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Reduction of Ferrylmyoglobin by Theanine and Green Tea Catechins. Importance of Specific Acid Catalysis

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ABSTRACT: Reduction of the hypervalent heme pigment ferrylmyoglobin by green tea catechins in aqueous solution of pH = 7.5 was investigated by stopped-flow spectroscopy. Reduction by the gallic acid esters epigallocatechin gallate (EGCG, k_2 = 1460 L mol^{-1} s^{-1}, 25.0 °C, 0.16 ionic strength) and epicatechin gallate (ECG, 1410 L mol^{-1} s^{-1}) was found faster than for epicatechin (EC, 300 L mol^{-1} s^{-1}) and epigallocatechin (EGC, 200 L mol^{-1} s^{-1}), even though the gallate ion (G, 330 L mol^{-1} s^{-1}) is similar in rate to EC. The rate for reduction by EC, EGC, ECG, EGCG, and G shows no correlation with their oxidation potentials or phenolic hydrogen−oxygen bond dissociation energy, but with the pK_a of the most acidic phenol group. Theanine, with an acidity similar to that of EC, reduces ferrylmyoglobin with a similar rate (200 L mol^{-1} s^{-1}), in support of general acid catalysis with an initial proton transfer prior to electron transfer.

KEYWORDS: *ferrylmyoglobin, green tea catechins, theanine, acid catalysis*

INTRODUCTION

Green tea extract is receiving increasing attention as a natural antioxidant for protection of meat and meat products, since green tea extract, apparently in contrast to some of the other extracts of plant material such as rosemary and white grape used by the meat industry, protects not only lipids against oxidation but also proteins against oxidative degradation.[1−5] Hypervalent meat pigments formed in muscles and meat by reaction of peroxides with myoglobin have now been shown to initiate protein polymerization, resulting in loss of meat tenderness, making studies of the interaction of green tea components and myoglobins highly relevant.[6] The mechanism behind protection by green tea extract of meat proteins against oxidative polymerization seems related to a deactivation of hypervalent iron pigments by green tea catechins alone or in combination with other reducing constituents of green tea. Radicals of meat proteins such as myosin may, however, also be scavenged directly by constituents of green tea as an alternative reaction path protecting meat against protein polymerization through disulfide and dityrosine cross-linking and loss of meat tenderness.[7,8]

The mechanism by which myoglobins are involved in lipid peroxidation and oxidative protein polymerization in living muscles or in meat products has long been associated with the hypervalent myoglobin species perferrylmyoglobin, $^{\bullet}$MbFe(IV)=O, and ferrylmyoglobin, MbFe(IV)=O, which both are powerful pro-oxidants resulting from reaction of native myoglobins with peroxides.[9−11] Hypervalent heme pigments have been shown to oxidize a number of compounds present in living cells or in meat and meat products during storage, causing cellular damage stemming from lipid and protein oxidation.[12−15] The reaction between metmyoglobin, MbFe(III), and hydrogen peroxide is known initially to form a protein radical, perferrylmyoglobin, prior to formation of MbFe(IV)=O by a rapid decay of the protein radical.[16,17] A subsequent reduction of MbFe(IV)=O to yield MbFe(III) without any external reducing agent, a so-called "autoreduction" modifying the myoglobin protein oxidatively, is very slow, and MbFe(IV)=O will rather react with proteins[15,16] in competition with reducing cellular components such as glutathione[18] or antioxidants such as ascorbate,[19,20] vanillin,[21] and flavonoids[22,23] when present, although the detailed mechanism by which the hypervalent myoglobin is deactivated or is initiating protein oxidation in parallel still seems poorly understood.

Plant polyphenols, as naturally occurring antioxidants, are an important group of compounds increasingly being examined for potential reductive effects on MbFe(IV)=O in relation to meat digestion and human health.[20−23] Tea, as the most consumed beverage in the world next to water, has attracted much attention in recent years due to claims of health benefits depending on antioxidant, antimicrobial, anticarcinogenic, anti-inflammatory, and antiarteriosclerotic properties.[24−26]

Green tea extract has further been demonstrated to have potential applications as an all-natural ingredient for various foods such as bread,[27] vegetable oil,[28] meat, sausages, and fish.[29−31] The bioactivities seem to be associated with the catechins as the principal polyphenols of green tea. Green tea contains the four primary catechins (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG); see Figure 1.[26] EGCG, quantitatively the most important and most well-studied among the four green tea catechins, has been reported to have a very high efficiency in deactivation of MbFe(IV)=O at physiological pH or at the lower pH of gastric fluids.[32] However, no further comparison of structural effects of the four primary catechins on deactivation of MbFe(IV)=O or of any possible interaction among the catechins as reductants in green tea extract was reported, although it was concluded that green tea extract was more efficient as reductant than the individual

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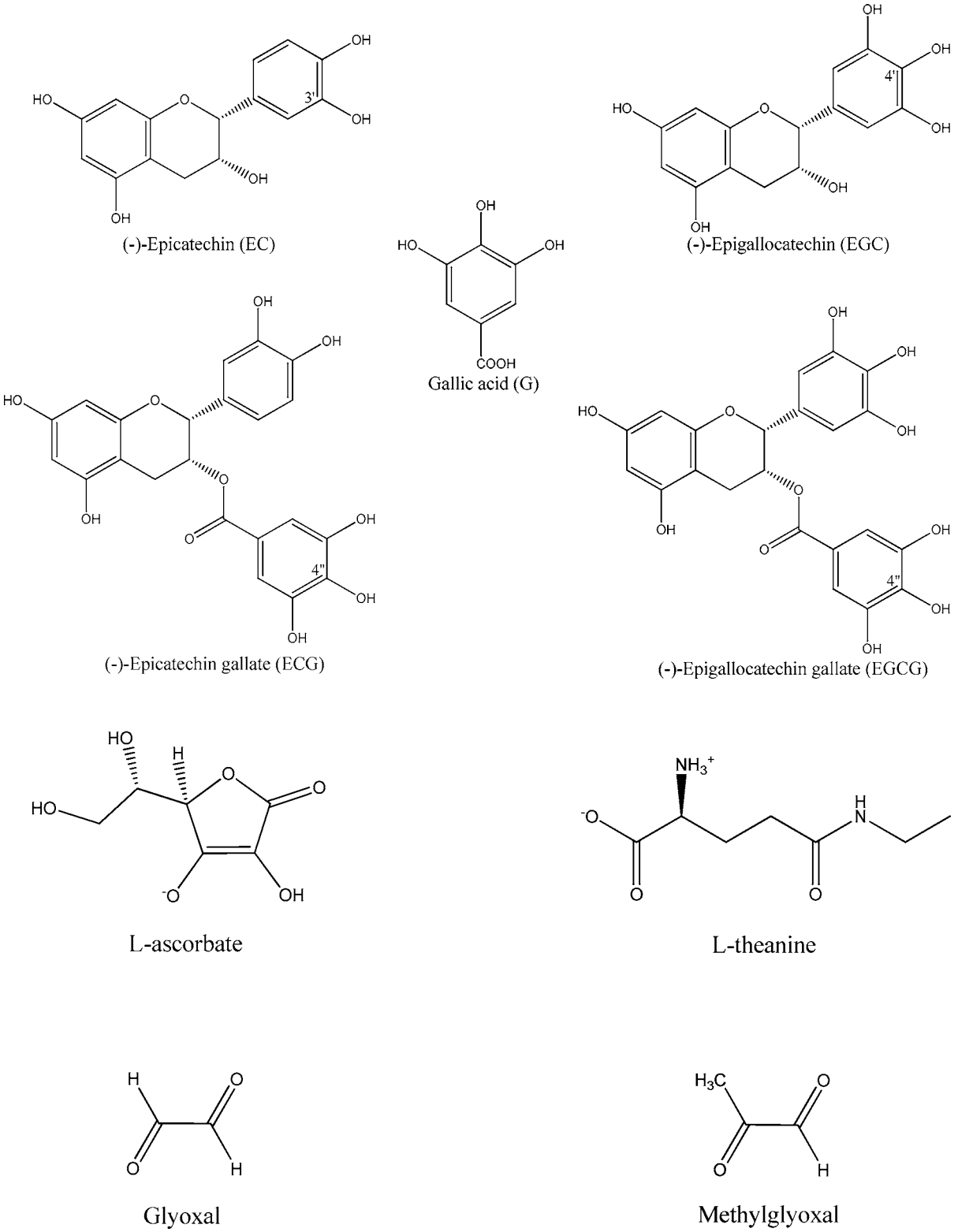

Figure 1. Chemical structures of green tea catechins, gallic acid, theanine, ascorbic acid, glyoxal, and methylglyoxal investigated as reductants in the present study. The most acidic phenolic group is labeled; see Table 2.

catechin.[32] Notably, green tea extract shows nonadditive antioxidative effects with other antioxidants such as α-tocopherol.[33]

The present study, based on stopped-flow spectroscopy for fast kinetics, was accordingly initiated with a focus on a mechanistic description of reduction of ferrylmyoglobin by each of the four green tea catechins and gallate alone and in combinations. Theanine, another bioactive constituent characteristic for green tea present in high amount and, somewhat surprisingly, also a biological antioxidant,[34] was included in the study together with a number of other reductants, either present in food or formed during heating and storage of food;

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Table 1. Observed Pseudo-First-Order Rate Constants for Reduction of MbFe(IV)=O by Tea Catechins, Ascorbate, Theanine, Glyoxal, and Methylglyoxal Alone and in Combinations with Tea Catechins (A) and Combinations of Epicatechin or Epigallocatechin Gallate with Ascorbate, Theanine, Glyoxal, and Methylglyoxal (B) in Aqueous Phosphate Buffer (25.0 °C, pH 7.5, I = 0.16)[a]

(A) k_{obs} (s^{-1})					
	+ EC	+ EGC	+ ECG	+ EGCG	+ G
EC	0.119 ± 0.009	0.125 ± 0.003	0.547 ± 0.016	0.628 ± 0.010	0.162 ± 0.007
EGC		0.081 ± 0.003	0.592 ± 0.002	0.707 ± 0.008	0.112 ± 0.003
ECG			0.565 ± 0.003	1.044 ± 0.062	
EGCG				0.584 ± 0.014	
G					0.133 ± 0.005

(B) k_{obs} (s^{-1})						
	+ EC	+ EGCG	+ ascorbate	+ T	+ GO	+ MGO
EC	0.108 ± 0.002		0.074 ± 0.002	0.124 ± 0.011	0.108 ± 0.003	0.108 ± 0.001
EGCG		0.577 ± 0.001	0.564 ± 0.014	0.583 ± 0.014	0.592 ± 0.010	0.554 ± 0.006
ascorbate			0.045 ± 0.001			
T				0.080 ± 0.005		
GO					0.077 ± 0.004	
MGO						0.046 ± 0.001

[a]The final concentration of each reductant alone and of each of the reductants in the combinations was in all cases 400 μM. (A) and (B) are two separate experimental series. EC: epicatechin, EGC: epigallocatechin, ECG: epicatechin gallate, EGCG: epigallocatechin gallate, G: gallic acid, T: theanine, GO: glyoxal, MGO: methylglyoxal.

see Figure 1. The primary goal was to obtain an understanding of the mechanism behind the obvious efficiency of green tea extract as a protector of proteins under conditions relevant for meat. However, such a mechanistic understanding clearly also is of relevance for a better understanding of the role of hypervalent iron in oxidative stress in relation to the many health claims for green tea as a beverage.[24,26]

MATERIALS AND METHODS

Chemicals. Horse heart myoglobin (type III), referred to as metmyoglobin (MbFe(III)), catalase (from bovine liver; 1680 units/mg solid), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG), gallic acid (G), methylglyoxal solution (40 wt % in H_2O), and glyoxal solution (40 wt % in H_2O) were from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid and iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) were from Merck (Darmstadt, Germany). Green tea extract (GTE) was from DuPont Nutrition and Biosciences ApS (formerly Danisco A/S, Brabrand, Denmark), containing 20% total catechins (natural green tea extract) and 80% salt, the same as used in recent studies with meat proteins and food emulsions.[2,33] L-Theanine was from Angene (Kowloon, Hong Kong). Aqueous solutions (ionic strength = 0.16) at different pH values were prepared from the corresponding analytical grade chemicals using Milli-Q water, which was purified through a Milli-Q purification train (Millipore, Bedford, MA, USA).

pH Measurement. pH was measured relative to concentration standards (0.0100 and 0.00100 M HCl, ionic strength 0.16 adjusted with NaCl), employing the definition pH = $-\log[H^+]$. pH was measured with a Metrohm 6.0234.100 combination glass electrode (Hamilton Bonaduz AG, Bonaduz, Switzerland) connected to a Metrohm 713 pH-meter (Metrohm, Herisau, Switzerland).

Synthesis of Ferrylmyoglobin. MbFe(III) dissolved in 30 mM phosphate buffer (ionic strength 0.16 adjusted with NaCl) was purified on a PD-10 Shephadex G-25 column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The eluted MbFe(III) was diluted with the phosphate buffer, and the concentration was determined based on ε_{525} = 7700 $M^{-1} \cdot cm^{-1}$ to yield the concentration in question. MbFe(IV)=O was formed by reaction of MbFe(III) with 2 times molar excess of H_2O_2 (concentration determined spectrophotometrically using ε_{240} = 39.4 $L \cdot mol^{-1} \cdot cm^{-1}$) for 3 min, and the identity of the MbFe(IV)=O was confirmed by absorption spectroscopy.[19]

Identification of the Reaction Product. The product from reactions between MbFe(IV)=O and tea catechins or other reducing agents was identified by recording absorption spectra in the region 450−700 nm using an HP8453 UV−vis diode array spectrophotometer (Hewlett-Packard Co., Palo Alto, CA, USA). The reaction of ferrylmyoglobin was followed after addition of green tea catechins or other reducing components for 4 min at room temperature before recording the absorption spectra. In accordance with the method described previously,[19] the reaction product was identified as MbFe(III) from the product spectrum.

Kinetic Experiments. The MbFe(IV)=O solution and an EC, EGCG, or G solution were placed in each syringe of a DX17MV stopped-flow spectrophotometer (Applied Photophysics, London, UK), and the reaction was followed by absorbance measurements at 580 nm. Each experiment was made at least in duplicate, and calculations of rate constants were done by nonlinear regression methods using Applied Photophysics software. For green tea extract the calculation of the second-order rate constant was based on the phenol content.[33] Solutions of various concentrations of EC, EGCG, or G were freshly prepared at pH 5.5, 6.0, 6.5, 7.0, and 7.5 (ionic strength 0.16 was adjusted with NaCl). The ferrylmyoglobin solution was prepared as described above to give a final concentration of 20 μM in the reaction mixture. The molar concentration ratio between MbFe(IV)=O and EC, EGCG, or G was 1:10, 1:20, 1:40, 1:60, and 1:80, corresponding to a final concentration of EC, EGCG, or G of 0.2, 0.4, 0.8, 1.2, and 1.6 mM. The reaction temperature for all measurements was set at 25 °C by a water bath.

Combinations of the four tea catechins and the mixtures of EC and EGCG with other reducing components (combination scheme shown in Table 1) to deactivate MbFe(IV)=O were investigated at pH 7.5, 25 °C. All of the individual compounds in the combinations and the individual compound alone were tested at the same final concentration, 400 μM, while the final concentration of MbFe(IV)=O was 20 times less than that, i.e., 20 μM in all experiments.

RESULTS AND DISCUSSION

The hypervalent iron pigment ferrylmyoglobin, MbFe(IV)=O, was found to be efficiently reduced by each of the four green tea catechins EC, EGC, ECG, and EGCG and by gallate (G) in aqueous solution, as seen from Figure 2, showing absorption spectra of MbFe(IV)=O during reaction with ECGC at pH = 7.5, ionic strength 0.16, at 25.0 °C. The reduction of

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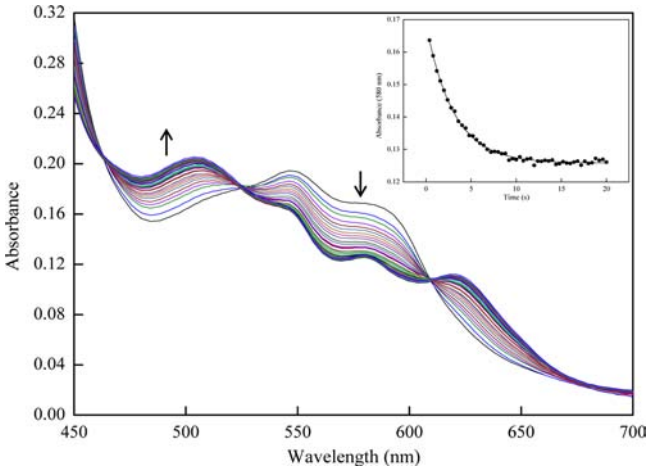

Figure 2. Absorption spectra in the visible region of an aqueous 20 μM MbFe(IV)=O and 0.40 mM EGCG solution in aqueous phosphate buffer (25.0 °C, pH 7.5, I = 0.16). The arrows indicate increasing or decreasing absorption during the reaction, for which spectra were recorded with the same time interval. Inset: Absorption recorded at 580 nm for a total of 20 s elapsed from initiation of the reaction to recording of the final spectrum with exponential fit for calculations of pseudo-first-order rate constant.

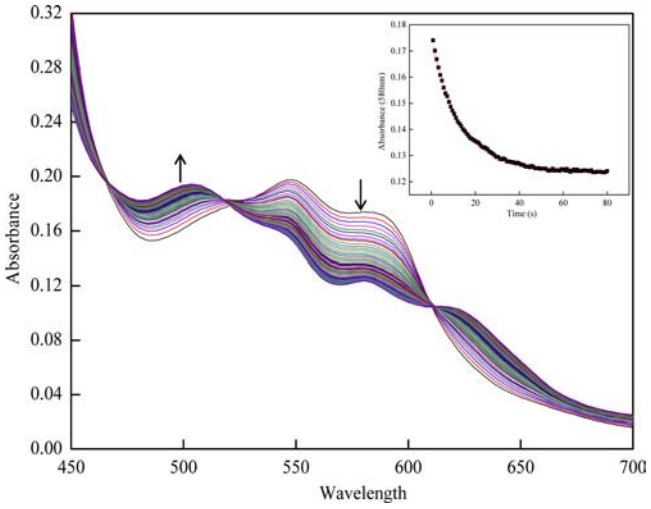

Figure 3. Absorption spectra in the visible region of an aqueous 20 μM MbFe(IV)=O and 0.40 mM theanine solution in aqueous phosphate buffer (25.0 °C, pH 7.5, I = 0.16). The arrows indicate increasing or decreasing absorption during the reaction, for which spectra were recorded with the same time interval. Inset: Absorption recorded at 580 nm for a total of 80 s elapsed from initiation of the reaction to recording of the final spectrum with exponential fit for calculations of pseudo-first-order rate constant.

MbFe(IV)=O to metmyoglobin, MbFe(III), as identified by the absorption spectrum after 20 s of reaction and confirming previous results,[32] was conveniently followed by stopped-flow spectroscopy. For all green tea constituents and the other reductants studied, the reduction to MbFe(III) was found to follow first-order kinetics in excess of the reductant, as seen in Figures 2 and 3 for EGCG and theanine, respectively. The pseudo-first-order rate constants calculated by nonlinear regression analysis, k_{obs}, was for EC, EGCG, and G found to depend linearly on the concentration of the reductant, as seen for EGCG in Figure 4:

$$k_{obs} = k_{auto} + k_2[\text{tea catechin}] \quad (1)$$

where k_{auto} is the first-order rate constant for "autoreduction" of ferrylmyoglobin and k_2 is the second-order rate constant for reduction by a tea catechin or another reductant. For EC, EGCG, and G, the pH-dependence of k_2 was investigated for neutral to moderately acidic conditions; see Figure 5. The rate of reduction increases with decreasing pH, corresponding to parallel reduction of MbFe(IV)=O and of protonated ferrylmyoglobin, MbFe(IV)=O, H^+, as has been already found for reduction by NADH[35] and ascorbate.[20] It is now generally accepted that protonation of ferryl heme controls the peroxidatic reactivity of globins, and a pK_a value around 4.7–4.9 has been assigned to the equilibrium[35,36]

$$\text{MbFe(IV)=O, } H^+ \rightleftharpoons \text{MbFe(IV)=O} + H^+ \quad (2)$$

The autoreduction of MbFe(IV)=O has $k_{auto} = 2 \times 10^{-4}$ s^{-1} in neutral solution at 25.0 °C, and the k_2 term is accordingly dominating for the conditions used to compare the individual catechins with other reductants.[35] The pH profile for the rate of reduction of EGCG, EC, and G confirms previous results for ascorbate, chlorogenate, and EGCG as reductants[20,32] and is related to the equilibrium of eq 2 and not to any protolytic active groups of the green tea catechins, since they all are fully protonated at pH < 7.0. Under these acidic conditions another mechanism for MbFe(IV)=O reduction including specific acid catalysis will dominate. The present study was focused on

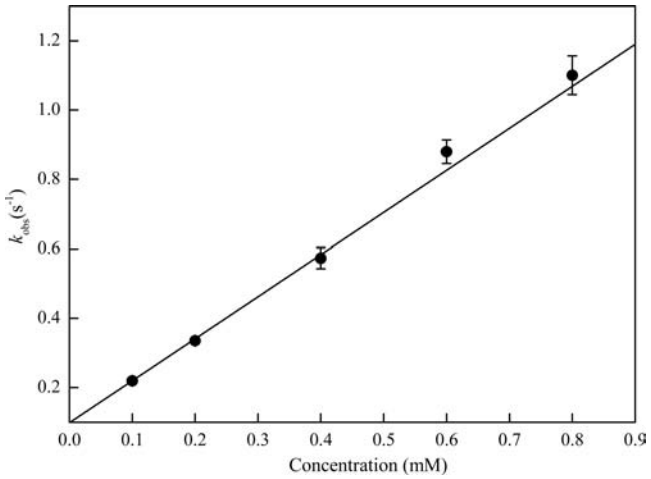

Figure 4. Observed pseudo-first-order rate constant for the reduction of MbFe(IV)=O in aqueous phosphate buffer (25.0 °C, pH 7.5, I = 0.16) for excess concentrations of EGCG.

conditions of neutral pH, and the other reductants and their combinations with tea catechins were studied under these conditions.

The comparison between the individual green tea catechins is based on the observed first-order rate constants presented in Table 1. As seen from the diagonal of Table 1A and Table 1B (two separate experimental series), the efficiency by which the green tea catechins reduce MbFe(IV)=O is decreased along EGCG > ECG > EC > EGC. G is more efficient than EGC and EC, but less than ECG and EGCG. In Table 2, the observed rate constants are converted to second-order rate constants, since k_{auto} in eq 2 may be neglected, and compared to the standard reduction potential of the one-electron-oxidized catechin and to the dissociation energy for the weakest phenolic oxygen–hydrogen bond.[37−46] Included in this comparison is also the second-order rate constants for

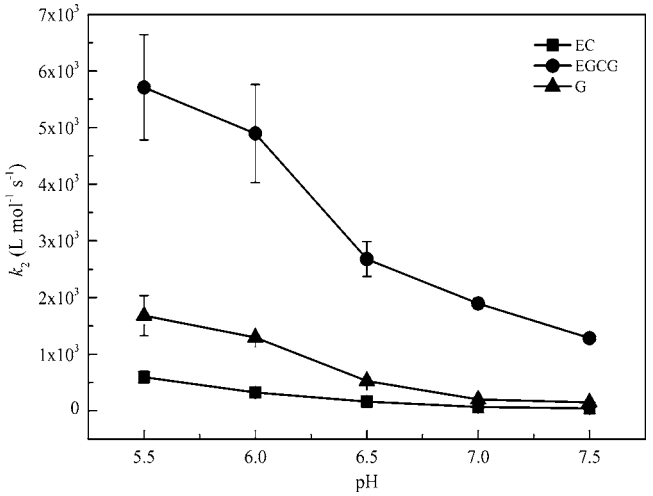

Figure 5. Second-order rate constant obtained for the deactivation of MbFe(IV)=O by an excess of green tea catechins EC or EGCG or gallic acid (G) in aqueous solutions at 25.0 °C, I = 0.16, of varying pH.

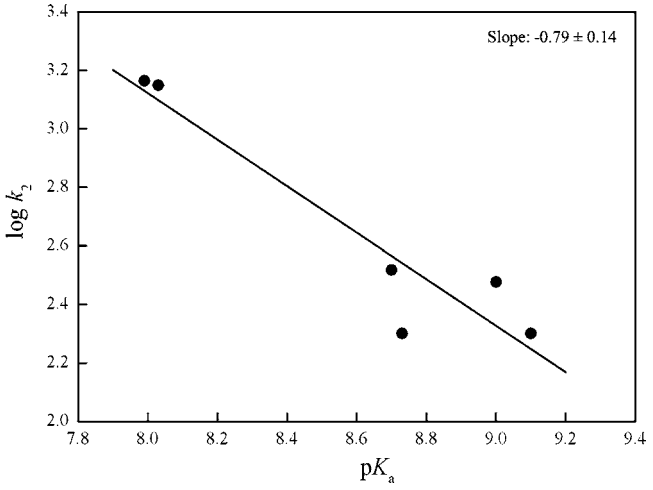

Figure 6. Brønsted–Pedersen correlation of the second-order rate constant (k_2 in L mol^{-1} s^{-1}) for acid-catalyzed reduction of ferrylmyoglobin by catechins, gallate, and theanine as main components of green tea extract. Data from Table 2.

ascorbate and theanine (Figure 3) and for glyoxal and methylglyoxal from Table 1 and a literature value for NADH.[35] Clearly, the k_2 shows no simple correlation with $E^{0\prime}$ or BDE but rather with pK_a for the most acidic phenolic group or for ascorbate the enol group corresponding to pK_{a2} for ascorbic acid.[40−42] For theanine, the pK_{a2} for glutamate is adapted and included in the comparison. For the main constituents of green tea extract, the correlation is shown in Figure 6 as a Brønsted–Pedersen plot, and the linearity with a slope around 0.8 is indicative of a common mechanism for these six reductants involving general acid catalysis.[49] The reduction of MbFe(IV)=O is accordingly induced by a proton transfer from the weakly acidic reductant prior to reduction. This initial proton transfer triggering reduction is in agreement with the faster reduction of the protonated MbFe(IV)=O,H$^+$ as compared to MbFe(IV)=O, and also for specific acid catalysis as known for the autoreduction of ferrylmyoglobin.[35,36] Theanine is not a reductant but still a biological antioxidant,[34] and the electron transfer may for the reduction by theanine involve the protein moiety of MbFe(IV)=O as electron donor to the iron center, while the phenolic compounds may donate an electron directly from the phenolate ion formed by proton transfer.

An initial proton transfer involves a specific reaction site on MbFe(IV)=O, which may be identified as the iron center with the oxygen as primary hydrogen ion acceptor.[35,36] The electron transfer may accordingly be classified as an inner-sphere mechanism, since the electron transfer depends on contact between specific sites on the oxidant and reductant. Competition between reductants for MbFe(IV)=O may provide further information related to this site on MbFe(IV)=O, as is evident for the observed rate constants of the six combinations of EC, EGC, ECG, and EGCG in Table 1A and Figure 7. For most of the combinations additivity or moderate

Table 2. Second-Order Rate Constant (k_2) for Reduction of Ferrylmyoglobin by Green Tea Catechins, Theanine, and Other Reductants at pH = 7.5, Ionic Strength = 0.16, and 25 °C Together with Standard Reduction Potential ($E^{0\prime}$) Corresponding to One-Electron Oxidation in Neutral Aqueous Solution, pK_a Value for the Most Acidic Phenolic Group, Bond Dissociation Energy (BDE) for Weakest Phenolic Oxygen Hydrogen Bond, and Antioxidative Capacity Measured As Trolox Equivalent (TEAC)

	k_2 (L mol^{-1} s^{-1})	$E^{0\prime}$ (V)	pK_a	BDE (kJ mol^{-1})	TEAC
epicatechin (EC)	300[a]	0.57[c]	9.00 (3′-OH)[f]	322 (4′-OH)[j]	2.5[o]
epigallocatcchin (EGC)	200[a]	0.43[c]	8.73 (4′-OH)[f]	297 (4′-OH)[j]	3.8[o]
epicatechingallate (ECG)	1410[a]	0.55[c]	8.03 (4″-OH)[f]	316 (4″-OH)[j]	4.9[o]
epigallocatechin gallage (EGCG)	1460[a]	0.43[c]	7.99 (4″-OH)[f]	309 (4′-OH)[j]	4.8[o]
gallate (G)	330[a]	0.44[d]	8.7 (−OH)[g]	344[k]	3.0[o]
ascorbate	110[a]	0.28[c]	11.6[h]	300[l]	1.0[p]
theanine (T)	200[a]		9.1[I]		
glyoxal (GO)	190[a]			393[m]	
methylglyoxal (MGO)	115[a]			411[m]	
NADH	1060[b]	0.93[e]		382[n]	
green tea extract (GTE)	1340[a]				

[a]Present work, and the value for green tea extract is based on phenol content determined by GAE (gallic acid equivalent). [b]From ref 35. [c]From ref 37. [d]From ref 38, pH = 6.15. [e]From ref 39. [f]From ref 40. Lowest pK_a values. [g]From ref 41, pK_{a2} of gallic acid. [h]From ref 42, pK_{a2} of ascorbic acid. [I]Value for glutamate, from ref 43, pK_{a2}. [j]Based on DFT calculations, from ref 40. [k]Based on DFT calculations, from ref 44. BDE for phenolic group in gallic acid. [l]Based on DFT calculations, from ref 45. [m]Based on DFT calculations, from ref 46. [n]Based on DFT calculations, from ref 39. [o]Trolox equivalent antioxidant capacity, from ref 47. [p]Trolox equivalent antioxidant capacity (TEAC), from ref 48.

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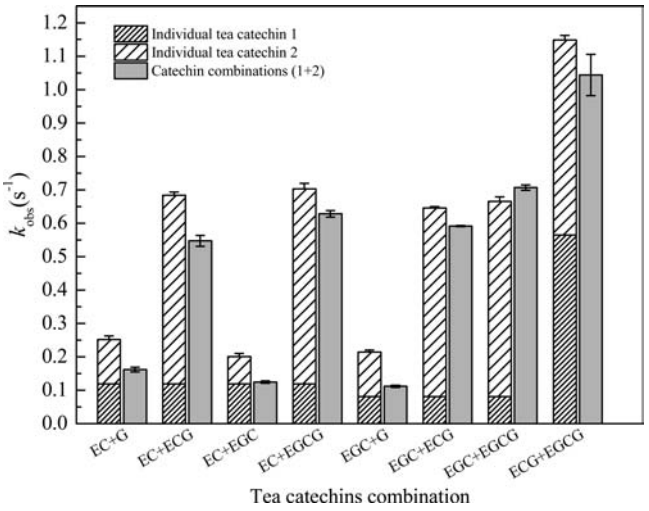

Figure 7. Observed pseudo-first-order rate constant for reduction of MbFe(IV)═O by tea catechin alone and tea catechin combinations in aqueous phosphate buffer (25.0 °C, pH 7.5, I = 0.16). The total concentration of each tea catechin alone and the total concentration of each tea catechin in the combinations was 400 μM: tea catechin 1 and tea catechin 2 were investigated individually followed by their combinations (e.g., 1 is EC and 2 is G in EC+G).

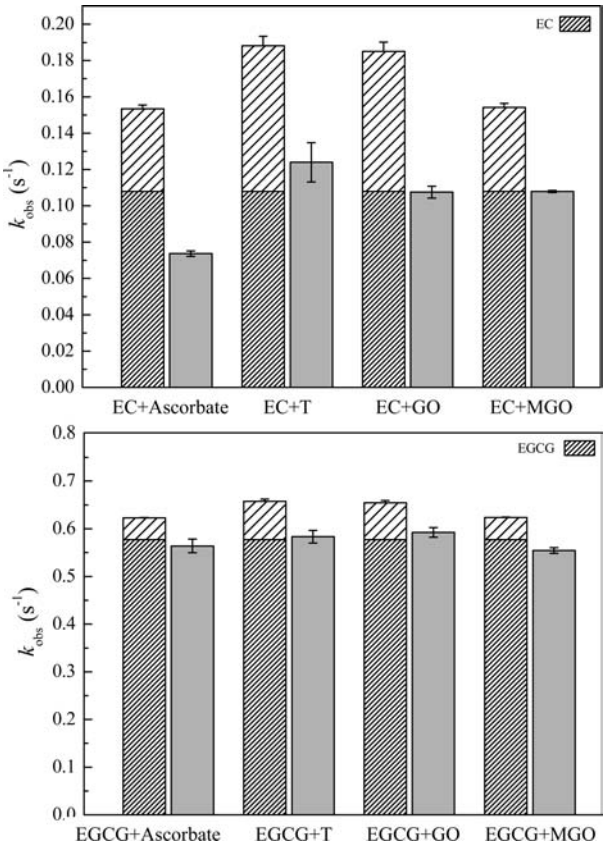

Figure 8. Observed pseudo-first-order rate constant for reduction of MbFe(IV)═O by EC, EGCG, ascorbate, theanine (T), glyoxal (GO), or methylglyoxal (MGO) alone and in combinations of tea catechins with other compounds in aqueous phosphate buffer (25.0 °C, pH 7.5, I = 0.16). The final concentration of each tested compound alone and of each individual compound in each of the combinations was 400 μM. (A) Combinations of EC and other reductants, (B) combinations of EGCG and other reductants. Other reductants: striped bars. Combinations: gray bars.

antagonism of rates is seen, indicating that the catechin binds with comparable affinity prior to proton and electron transfer. However, for the combinations EC + ECG and EC + EGC, a significant antagonism is evident. This leads to the conclusion that while reduction is faster for the catechins with the more acidic gallate esters, the smaller EC, being a less efficient reductant, may block the access for the efficient reductants EGCG and ECG, an effect not seen for the larger EGC. G is comparable in efficiency to EC, but addition of an equimolar concentration of G to EC or to EGC increases the rate less than additive, indicating that a proton transfer from the less acidic G is not as efficient as a transfer from the more acidic ECG and EGCG to initiate electron transfer (Figure 7). This seems to suggest a certain degree of concerted action for proton and electron transfer. Ascorbate as an important biological reductant is less acidic than the green tea catechins and is less efficient, in agreement with the suggested mechanism with general acid catalysis. Glyoxal and methylglyoxal, reductants both formed during the Maillard reaction in food, likewise are less efficient; see Table 1B, Table 2, and Figure 8. These reductants seem not to interact with EC and EGCG during reduction of MbFe(IV)═O. For the very efficient EGCG, neither glyoxal, methylglyoxal, ascorbate, or theanine affects the reduction, indicating that EGCG is efficient in proton transfer to the reactive site on MbFe(IV)═O, not leaving access for the other less efficient reductants. The k_2 value for green tea extract based on the phenolic content as included in Table 2 is comparable to the value for EGCG and ECG.

NADH reduces MbFe(IV)═O with a rate comparable to ECG and EGCG even though NADH is not an acid or a good electron donor.[35,50] Notably, NADH reduces MbFe(IV)═O to deoxymyoglobin, MbFe(II), or its oxygenated form, as another product besides MbFe(III), indicating competitive hydride ion transfer and electron transfer. The iron(III)/iron(II) ratio depends on pH for reduction by NADH, and clearly an alternative mechanism is operating for NADH as compared to the catechins.

EGCG is the most efficient in deactivating MbFe(IV)═O. It is of interest to note that the antioxidant capacity of the catechins shows a similar pattern, with EGCG and ECG having larger TEAC values corresponding to the higher number of phenolic groups in EGCG and in ECG as compared to EC and EGC and to gallate and ascorbate (see Table 2).[47,48] Other factors such as the increased hydrophobicity of the two gallic acid esters EGCG and ECG may also contribute to the increased activity due to better protein binding.[51] The tea catechin is a unique series of plant phenols for which a low standard reduction potential of the one-electron-oxidized catechin depends on the presence of a B-ring with vicinal triphenolics. However, the efficiency as a reductant for hypervalent iron from a kinetic point of view rather depends on the presence of a gallate ester of higher acidity, making it capable of efficient proton transfer prior to electron transfer, which indicates an important coupling between phenol acidity and antioxidant capacity of this series of plant phenols.[52] EGCG seems to be the most efficient reductant for MbFe(IV)═O to fully account for the activity of green tea extract.

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Notes

The authors declare no competing financial interest.

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