Mechanisms of Protein Precipitation for Two Tannins, Pentagalloyl Glucose and Epicatechin$_{16}$ (4→8) Catechin (Procyanidin)

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The precipitates that form when purified pentagalloylglucose or a purified procyanidin [epicatechin$_{16}$ (4→8) catechin; EC$_{16}$-C] are mixed with bovine serum albumin were quantitatively analyzed. EC$_{16}$-C is a more efficient protein precipitating agent than pentagalloylglucose on a molar or a mass basis. EC$_{16}$-C precipitates protein independently of temperature and presence of organic solvent. Precipitation by pentagalloylglucose increases as temperature is increased and decreases when alcohols are present. When tannin is in excess, up to 40 mol of pentagalloylglucose is bound per mole of protein precipitated, but only 20 mol of EC$_{16}$-C is bound per mole of protein precipitated. The data support different models of precipitation for the two types of tannin: pentagalloylglucose, which is very nonpolar, precipitates by forming a hydrophobic coat around the protein, whereas the much more polar EC$_{16}$-C forms hydrogen-bonded cross-links between protein molecules.

Keywords: *Polyphenol−protein interaction; tannin; pentagalloylglucose; procyanidin; condensed tannin; bovine serum albumin*

INTRODUCTION

The ability to precipitate protein is the defining characteristic of the compounds known as tannins or plant polyphenols. The definition of tannins as "water-soluble phenolic compounds having molecular weights between 500 and 3000...[with] the ability to precipitate alkaloids, gelatin and other proteins" was articulated by Bate-Smith and Swain over 35 years ago (Bate-Smith and Swain, 1962) and still provides us with the most useful functional understanding of these chemically diverse compounds. Tannins are widely distributed in plants including those used for foods, feeds, and medicines. The tendency of tannins to interact with proteins thus influences the appearance and taste of foods and beverages (Shahidi and Naczk, 1995), the function of ecological (Hagerman and Butler, 1991) and agricultural (Kumar and Singh, 1984) systems, and the discovery and application of new pharmaceutical agents (Haslam et al., 1989; Zhu et al., 1997). Understanding the mechanism of interaction between tannin and protein should help control and exploit the properties of polyphenols in these diverse systems.

Examination of tannin−protein interactions using modern techniques including competitive binding methods and spectroscopic methods has clearly demonstrated that protein structure plays an essential role in complex formation. In general, flexible proline-rich proteins have high affinity for tannins. The first studies that demonstrated specificity for protein structure were conducted with a condensed tannin, or procyanidin (Hagerman and Butler, 1981), and suggested that the tannin interacted with protein mainly through hydrogen bond formation between the phenolic donor and the peptide acceptor. Proline-rich proteins formed particularly strong hydrogen bonds and were conformationally flexible, leading to accessibility of hydrogen bonding

sites. Subsequent studies suggested that the interaction of the gallotannin, β-1,2,3,4,5-penta-*O*-galloyl-D-glucose (PGG), with proline-rich peptides involved hydrophobic stacking of the planar phenolic ring against the pyrrolidine ring of the proline (Murray et al., 1994; Luck et al., 1994). The relative importance of hydrogen bonding versus hydrophobic interactions has not been clearly established (Hagerman, 1992; Haslam, 1993).

The role of tannin structure in the precipitation reaction has been less systematically studied. The tannins are chemically very heterogeneous (Porter, 1989), and it is only relatively recently that isolation and structure determination have become routine (Hagerman et al., 1997). In terrestrial plants the tannins can be divided into two groups: the flavonoid-derived proanthocyanidins (condensed tannins) (Porter, 1992) and the gallic acid ester-derived hydrolyzable tannins (Okuda et al., 1995). A third major group of tannins, the phlorotannins, are found only in marine algae (Ragan and Glombitza, 1986). Within each of these groups there is substantial structural variation in features including pattern of substitution, stereochemistry, degree of oxidation, and molecular size. Most studies on the importance of tannin structure in the tannin−protein interaction have involved within-group comparisons. For example, it has been demonstrated that both substitution patterns (Asano et al., 1984) and polymer size (Porter and Woodruffe, 1984) influence the precipitation of proteins by condensed tannins. The ability of hydrolyzable tannins to precipitate protein has been related to the degree of esterification (Hagerman et al., 1992; Kawamoto et al., 1996) or structural flexibility (Haslam et al., 1992) of the polyphenol. Very few attempts have been made to use protein precipitation to directly compare condensed tannins to hydrolyzable tannins (Hagerman, 1992).

The specific objective of the work described here was to quantitatively compare the protein-precipitating activities of two well-characterized polyphenolics that

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have significant differences in chemical and structural properties. The compounds selected were PGG, which has been the focus of Haslam's extensive and elegant studies on tannin−protein interactions [e.g., Haslam (1989)] and epicatechin$_{16}$ (4→8) catechin (EC$_{16}$-C), which has been used in much of Hagerman's work [e.g., Hagerman (1992)]. It was hoped that one outcome of this study would be the development of structure−activity relationships so that the ability of other polyphenols to precipitate protein could be predicted. Furthermore, it was anticipated that the studies might help to differentiate the roles of hydrogen bonding and hydrophobic forces in the interaction between tannins and proteins.

MATERIALS AND METHODS

Materials. PGG (M_r 940) was purified from commercial tannic acid by solvent extraction after methanolysis and was characterized by proton NMR and negative ion FAB MS (Hagerman et al., 1997). EC$_{16}$-C [epicatechin$_{16}$ (4→8) catechin, M_r 4930] was purified from *Sorghum bicolor* (Moench) grain (IS8260, provided by Dr. John Axtell, Purdue University) (Hagerman and Butler, 1980b) and was characterized by HPLC after phloroglucinol degradation (Koupai-Abazani et al., 1992; Schofield et al., 1998) and by ^{13}C NMR (Czochanska et al., 1980). The other polyphenols (listed in Table 2) were provided by Lipton Tea Co. (Newark, NJ) or were purified by preparative scale normal phase HPLC (Hagerman et al., 1997); compositions of the polyphenolic preparations were determined by reversed phase HPLC as described below. Bovine serum albumin (BSA; fraction V, fatty acid free) was purchased from Sigma Chemical Co. (St. Louis, MO) and was radioiodinated with the chloramine T method (Hagerman and Butler, 1980a).

Analytical Methods. The precipitation method described previously (Hagerman and Butler, 1980a) was scaled down. The polyphenol was dissolved in 100 μL of methanol or metal-free water (Barnstead Nanopure System, Dubuque, IA) and was added to protein dissolved in 300 μL of buffer A (0.2 M acetate, pH 4.9, containing 0.17 M NaCl). The mixtures were vortexed vigorously, incubated for 30 min, and centrifuged at 4 °C for 5 min at 11000g. The supernatants were removed by aspiration, and the precipitates were gently washed with 100 μL of buffer and were centrifuged again for 1 min. The samples were again aspirated and the precipitates analyzed. Precipitated protein was determined by gamma counting.

To determine precipitated PGG, the precipitates were dissolved in 300 μL of 1.0% aqueous sodium dodecyl sulfate solution and analyzed by HPLC (Kawamoto et al., 1996). A C$_{18}$ (ODS) column (Beckman Ultrasphere, 4.6 mm × 25 cm, 5 μm particles) and similar C$_{18}$ precolumn were used for the separation. Samples were eluted with a gradient consisting of 0.1% aqueous trifluoroacetic acid (TFA) and 0.1% TFA in acetonitrile (4:1 to 3:2 over 7 min) at a flow rate of 1 mL min^{-1}. Analytes were detected at 220 nm, and peaks were integrated with HP Chemstation A.4.02 software (Hewlett-Packard Co., Wilmington, DE). The retention time of PGG was 5.9 min.

To determine precipitated EC$_{16}$-C, the precipitates were dissolved in 100 μL of sodium dodecyl sulfate/triethanolamine solution before reaction with 50 μL of FeCl$_3$; the value of A_{510} was then read (Hagerman and Butler, 1978).

Octanol/water partition ratios were determined by partitioning 0.001 M aqueous solutions of the phenolics against an appropriate volume of octanol (Leo et al., 1971). After partitioning, most polyphenols were determined by HPLC using the parameters described above. EC$_{16}$-C was determined by direct spectrophotometry of the octanol phase at 280 nm.

Molecular Modeling. The program Alchemy 2000 (Tripos Inc., St. Louis, MO) was used to geometry optimize simple polyphenolic structures. Molecular dimensions were calculated with the software after the geometry optimization.
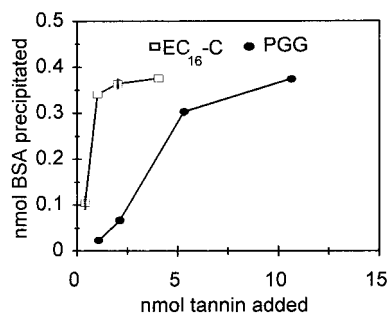

Figure 1. Precipitation of protein (BSA) by PGG or EC$_{16}$-C. Tannin dissolved in water was added to 300 μL of buffer A containing 0.46 nmol of radiolabeled BSA, and the mixture was incubated at room temperature. Tannin concentrations were set so that up to 10 nmol of tannin was added in the 100 μL aliquot of aqueous solution. The amount of BSA precipitated was determined by counting the precipitate. Values are the mean of three determinations; error bars indicate the standard deviations.

RESULTS

Preliminary experiments were performed to establish optimum conditions for precipitation for the two polyphenolics. The pH optimum for BSA precipitation was between pH 4 and 5 for EC$_{16}$-C or PGG (data not shown), confirming earlier suggestions that precipitation is maximized near the isoelectric point of many proteins (Hagerman and Butler, 1978). All reactions were therefore run at pH 4.9 using a moderate ionic strength acetate buffer. Protein precipitation occurred rapidly, with the amount of protein precipitated independent of time for times ranging from 5 min to at least 60 min after protein was mixed with EC$_{16}$-C or PGG.

EC$_{16}$-C was a more effective protein-precipitating agent than was PGG on a molar basis (Figure 1). The amount of BSA precipitated by EC$_{16}$-C increased linearly with increasing polyphenolic until all of the BSA present was precipitated. About 1 nmol (5 μg) of EC$_{16}$-C was required to precipitate essentially all of the BSA present (0.40 nmol) (Figure 1). When PGG was incubated with protein under these conditions, very little BSA was precipitated unless at least 2.5 nmol (2.5 μg) of PGG was added (Figure 1). The amount of protein precipitated then increased gradually so that 10 nmol (10 μg) of PGG was required to precipitate all of the BSA (Figure 1). Precipitation of phenolics (PGG or EC$_{16}$-C) paralleled precipitation of protein (data not shown).

Protein precipitation by PGG was sensitive to temperature, with a 4-fold increase in precipitated protein noted when the temperature was increased from 4 ° to 40 °C (Figure 2). The precipitation by EC$_{16}$-C was temperature insensitive (Figure 2), as previously reported (Hagerman and Butler, 1978). Precipitation of EC$_{16}$-C or PGG paralleled precipitation of the protein at various temperatures (data not shown).

The amount of protein and polyphenol precipitated in the PGG reaction mixtures was substantially decreased by the presence of alcohol in the reaction mixture (Figures 3 and 4). As little as 10% (v/v) of alcohol in the reaction mixture decreased the amount of protein precipitated by PGG, and when the reaction mixture contained 25% methanol (by volume), precipitation was almost completely suppressed (Figure 3). As previously demonstrated (Hagerman and Butler, 1980a), alcohol does not affect precipitation of protein by EC$_{16}$-C (Figure 3).
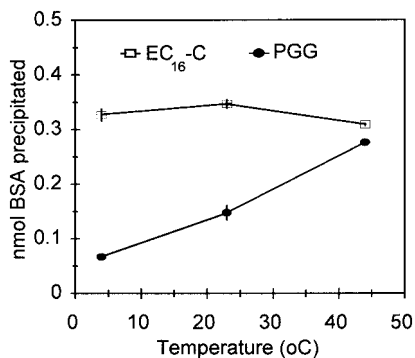
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Figure 2. Effect of temperature on precipitation of protein (BSA) by PGG or EC_{16}-C. Solutions were brought to the indicated temperature (4 °C, room temperature, or 44 °C), and tannin was added to 300 μL of buffer A containing 0.45 nmol of radiolabeled BSA. Tannin concentrations were set so that 5.32 nmol of PGG or 1.01 nmol of EC_{16}-C was added in the 100 μL aliquot of aqueous solution. The amount of BSA precipitated was determined by counting the precipitate. Values are the mean of three determinations, with error bars indicating standard deviations.
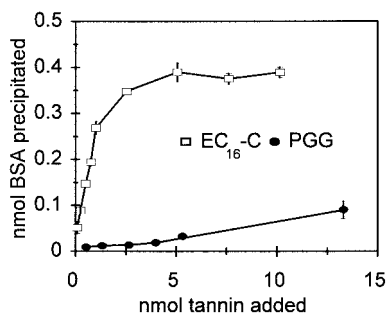

Figure 3. Precipitation of protein (BSA) by PGG or EC_{16}-C in the presence of 25% methanol. Tannin dissolved in methanol was added to 300 μL of buffer A containing 0.40 nmol of radiolabeled BSA, and the mixture was incubated at room temperature. Concentrations of the tannin solutions were set so that up to 15 nmol of tannin could be added in the 100 μL aliquot of methanol. The amount of BSA precipitated was determined by counting the precipitate. Values shown are the means of triplicate determinations; error bars show standard deviations.

In the alcohol-containing reaction mixtures, very little PGG coprecipitated with the protein. Only 2−3 nmol of PGG was precipitated even when as much as 53 nmol of PGG was used to drive the precipitation reaction (Figure 4). Recovery experiments were performed by analyzing both supernatants and precipitates to ensure that there was no unexpected loss or degradation of PGG during the precipitation reaction. The average recovery was 111% of the added PGG, with >95% of the polyphenol found in the supernatant. Under similar conditions, EC_{16}-C is quantitatively precipitated.

In the presence of methanol, the pattern of temperature sensitivity was reversed. If the reaction mixture contained 25% methanol (by volume), the amount of BSA precipitated by PGG *decreased* ~4-fold when the temperature was increased from 4° to 40 °C (data not shown). EC_{16}-C precipitation was temperature insensitive even in alcohol-containing reaction mixtures.

The stability of the BSA−polyphenol precipitates depended on both the nature of the polyphenol and on the reaction conditions (Table 1). The EC_{16}-C−BSA precipitates were quite stable, and insignificant amounts of protein were resolubilized when the precipitates were resuspended in fresh buffer A. The PGG−BSA precipi-
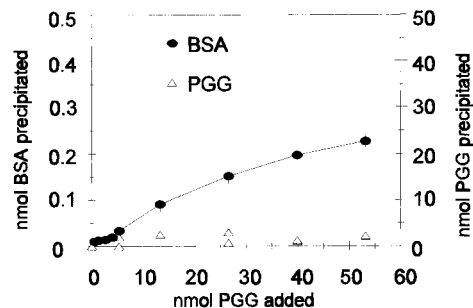

Figure 4. Precipitation of protein (BSA) and polyphenolic (PGG) in solutions containing 25% methanol. PGG dissolved in methanol was added to 300 μL of buffer A containing 0.40 nmol of radiolabeled BSA, and the mixture was incubated at room temperature. Concentrations of PGG solutions were set so that up to 55 nmol of PGG could be added in the 100 μL aliquot of methanol. The amount of BSA precipitated was determined by counting the precipitate. The amount of PGG precipitated was determined by HPLC analysis of the precipitate. For BSA precipitation, values shown are the means of triplicate determinations; error bars show standard deviations. For PGG precipitation, duplicate determinations are shown.

tates formed in pure aqueous solution were similarly very stable. However, the PGG−BSA precipitates formed in solutions containing 25% methanol were very unstable, so that a substantial amount of the precipitated protein was redissolved when the precipitates were resuspended in fresh methanol-containing buffer A. The addition of the strong reducing agent dithiothreitol (DTT) to the methanol-containing reaction solutions did not alter the amount of protein precipitated by PGG or by EC_{16}-C and did not alter the stability of the precipitates (Table 1).

By using enough polyphenol (10 nmol of EC_{16}-C or 53 nmol of PGG) to drive the precipitation reaction to completion, it was possible to determine the stoichiometry of the polyphenol−protein precipitate. When the polyphenol/BSA ratio (t:p) in the reaction mixture was low, the composition of the precipitate was identical to that of the initial solution (Figure 5). When the initial concentration of EC_{16}-C was >21 times that of the BSA, the composition of the precipitate became fixed at a value of 21.4 mol of EC_{16}-C/mol of BSA (Figure 5). When the initial concentration of PGG was >42 times that of the BSA, the composition of the precipitate became fixed at a value of 42.7 mol of PGG/mol of BSA (Figure 5). This ratio is achieved only when the PGG precipitation reaction is run in the absence of methanol. Such small amounts of PGG are precipitated in the presence of 25% methanol that ratios of PGG-to-BSA in precipitates cannot be calculated (Figure 4).

The limited solubility of PGG in aqueous solution (Hagerman et al., 1997) is reflected in its very large octanol/water partition ratio (Table 2). In contrast, the octanol/water partition ratio for EC_{16}-C is very low, consistent with it high solubility in water (Table 2). Octanol/water partition coefficients reflect molecular polarity. To better understand differences in polarity, we used a geometry optimizing program (Alchemy 2000) to calculate preferred structures for simple polyphenolics. PGG is geometry optimized as a roughly spherical molecule. Its symmetry is consistent with its low polarity despite the presence of numerous polar phenolic hydroxyl groups on the molecule. Addition of galloyl groups to the core PGG decreases molecular symmetry and increases polarity; the octanol/water partition ratio (Table 2) reflects that change. The high polarity of EC_{16}-C apparently is a result of the elongated shape of

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Table 1. Amount of Protein Precipitated by Polyphenols before and after Vigorous Resuspension of Precipitate[a]

	no DTT		DTT-containing buffer	
	initial precipitate (nmol of BSA)	precipitate after 30 min resuspension (nmol of BSA)	initial precipitate (nmol of BSA)	precipitate after 30 min resuspension (nmol of BSA)
EC$_{16}$-C/methanol	0.233 ± 0.022a	0.223 ± 0.021a	0.289 ± 0.012a	0.255 ± 0.013a
PGG/aqueous	0.348 ± 0.006b	0.346 ± 0.004b	ND	ND
PGG/methanol	0.193 ± 0.006c	0.125 ± 0.015d	0.209 ± 0.018c	0.085 ± 0.006d

[a] Tannin (53.2 nmol of PGG or 1.01 nmol of EC$_{16}$-C) was added to 0.48 nmol of radiolabeled BSA in buffer ± 150 mM DTT. After the mixture was incubated at room temperature, the precipitates were isolated by centrifugation, radiochemically counted, and resuspended by vigorous mixing for 30 min. The precipitates were again isolated by centrifugation and counted. Values are the mean of three determinations ± standard deviation. Different letters indicate significant difference ($p < 0.05$); ND means not determined.
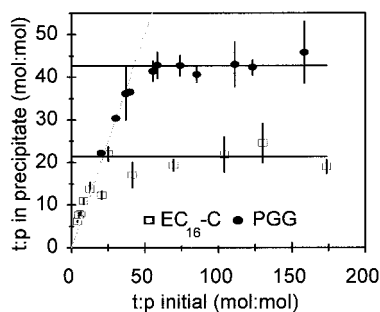

Figure 5. Ratios of polyphenolic to protein (t:p) in the precipitate as a function of the t:p in the initial reaction mixture. PGG or EC$_{16}$-C was added to 300 μL of buffer A containing up to 3 nmol of radiolabeled BSA, and the mixture was incubated at room temperature. PGG concentration was set so that 53.2 nmol of PGG was added in the 100 μL aliquot of aqueous solution. EC$_{16}$-C concentration was set so that 10.1 nmol of EC$_{16}$-C was added in the 100 μL aliquot of methanol solution. The amount of BSA precipitated was determined by counting the precipitate. The amount of PGG precipitated was determined by HPLC analysis of the precipitate. The amount of EC$_{16}$-C precipitated was determined by spectrophotometric analysis of the precipitate. Values are the mean of three determinations, with error bars indicating standard deviations.

4→8 procyanidins (Fletcher et al., 1977; Helfer and Mattice, 1995). An extended, rodlike procyanidin polymer would have a much larger dipole than the flavan-3-ol subunits, which are roughly spherical molecules.

DISCUSSION

PGG is an effective protein-precipitating agent only in pure aqueous solutions. Under those conditions, this polyphenol forms strong noncovalent bonds to BSA, as demonstrated by the stability of the precipitates to disruption by buffer A (Table 1) and sensitivity of the precipitates to treatment with protein denaturants such as the sodium dodecyl sulfate solution used to redissolve the samples for HPLC analysis. The interaction between PGG and BSA appears to be dominantly a hydrophobic interaction with the characteristic temperature (Figure 2) and organic solvent (Figure 1 versus Figure 3) sensitivity of hydrophobic precipitation (Kennedy, 1990). When organic solvents, which suppress hydrophobic interactions, are present, the temperature dependence of precipitation by PGG reverses (data not shown), suggesting that precipitation involves very weak hydrogen bonding interactions. These interpretations are consistent with earlier investigations of the PGG−protein interaction, which relied on thermodynamic or spectroscopic measurements (McManus et al., 1985; Murray et al., 1994).

EC$_{16}$-C is an effective protein-precipitating agent in aqueous solution or in mixed solvents and forms stable noncovalent bonds to BSA. The bonds involved in complex formation are strong, as demonstrated by the

stability of the complexes when the precipitate is resuspended in buffer A (Table 2) or in the presence of additional protein. The noncovalent nature of the complexes is demonstrated by release of both protein and polyphenolic when the precipitate is treated with protein denaturants such as detergents or strong base (Hagerman and Butler, 1980b). The precipitation reaction is insensitive to temperature (Figure 2) and to the presence of simple alcohols (Hagerman and Butler, 1980a; Figure 1 versus Figure 3), suggesting that hydrophobic forces do not have an important role in the EC$_{16}$-C−BSA interaction. This interpretation is consistent with earlier work which suggested that hydrogen bonding is the primary mode of interaction between EC$_{16}$-C and protein (Hagerman and Butler, 1981).

None of the data provided any evidence for oxidative reactions yielding covalent bonds between PGG or EC$_{16}$-C and BSA (Pierpoint, 1969; Beart et al., 1985). Under similar reaction conditions phlorotannins form irreversible covalent complexes with BSA, unless a reducing agent is present to prevent oxidative reactions (Stern et al., 1996). All of the complexes formed by PGG or EC$_{16}$-C were noncovalent and could be completely dissociated by protein-denaturing conditions. The addition of a reducing agent did not affect the amount of protein or polyphenol precipitated and did not affect the stability of the precipitate that formed (Table 1). These results suggest that under the mild conditions employed here EC$_{16}$-C and PGG do not oxidatively damage BSA.

Haslam et al. (1992) speculated that the efficacy of precipitation by polyphenols was inversely related to their water solubility. Our data show the opposite trend; EC$_{16}$-C, which is very water soluble (Table 2), was a more efficient protein precipitant than PGG, which is virtually water insoluble (Table 2). We propose that polyphenol polarity can be used to predict whether a given tannin preparation will precipitate via hydrophobic forces or hydrogen bonding. For example, preliminary precipitation experiments indicated that for heptagalloylglucose, which is more polar than pentagalloylglucose, hydrogen bonding was the dominant force involved in the interaction with BSA. For the relatively nonpolar polyphenolics theaflavin and epicatechin gallate (Table 2), hydrophobic forces dominated the reaction.

The realization that tannin structure dictates the mechanism of interaction with protein should facilitate understanding of the behavior of polyphenols in a variety of systems. Our work suggests that recent attempts (Siebert et al., 1996) to model haze formation in beverages using commercial tannic acid, a mixture of low molecular weight, nonpolar gallotannins (Hagerman et al., 1992), may be especially useful for beverages such as green tea, which contains largely nonpolar polyphenols (Balentine, 1992). However, black teas, red

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Table 2. Octanol/Water Partition Ratios of Polyphenols[a]

compound	partition ratio	compound	partition ratio	compound	partition ratio
EC$_{16}$-C	2.12×10^{-3}	hexagalloylglucose	1.51	theaflavin	2.23×10^1
epigallocatechin	2.81×10^{-1}	epicatechin	2.43	epicatechin gallate	4.80×10^1
heptagalloylglucose	1.03	epigallocatechin gallate	1.21×10^1	PGG	1.29×10^2

[a] Compounds (1 mM) were partitioned between water and octanol. The polyphenol was determined by HPLC or spectrophotometrically at 280 nm (EC$_{16}$-C only). The values shown are averages of three replicates. The average standard deviation was 3.3% of the mean for these determinations.

wines, and fruit juices contain polar polyphenols including condensed tannins and poorly characterized polymeric compounds (Foo and Porter, 1981), which interact with protein on a very different basis from the small gallotannins. These findings provide a mechanistic basis for the specificity of action of tannins in biological systems (Hagerman and Robbins, 1993; Ayres et al., 1997). The realization that alcohol inhibits protein precipitation by certain polyphenols should prompt reevaluation of precipitation assays appropriate for assessment of crude plant extracts.

This work extends the stoichiometric studies of Kawamoto et al. (1996). We found that when tannin is in excess over protein, precipitates having a fixed ratio of tannin to protein form. For EC$_{16}$-C the ratio is ∼20 mol of polyphenol/mol of BSA. For PGG the ratio is ∼40 mol of polyphenol/mol of BSA (Figure 5). These stoichiometries clearly indicate that polyphenols are not selectively bound by the hydrophobic ligand binding sites found in domains IIA and IIIA of BSA (He and Carter, 1992), since each molecule of protein has only two of these hydrophobic sites. The ratios may reflect the sizes of the tannin molecules and available surface area of the protein. Simple geometry-optimized molecular modeling indicates that PGG is a spherical molecule with $d = 11$ Å, so the maximum amount of PGG which could be bound on the surface of serum albumin, a triangular protein (80 Å on a side, 30 Å deep) (He and Carter, 1992), would be ∼100 mol of PGG/mol of BSA. The approximate agreement of the experimental value with this maximum theoretical value suggests that Haslam's (1989) model for polyphenol−protein interactions, in which the hydrophobic polyphenol nonspecifically coats the surface of the protein molecule, may be correct for PGG−BSA interactions.

The ratio of EC$_{16}$-C to BSA is about half that obtained for PGG, presumably due to size considerations and/or number of binding sites on the protein. Differentiating between these two possibilities is difficult because the size and shape of polymeric proanthocyanidins is not accurately known (Helfner and Mattice, 1995). We are attempting to address that question by extending our studies to include other polyphenolics. For example, heptagalloylglucose is about the same size as PGG but has a polarity more similar to that of EC$_{16}$-C. Preliminary data suggest that only ∼20 mol of heptagalloylglucose bind per mole of BSA. This suggests that the number of sites, rather than the size of the polyphenolic, dictates the stoichiometry of the complexes for hydrogen bonding polyphenolics.

This work clearly demonstrates that the characteristics of tannin−protein complexes are strongly influenced by the structure of the tannin. This information complements our understanding of how protein structure affects the interaction (Hagerman and Butler, 1981). The results suggest that future effort should be focused on systems of well-characterized polyphenolics and well-characterized proteins so that a better understanding of specificity and structure−activity relationships can be achieved.

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