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Reaction pH and protein affect the oxidation products of β-pentagalloyl glucose

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Abstract
To better understand the biochemical consequences when polymeric polyphenols serve as biological antioxidants, we studied how reaction pH (pH 2.1–7.4) and protein affected the oxidation of pentagalloyl glucose (PGG) by $NaIO_4$ in aqueous solution. PGG oxidation produced an *o*-semiquinone radical intermediate, which tended to form polymeric products at pH values below 5, and *o*-quinones at higher pH. The model protein bovine serum albumin promoted the formation of quinone even at low pH. Two other polyphenols, procyanidin (epicatechin$_{16}$-(4 → 8)-catechin) and epigallocatechin gallate, had similar pH-dependent oxidation patterns.

Keywords: *Polyphenolic antioxidants, tannin, tannin oxidation, oxidized polyphenol–protein interactions, polyphenol oxidation*

Abbreviations: *ROS, reactive oxygen species; BSA, bovine serum albumin; PGG, β-pentagalloyl glucose; PC, Sorghumprocyanidin (epicatechin$_{16}$-(4(→ 8)-catechin); EGCG, epigallocatechin gallate; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; EPR, electron paramagnetic resonance*

Introduction

Tannins are polymeric polyphenols that interact with protein [1,2]. They are widespread in plant-derived foods, beverages and medicines [3]. A typical Western diet includes about 1 g of polyphenols per day, comprising about 50% tannins such as the galloyl ester-derived hydrolyzable tannins and the flavan-3-ol-based condensed tannins (Figure 1). Epigallocatechin gallate (EGCG), the main polyphenol in green tea, is the smallest tannin[4] (Figure 1).

Reactive oxygen species (ROS) are involved in human diseases and aging [5,6]. The digestive tract is constantly exposed to ROS derived from oxidized dietary lipids, transition metals and pathogens [7–11]. Both nutritional (e.g. ascorbic acid, α-tocopherol) and non-nutritional antioxidants (e.g. polyphenols) may augment the endogenous antioxidants such as glutathione [12–15].

Tannins are potent ROS scavengers, suggesting they may be beneficial dietary antioxidants [16,17]. It has been difficult to attribute effective antioxidant activity to dietary tannins, in part because their fate after ingestion is undefined [18,19]. Polyphenols may be oxidized by ROS to form more reactive species, or may be oxidized to harmless products. It is clear that factors including solvent composition, pH, protein, and metal ions affect the ROS-scavenging activity of polyphenols [20–26]. However, factors that control the oxidation products of polyphenols are unknown. We hypothesized that it was particularly important to explore how protein and pH, two prominent factors in the digestive tract, affect oxidation pathways of dietary constituents such as tannin. In the digestive tract, dietary and endogenous proteins constantly interact with dietary tannins. There are significant changes in pH along the digestive tract, with the pH in the human mouth, stomach and gastrointestinal (GI) tract estimated to be 6, 2 and 7.4, respectively [27,28].

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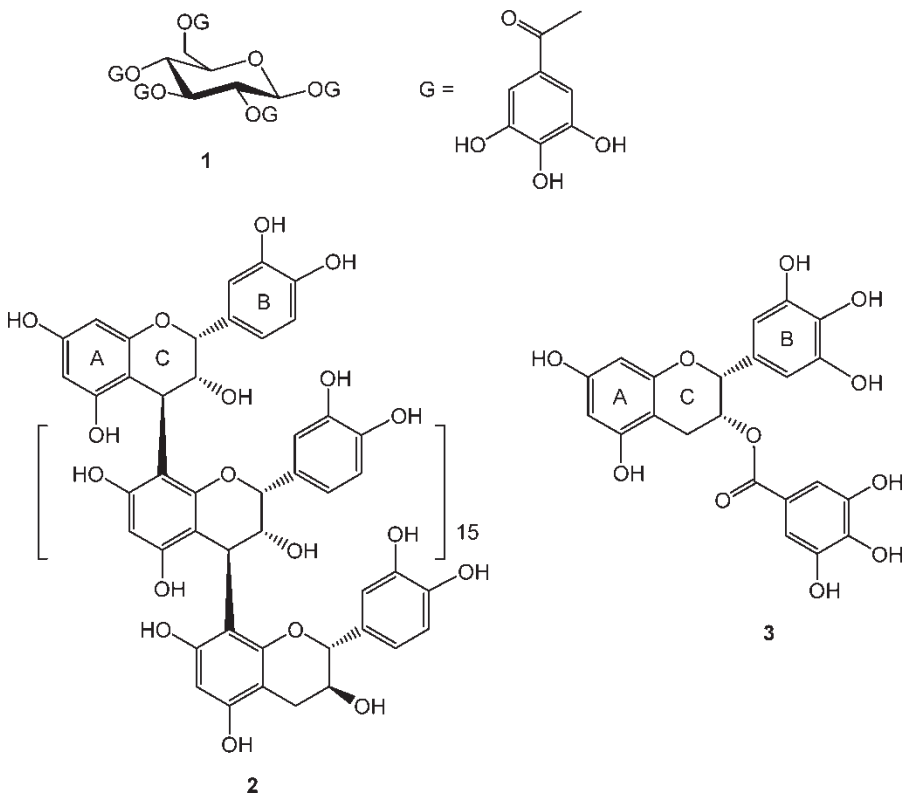


Figure 1. β-Pentagalloyl glucose (**1**) is a typical hydrolyzable tannin; *Sorghum* procyanidin (epicatechin$_{16}$-(4 → 8)-catechin) (**2**) is a representative condensed tannin; ECGC (**3**) is a flavan-3-ol gallate ester.

We used β-pentagalloyl glucose (PGG), bovine serum albumin (BSA) and $NaIO_4$ as the model tannin, protein and oxidant, and characterized how biological pH and protein affected PGG oxidation through *in vitro* chemical analysis. Furthermore, we examined the interactions between PGG oxidation products and protein.

Materials and methods

Fraction V fatty acid free BSA, trichloroacetic acid (TCA) solution (100%, w/v), β-mercaptoethanol, and electrophoresis grade sodium dodecyl sulfate (SDS) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). PGG was purified from tannic acid (The Coleman & Bell Co., Norwood, OH) [2]. [^{14}C]PGG with a specific radioactivity of 2 mCi/mmol was synthesized [29]. [^{125}I]BSA was prepared using chloramine T [30]. *Sorghum* procyanidin (PC, epicatechin$_{16}$-(4 → 8)-catechin) was purified from *Sorghum bicolor* grain [24,31]. EGCG was a gift from Doug Balentine (Lipton Tea, NJ). All other chemicals were reagent grade or the best available. Solutions were prepared in buffers (5 mM containing 4.25 mM NaCl, comprised of phosphate, pH 2.1, 6.0, 7.4; phosphate/citrate, pH 3.0; phosphate/citrate/acetate, pH 4.0; acetate, pH 4.9) prepared in Barnstead nanopure water (Dubuque, IA). PGG is quite water insoluble but can be dissolved by incubating the sample at 100°C for 1 min. The concentrations of PGG ($\varepsilon^{280} = 5.4 \times 10^4\ M^{-1}\ cm^{-1}$) or BSA ($\varepsilon^{280} = 4.37 \times 10^4\ M^{-1}\ cm^{-1}$) solutions were spectrophotometrically determined. Centrifugation was performed at 11,000*g* at 4°C for 10 min. To radiochemically measure PGG, samples were mixed with 5 ml of ScintiVerse BD (Fisher Chemicals, Fair Lawn, NJ) and counted on a Wallac 1409 liquid scintillation analyzer (Turku, Finland). Radiochemical measurements of BSA were performed on a gamma counter (Cobra-Auto-Gamma, Packard Instrument Company, Downers Grove, IL). UV–vis experiments were performed with an Agilent 8453 UV–visible spectrophotometer (Waldbronn, Germany). Electron paramagnetic resonance (EPR) experiments were performed with a Bruker EMX EPR spectrometer equipped with an AquaX sample cell (Bruker Instruments, Inc., Billerica, MA).

PGG oxidation products

To examine the effects of pH on the formation of oxidation products, PGG (10.6 nmol, 200 μl) was oxidized with $NaIO_4$ (159 nmol, 75 μl) at various pH values. Chromophore production was measured at 520 nm. To quantitate precipitable oxidation products, [^{14}C]PGG was reacted with $NaIO_4$ at room temperature for 2 min, the mixture was vortexed with SDS (1%, w/v, 100 μl) and was centrifuged. The amount of PGG in the precipitate was indirectly measured by counting 150 μl of the supernatant. To examine the effects of protein on PGG oxidation,

the mixture of PGG (10.6 nmol, 100 μl) and BSA (10.6 nmol, 100 μl) was incubated at room temperature for 15 min before treating with $NaIO_4$ and analyzing as described above.

Radical intermediates were detected using conditions described above, and transferring the samples to the EPR cell immediately after mixing. Typical instrument settings were center field 3490 G, sweep width 25 G, frequency 9.80 GHz, power 25 mW, receiver gain 1×10^6, modulation amplitude 0.4 G, conversion time 82 ms, sweep time 84 s, three scans. To assess Zn^{2+}-stabilized radicals, pH 2.5 acetate buffers were prepared with sodium and zinc acetate to achieve 2 M acetate and zinc concentrations ranging from 0 to 0.5 M. Spectra were taken as described above, but with modulation amplitude (1.2 G), conversion time (41 ms), sweep time (42 s), thirty scans. The amounts of radical intermediate at different Zn^{2+} concentrations were compared using the double integrations of the EPR signals. In the same zinc acetate buffers, [^{14}C]PGG (10.6 nmol, 100 μl) was reacted for 60 s with $NaIO_4$ (159 nmol, 75 μl) in the filtration chamber of a Spin-X tube (0.45 μm nylon membrane) (Corning Inc., Corning, NY) before centrifuging and determining precipitable PGG product by counting 100 μl of the filtrate.

BSA-precipitating capacity

To evaluate the ability of PGG oxidation products to precipitate protein, PGG (10.6 nmol, 100 μl) was reacted with $NaIO_4$ (159 nmol, 75 μl) for 2 min at various pH values. This fifteen-fold molar excess of periodate ensured that the reaction went to completion [32]. The reactions were stopped by the addition of 2 μl of β-mercaptoethanol and the mixtures were treated with [^{125}I]BSA (10.6 nmol, 100 μl) in 0.2 M acetate buffer (pH 4.9), incubated at room temperature for 15 min, and centrifuged. The supernatants were removed by aspiration and the precipitates were counted.

Quantitation of SDS-resistant PGG-BSA complexes

To determine the SDS-dissociability of the complexes of oxidized PGG and protein, PGG was oxidized at various pH values before reacting with BSA [32]. Briefly, [^{14}C]PGG (10.6 nmol, 100 μl) was mixed with $NaIO_4$ (159 nmol, 75 μl) and reacted with BSA (10.6 nmol, 100 μl) for 2 min. Successive additions of SDS (1%, w/v, 100 μl) and TCA (100%, w/v, 100 μl) were accompanied by vortexing and were followed by a 10-min incubation on ice, centrifugation and removal of 100 μl of the supernatant for counting. In complementary reactions, [^{14}C]PGG was incubated with BSA for 15 min before the addition of $NaIO_4$ or buffer and treating as described above.

Oxidation of PC or EGCG

PC (16.3 nmol, 100 μl) or EGCG (100 nmol, 100 μl) was reacted with $NaIO_4$ (1272 nmol, 75 μl or 600 nmol, 100 μl, respectively). Chromophore was detected at the λ_{max}, 390 nm for PC or 520 nm for EGCG. To quantitate the precipitable oxidation products, reagents were mixed in the filtration chambers of Spin-X tubes. After 2 min, the reaction solutions were removed by vacuum filtration, and the amounts of precipitable products were photodensitometrically quantitated using an Alpha Imager 2000, Version 3.3b Documentation and Analysis System (Alpha Innotech Corp., USA).

Results

Reaction pH affects PGG oxidation

We tested $NaIO_4$, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$), 2,2′-azobis(isobutyramidine) dihydrochloride (AAPH) and $S_2O_8^{2-}/SO_3^{2-}$ as oxidants. $NaIO_4$ was selected for additional experiments because it has no absorbance above 260 nm, allowing direct spectrophotometric studies of polyphenol oxidation [33,34]. When PGG was reacted with $NaIO_4$ at pH 7.4, a pink chromophore formed ($\lambda_{max} = 520$ nm) (Figure 2A). We attributed the chromophore to *o*-quinone formation, based on spectral similarity to the quinones produced by substituted catechols [35]. Reactivity of the product with nitroblue tetrazolium and sodium glycinate provided further evidence for quinone formation [36]. Oxidation of PGG at pH 2.1 produced precipitate that could be isolated by centrifugation or filtration, suggesting it was a polymer. The amount of chromophore that formed increased and the amount of polymer that formed decreased as reaction pH was increased from 2.1 to 7.4 (Figure 2B).

Radical intermediate in PGG oxidation

We speculated that PGG oxidation might involve radical intermediates that could be detected by EPR spectrometry. A radical intermediate was readily detected when PGG was oxidized in the pH range of 6.0–7.4, but none could be detected when PGG was oxidized at lower pH values (Figure 3). In our EPR experiments, it took about 30 s to transfer reagents into the EPR cell. The polymer formed immediately upon mixing PGG and $NaIO_4$ at pH 2.1 (data not shown), suggesting that the short lifetime of the intermediate formed at low pH might prevent its detection. At low pH Zn^{2+} can be used to trap radical intermediates [37–39]. When we oxidized PGG with $NaIO_4$ at pH 2.5, as Zn^{2+} concentrations were increased from 0 to 0.5 M, the intensities of EPR signals increased while

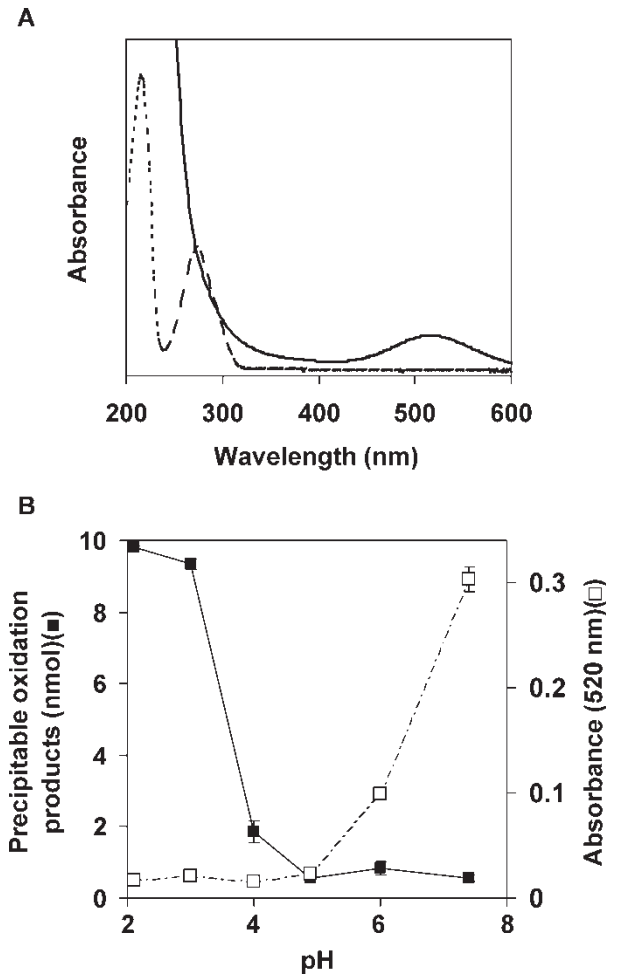


Figure 2. PGG oxidation products. (A) When PGG (10.6 nmol, 200 μl) was reacted with $NaIO_4$ (159 nmol, 75 μl) at pH 7.4, a chromophore (solid line) was formed. The spectrum of unoxidized PGG (dotted line) was taken at a 1 μM to show all its spectroscopic features. (B) [^{14}C]PGG (10.6 nmol, 200 μl) was reacted with $NaIO_4$ (159 nmol, 75 μl). After 2 min, the mixture was treated with SDS (1%, w/v, 100 μl), vortexed and centrifuged. The amount of PGG in the precipitate (■) was radiochemically determined. The amount of chromophore (□) was monitored at 520 nm. Values are the mean of three determinations; error bars indicate the standard deviations.

the amount of polymeric oxidation product decreased (Figure 4). When we mixed PGG and $NaIO_4$ in zinc acetate buffers, we readily detected the stabilized radical at all pH values between 2.1 and 4.9 (data not shown).

Protein affects PGG oxidation

To reveal how protein affected PGG oxidation, we monitored the oxidation of PGG (10.6 nmol, 100 μl) in the presence or absence of BSA (10.6 nmol, 100 μl). Protein promoted the formation of quinone (Figure 5A), but inhibited the formation of polymer (Figure 5B). As little as 1.33 nmol of BSA completely inhibited polymerization of 10.6 nmol of

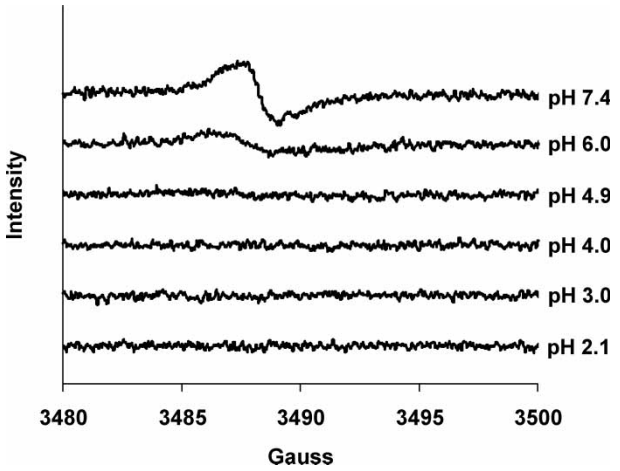


Figure 3. EPR detection of a radical intermediate in PGG oxidation. PGG (53 nmol, 1000 μl) was mixed with $NaIO_4$ (795 nmol, 375 μl). The mixture was transferred into EPR cell immediately for data collection. Three scans were taken for each spectrum.

PGG at pH 2.1. In the absence of BSA under the same conditions over 90% of the PGG was oxidized to polymer. These results suggested that protein was more important than pH in determining the nature of the PGG oxidation products.

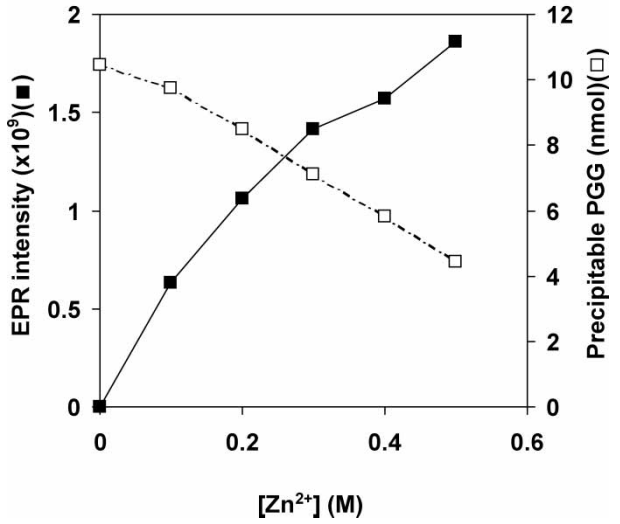


Figure 4. Zn^{2+} inhibits formation of the precipitable product during PGG oxidation. Reagents were prepared in 2 M acetate buffers (pH 2.5) with increasing concentrations of Zn^{2+} (0–0.5 M). PGG (106 nmol, 1000 μl) and $NaIO_4$ (1590 nmol, 750 μl) were mixed and transferred into EPR cell. Thirty scans were taken for each spectrum. The intensity (■) was the double integration of EPR signal. For precipitable oxidation product formation, [^{14}C]PGG (10.6 nmol, 100 μl) and $NaIO_4$ (159 nmol, 75 μl) were mixed in the filter chamber of a microspin filtration device. After 60 s, the mixtures were centrifuged. The amount of precipitable oxidation product isolated by filtration was radiochemically determined. Values for polymeric oxidation products (□) are the mean of three determinations. Standard deviations are smaller than the markers.

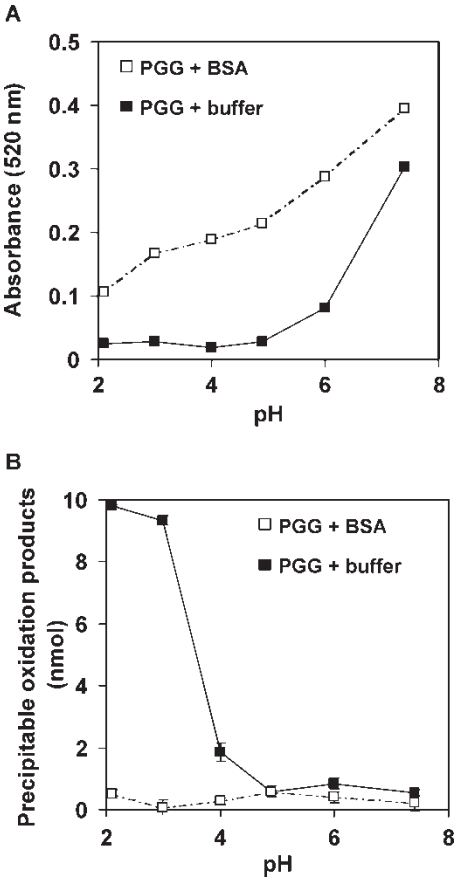


Figure 5. BSA affects PGG oxidation. (A) BSA promoted quinone formation. PGG (10.6 nmol, 100 μl) was incubated with BSA (10.6 nmol, 100 μl) (□) or the sample buffer (■) for 15 min at room temperature. UV spectra were taken after the addition of $NaIO_4$ (159 nmol, 75 μl). Absorbance at 520 nm is a single determination. (B) BSA inhibited formation of precipitable PGG oxidation products. Reactions were set up as above using [^{14}C]PGG. Two minutes after the addition of $NaIO_4$, the mixture was treated with SDS (1%, w/v, 100 μl), vortexed and centrifuged. The amount of PGG in the precipitate was radiochemically determined. Values are the mean of three determinations; error bars indicate the standard deviations.

Interactions between PGG oxidation products and protein

We monitored protein precipitation by oxidized PGG to evaluate the effects of oxidation on PGG–protein interactions. Because precipitation of BSA by PGG has a sharp pH-dependence with maximal precipitation at pH 4.9 [40], we adjusted the pH of each sample to 4.9 after oxidation [1]. When PGG was oxidized at pH values lower than 6, the product precipitated more BSA than unoxidized PGG (Figure 6). In contrast, oxidation of PGG at pH values greater than 6 yielded a product that precipitated less BSA than unoxidized PGG (Figure 6).

We also examined how oxidation affected the stability of PGG-BSA complexes. The complexes between oxidized PGG and BSA could not be dissociated by treatment with SDS, although unoxidized PGG-BSA complexes were completely

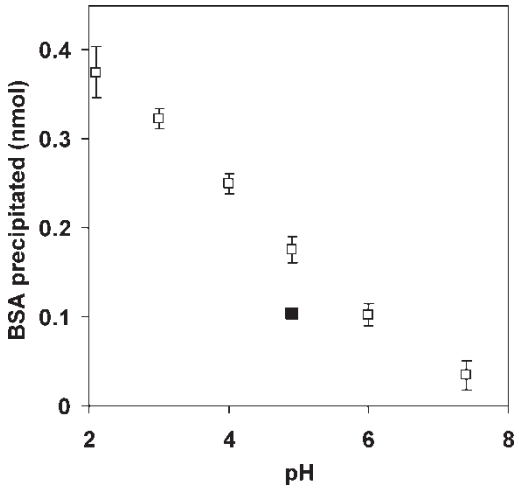


Figure 6. BSA precipitation at pH 4.9 by PGG oxidation products. At various pH values, PGG (10.6 nmol, 100 μl) was reacted with $NaIO_4$ (159 nmol, 75 μl). After 2 min, the reaction was stopped by the addition of 2 μl of β-mercaptoethanol, and [^{125}I]BSA (10.6 nmol, 100 μl) in 0.2 M acetate buffer (pH 4.9) was added. The mixtures were incubated at room temperature for 15 min, and centrifuged. The amount of BSA in the precipitates was radiochemically determined (□). For the control experiment at pH 4.9, $NaIO_4$ was substituted with the sample buffer (■); all other treatments remained the same. Values are the mean of three determinations; error bars indicate the standard deviations.

dissociated by SDS (Table I). Treatment of native PGG-BSA complexes with oxidizing agent yielded some SDS-resistant complexes, but less than when oxidation preceded the reaction with protein (Table I).

Reaction pH affects the oxidation of PC or EGCG

Oxidation of PC or EGCG at pH 7.4 produced chromophores (PC, $\lambda_{max} = 390$ nm; EGCG, $\lambda_{max} = 520$ nm) that are spectrally similar to catechol quinones [33,35]. Oxidation of PC and EGCG at low

Table I. Formation of SDS-resistant PGG-BSA complexes.

	SDS-resistant PGG-BSA complexes (nmol)*		
Sample	pH 4.9	pH 6.0	pH 7.4
Oxidized PGG + BSA†	10.4 ± 0.1	9.8 ± 0.1	7.4 ± 0.2
PGG + BSA with subsequent oxidation‡	6.4 ± 0.1	6.1 ± 0.2	6.7 ± 0.1
PGG + BSA without oxidation	−0.2 ± 0.4	ND	ND

*Values are the mean ± standard deviation of three determinations. ND stands for not determined. †[^{14}C]PGG (10.6 nmol, 100 μl) was mixed with $NaIO_4$ (159 nmol, 75 μl) before reacting with BSA (10.6 nmol, 100 μl) for 2 min, followed by SDS (1%, w/v, 100 μl) and TCA (100%, w/v, 100 μl). After 10 min incubation on ice the samples were centrifuged and 100 μl of the supernatant was counted. ‡In complementary reactions, [^{14}C]PGG was incubated with BSA for 15 min before the addition of $NaIO_4$ or buffer and treating as described above.

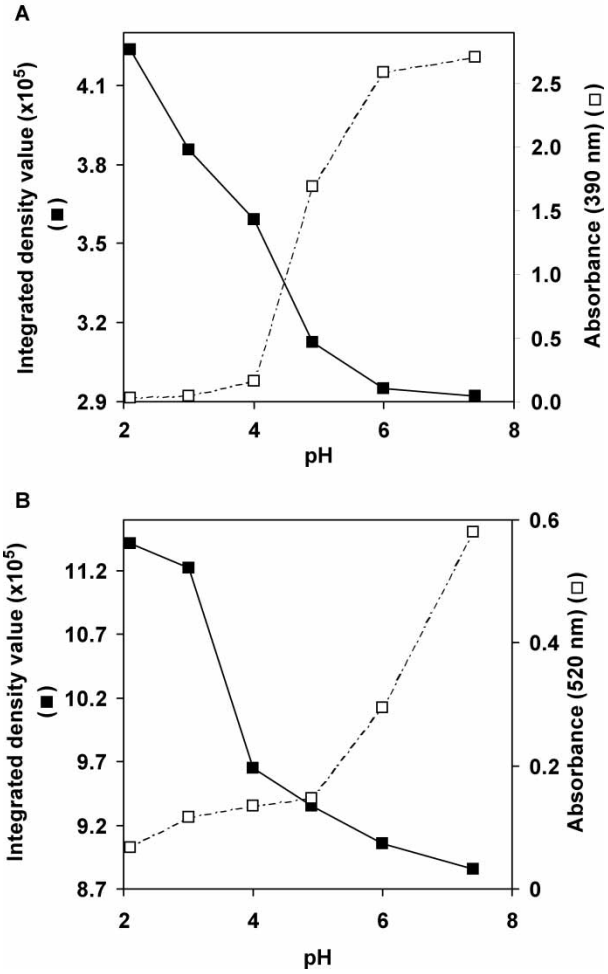


Figure 7. Reaction pH affects the oxidation of PC or EGCG. PC (16.3 nmol, 100 μl) was reacted with $NaIO_4$ (1272 nmol, 75 μl; NaIO4/catechin unit = 4.6) (A), and EGCG (100 nmol, 100 μl) was reacted with $NaIO_4$ (600 nmol, 100 μl; $NaIO_4$/EGCG = 6) (B). The formation of chromophore (□) was detected at 390 nm for PC or at 520 nm for EGCG. To quantitate the precipitable oxidation products, reagents were mixed in filter chambers in microspin filtration devices. After 2 min, the reaction solutions were removed by vacuum filtration. The amounts of precipitable oxidation products were photodensitomerically quantitated (■). Each data point is one measurement.

pH produced precipitates that we collected on nylon membranes (0.45 μm) and photodensitometrically determined. The amount of quinone increased while the amount of precipitate decreased as reaction pH was increased from 2.1 to 7.4 (Figure 7).

Discussion

Reaction pH and protein determined PGG oxidation products

A radical intermediate formed when PGG (Figure 1) was oxidized at pH values ranging from pH 2.1 to 7.4 (Figures 3 and 4). The radical was identified as an *o*-semiquinone radical based on its stabilization by Zn^{2+} [37–39]. The pK_a value for the *o*-semiquinone radical of methyl gallate is 4.4 [41], and our data suggest a similar pK_a for PGG *o*-semiquinone radical. At pH values below about 4, the PGG oxidation products are predominantly polymers, but at pH values above about 5 the products are predominantly quinones. We propose that at pH values below the apparent pK_a of the *o*-semiquinone radical, coupling of unionized PGG *o*-semiquinone radicals forms polymers (Scheme 1). At pH values above the apparent pK_a, ionization of PGG *o*-semiquinone radicals produces radical anions that do not polymerize due to electrostatic repulsion, but oxidize further to produce quinones (Scheme 1). At pH 2.5 stabilization of PGG *o*-semiquinone radical by Zn^{2+} inhibited polymer formation (Figure 4), supporting the idea that the unionized *o*-semiquinone radical was the precursor for the polymer.

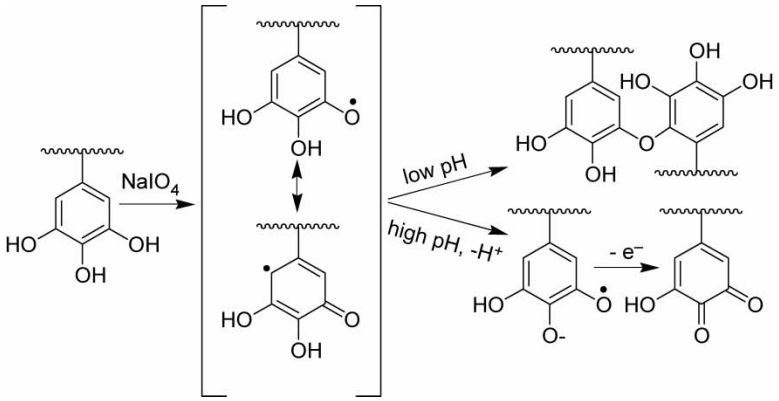
We also examined PC and EGCG, flavanol-based polyphenols that form *o*-semiquinone radicals with pK_a values of about 4.6 [39,41]. In the oxidation of PC or EGCG, low pH favored polymer formation, but high pH favored quinone formation (Figure 7). The similarity of oxidation pathways for three polyphenols, PGG, PC and EGCG, indicated that the same oxidation mechanism (Scheme 1) might apply to many polyphenols that have catechol or pyrogallol substitution patterns.

A representative protein, BSA, promoted the formation of *o*-quinone and inhibited the formation of polymer (Figure 5). Protein seemed be more important than pH in determining the products of PGG oxidation.

Changed biochemical activities of PGG after oxidation

Under non-oxidizing conditions, an important bioactivity of polymeric polyphenols is protein precipitation [1,42], which may reduce protein digestibility and thus diminish the consumption of tannin-containing plants by herbivores [43]. Polymeric PGG oxidation products that were formed at pH values lower than 6 had higher protein precipitating capacity than PGG (Figure 6), indicating they might be more effective in reducing proteindigestibility than unoxidized PGG. In contrast, oxidation of PGG to quinones appears to suppress its ability to precipitate protein. Further study is required to determine whether oxidation of polyphenols in the GI tract has deleterious or beneficial effects on protein nutrition.

PGG oxidation products reacted with proteins to form SDS-resistant complexes that might be covalently stabilized (Table I) [44]. SDS-resistant complexes were obtained when pre-oxidized PGG was mixed with protein, or when PGG-protein complexes were treated with oxidant (Table I). Likely reactions involve either attack by the semiquinone radical on susceptible amino acid residues[45] or attack of the quinone on nucleophilic residues such as lysine,



Scheme 1. Proposed pathway for PGG oxidation.

cysteine, methionine and tryptophan [46,47]. Covalent modification of proteins by small polyphenols such as chlorogenic acid reduces the digestibility of the modified protein [46], and covalent modification by tannins may have the same effect. We are characterizing the oxidized polyphenol-protein conjugates using electrophoresis, HPLC and MALDI-TOF MS [2].

We have demonstrated that pH and protein affect the oxidation of polyphenols through use of model compounds and *in vitro* chemical analysis. Polyphenols interact with metal ions[23] and lipids [48,49], and the effects of these factors on polyphenol oxidation could be explored in our system. Our approach could also be employed to examine other dietary polyphenolic antioxidants and model oxidants, ultimately achieving an understanding of the fates of polyphenols in the GI tract.

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References

[1] Hagerman AE, Rice ME, Ritchard NT. Mechanisms of protein precipitation for two tannins, pentagalloyl glucose and epicatechin$_{16}$-(4 → 8)-catechin (procyanidin). J Agric Food Chem 1998;46:2590–2595.

[2] Chen Y, Hagerman AE. Characterizing soluble non-covalent complexes between bovine serum albumin and β-1,2,3,4,6-penta-*O*-galloyl-D-glucopyranose by MALDI-TOF mass spectrometry. J Agric Food Chem 2004;52:4008–4011.

[3] Haslam E, Lilley TH, Cai Y, Martin R, Magnolato D. Traditional herbal medicines—the role of polyphenols. Planta Med 1989;55:1–8.

[4] Wroblewski K, Muhandiram R, Chakrabartty A, Bennick A. The molecular interaction of human salivary histatins with polyphenolic compounds. Eur J Biochem 2001; 268:4384–4397.

[5] Raha S, Robinson BH. Mitochondria, oxygen free radicals, disease and aging. Trends Biochem Sci 2000;25:502–508.

[6] Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47–95.

[7] Vandervliet A, Bast A. Role of reactive oxygen species in intestinal diseases. Free Radic Biol Med 1992;12:499–513.

[8] Blau S, Rubinstein A, Bass P, Singaram C, Kohen R. Differences in the reducing power along the rat GI tract: lower antioxidant capacity of the colon. Mol Cell Biochem 1999;194:185–191.

[9] Aw TY. Molecular and cellular responses to oxidative stress and changes in oxidation-reduction imbalance in the intestine. Am J Clin Nutr 1999;70:565–577.

[10] Srigiridhar K, Nair KM. Supplementation with alpha-tocopherol or a combination of alpha-tocopherol and ascorbic acid protects the gastrointestinal tract of iron-deficient rats against iron-induced oxidative damage during iron repletion. Br J Nutr 2000;84:165–173.

[11] Anton PM, Theodorou V, Bertrand V, Eutamene H, Aussenac T, Feyt N, Fioramonti J, Bueno L. Chronic ingestion of a potential food contaminant induces gastrointestinal inflammation in rats—role of nitric oxide and mast cells. Dig Dis Sci 2000;45:1842–1849.

[12] Zheng W, Sellers TA, Doyle TJ, Kushi LH, Potter JD, Folsom AR. Retinol, antioxidant vitamins, and cancers of the upper digestive-tract in a prospective cohort study of postmenopausal women. Am J Epidemiol 1995;142:955–960.

[13] Rice-Evans C. Flavonoid antioxidants. Curr Med Chem 2001;8:797–807.

[14] Bagchi M, Milnes M, Williams C, Balmoori J, Ye XM, Stohs S, Bagchi D. Acute and chronic stress-induced oxidative gastrointestinal injury in rats, and the protective ability of a novel grape seed proanthocyanidin extract. Nutr Res 1999;19:1189–1199.

[15] Halliwell B, Zhao KC, Whiteman M. The gastrointestinal tract: a major site of antioxidant action? Free Radic Res 2000;33:819–830.

[16] Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 1998;46:1887–1892.

[17] Bors W, Foo LY, Hertkorn N, Michel C, Stettmaier K. Chemical studies of proanthocyanidins and hydrolyzable tannins. Antioxid Redox Signal 2001;3:995–1008.

[18] Hagerman AE. Polymeric phenolics as antioxidants. Polyphenols Actualites 2001;:17–22.

[19] Santos-Buelga C, Scalbert A. Proanthocyanidins and tannin-like compounds—nature, occurrence, dietary intake and effects on nutrition and health. J Sci Food Agric 2000; 80:1094–1117.

[20] Gunckel S, Santander P, Cordano G, Ferreira J, Munoz S, Nunez-Vergara LJ, Squella JA. Antioxidant activity of gallates: an electrochemical study in aqueous media. Chem Biol Interact 1998;114:45–59.
[21] Barclay LRC, Edwards CE, Vinqvist MR. Media effects on antioxidant activities of phenols and catechols. J Am Chem Soc 1999;121:6226–6231.
[22] Snelgrove DW, Lusztyk J, Banks JT, Mulder P, Ingold KU. Kinetic solvent effects on hydrogen-atom abstractions: reliable, quantitative predictions via a single empirical equation. J Am Chem Soc 2001;123:469–477.
[23] Kumamoto M, Sonda T, Nagayama K, Tabata M. Effects of pH and metal ions on antioxidative activities of catechins. Biosci Biotechnol Biochem 2001;65:126–132.
[24] Riedl KM, Hagerman AE. Tannin-protein complexes as radical scavengers and radical sinks. J Agric Food Chem 2001;49:4917–4923.
[25] Arts MJTJ, Haenen GRMM, Voss HP, Bast A. Masking of antioxidant capacity by the interaction of flavonoids with protein. Food Chem Toxicol 2001;39:787–791.
[26] Foti MC, Barclay LRC, Ingold KU. The role of hydrogen bonding on the H-atom-donating abilities of catechols and naphthalene diols and on a previously overlooked aspect of their infrared spectra. J Am Chem Soc 2002; 124:12881–12888.
[27] Bilia A, Carelli V, Dicolo G, Nannipieri E. *In vitro* evaluation of a pH-sensitive hydrogel for control of gi drug delivery from silicone-based matrices. Int. J Pharm 1996;130:83–92.
[28] Davidson JM, Linforth RST, Taylor AJ. In-mouth measurement of pH and conductivity during eating. J Agric Food Chem 1998;46:5210–5214.
[29] Chen Y, Hagerman AE, Minto RE. Preparation of 1,2,3,4,6-penta-*O*-galloyl-[U-^{14}C]-D-glucopyranose. J Label Compd Radiopharm 2003;46:99–105.
[30] Hagerman AE, Butler LG. Determination of protein in tannin-protein precipitates. J Agric Food Chem 1980; 28:944–947.
[31] Hagerman AE, Butler LG. Condensed tannin purification and characterization of tannin-associated proteins. J Agric Food Chem 1980;28:947–952.
[32] Chen Y, Hagerman AE. Quantitative examination of oxidized polyphenol-protein complexes. J Agric Food Chem 2004; 52:6061–6067.
[33] Yuu ME, Hwang JY, Deming TJ. Role of l-3,4-dihydroxyphenylalanine in mussel adhesive proteins. J Am Chem Soc 1999;121:5825–5826.
[34] Dangles O, Dufour C, Bret S. Flavonol-serum albumin complexation. Two-electron oxidation of flavonols and their complexes with serum albumin. J Chem Soc Perk Trans 1999;2:737–744.
[35] Liberato DJ, Byers VS, Dennick RG, Castagnoli N. Regiospecific attack of nitrogen and sulfur nucleophiles on quinones derived from poison oak-ivy catechols (urushiols) and analogs as models for urushiol-protein conjugate formation. J Med Chem 1981;24:28–33.
[36] Paz MA, Fluckiger R, Boak A, Kagan HM, Gallop PM. Specific detection of quinoproteins by redox-cycling staining. J Biol Chem 1991;266:689–692.
[37] Kalyanaraman B, Premovic PI, Sealy RC. Semiquinone anion radicals from addition of amino-acids, peptides, and proteins to quinones derived from oxidation of catechols and catecholamines—an electron-spin-resonance spin stabilization study. J Biol Chem 1987;262:11080–11087.
[38] Bors W, Michel C, Stettmaier K. Electron paramagnetic resonance studies of radical species of proanthocyanidins and gallate esters. Arch Biochem Biophys 2000;374:347–355.
[39] Hagerman AE, Dean RT, Davies MJ. Radical chemistry of epigallocatechin gallate and its relevance to protein damage. Arch Biochem Biophys 2003;414:115–120.
[40] Kawamoto H, Nakatsubo F. Effects of environmental factors on two-stage tannin-protein co-precipitation. Phytochemistry 1997;46:479–483.
[41] Jovanovic SV, Hara Y, Steenken S, Simic MG. Antioxidant potential of gallocatechins—a pulse-radiolysis and laser photolysis study. J Am Chem Soc 1995;117:9881–9888.
[42] Hagerman AE, Butler LG. The specificity of proanthocyanidin–protein interactions. J Biol Chem 1981;256:4494–4497.
[43] Bi JL, Felton GW, Murphy JB, Howles PA, Dixon RA, Lamb CJ. Do plant phenolics confer resistance to specialist and generalist insect herbivores? J Agric Food Chem 1997;45:4500–4504.
[44] Khan FH, Saha M, Chakrabarti S. Dopamine induced protein damage in mitochondrial-synaptosomal fraction of rat brain. Brain Res 2001;895:245–249.
[45] Dean RT, Fu SL, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. Biochem J 1997;324:1–18.
[46] Kroll J, Rawel HM, Seidelmann N. Physicochemical properties and susceptibility to proteolytic digestion of myoglobin-phenol derivatives. J Agric Food Chem 2000;48:1580–1587.
[47] Peter MG. Chemical modifications of bio-polymers by quinones and quinone methides. Angew Chem Int Ed Engl 1989;28:555–570.
[48] Simon SA, Disalvo EA, Gawrisch K, Borovyagin V, Toone E, Schiffman SS, Needham D, Mcintosh TJ. Increased adhesion between neutral lipid bilayers—interbilayer bridges formed by tannic-acid. Biophys J 1994;66:1943–1958.
[49] Huh NW, Porter NA, Mcintosh TJ, Simon SA. The interaction of polyphenols with bilayers: conditions for increasing bilayer adhesion. Biophys J 1996;71:3261–3277.