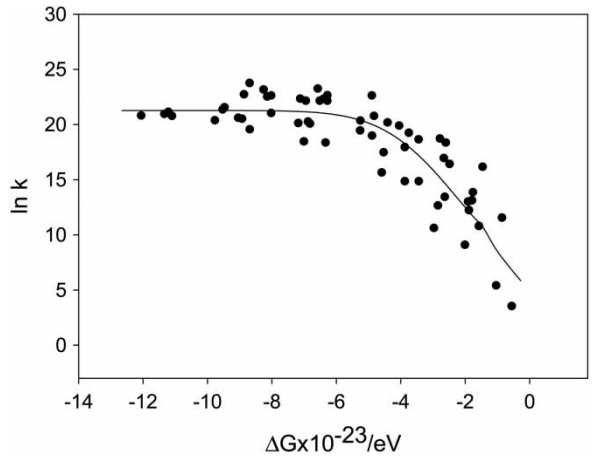

Figure 4 Correlation between the rate constant k and $\Delta G°$ for the data shown in Table 2. The symbol (black circle) represents the experimental data and the full lines the calculated fits according to Equation 1.

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