Interactions of Grape Seed Tannins with Salivary Proteins

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To evaluate the amount and type of condensed tannins binding salivary proteins, which are supposed to be involved in astringent sensation, model systems allowing further analyses of proteins and condensed tannins were developed. The precipitates formed after addition of grape seed tannins to salivary proteins indicate that a binding interaction occurs. Dissociation of insoluble complexes was achieved by sodium dodecyl sulfate treatment. Thiolysis reaction allowed the quantification and characterization of proanthocyanidins on both the resulting pellet and the supernatant. Binding proteins were investigated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis. The higher polymerized tannins predominantly precipitated together with the salivary proteins. The condensed tannins remaining in solution were low molecular weight polymers.

Keywords: *Condensed tannins; grape; procyanidins; saliva; salivary proteins; thiolysis; electrophoresis; tannin−protein interaction; tannin−protein binding*

INTRODUCTION

Grape condensed tannins (i.e., proanthocyanidins) are polymeric flavonoid compounds in which monomeric units consist of flavan-3-ols. The monomeric units are linked by interflavan bonds from C4 of the upper unit to C8 and to a lesser extent C6 of the lower unit (B-type) or are doubled linked with an additional ether linkage (A-type). The B-ring of the monomeric unit may have different hydroxylation patterns leading to different proanthocyanidin classes. These include, in particular, procyanidins, deriving from (epi)catechin (3′,4′-OH), and prodelphinidins, consisting of (epi)gallocatechin units (3′,4′,5′-OH).

Interest in grape and wine proanthocyanidins is related to their quantitative and qualitative importance for wine organoleptic properties, due to their great structural diversity and their high reactivity. Grape seed and skin proanthocyanidins have already been investigated and elucidated (Ricardo da Silva et al., 1991; Prieur et al., 1994; Souquet et al., 1996). Only B-type polymers are present in grapes, with small amounts of dimers and trimers. Whereas seed tannins are only procyanidins, (Czochanska et al., 1979; Prieur et al., 1994), recent studies have shown that grape skins contain procyanidin and prodelphinidin units (Souquet et al., 1996).

Astringency, which is reported as a major wine property, is defined as the complex of sensations due to shrinking, drawing, or puckering of the epithelium as a result of exposure to substances such as alums or tannins (ASTM, 1989). It is well-known that tannins interact with proteins, leading to inhibition of enzymes (Guyot et al., 1996; Oh and Hoff, 1986) and decrease of protein digestibility (Horigome et al., 1988; Ozawa et al., 1987). The organoleptic properties and reactivity of tannins, including protein-binding ability, certainly depend on their structure. Until now, hydrophobic interactions and hydrogen bonding were the two mechanisms proposed to explain tannin−protein complex formation. First evaluated as independent mechanisms, they are now approached as cooperative features leading to the associations (Haslam, 1996).

Protein-binding ability of tannins is believed to be involved in the astringent sensation perceived in the buccal cavity when wine is absorbed. However, the actual knowledge does not allow the determination of which proteins, tannins, and/or tannin combinations are really responsible for this phenomenon. The difficulties experienced in the determination of precipitated tannins and proteins are related to the lack of suitable analytical methods. In attempts to quantify tannin−protein precipitates, numerous methods have been used, including turbidimetry (Calderon et al., 1968; McMurrough and Hennigan, 1984), HPLC analysis, Lowry assay for proteins, or vanillin−HCl assay for tannins (Terril et al., 1992). Moreover, precipitations were generally studied with model tannins (tannic acid; penta-*O*-galloyl-D-glucose) and model proteins (bovin serum albumin, BSA) (McManus et al., 1985). However, the principal tannins encountered in food are condensed tannins, which are greatly different from tannic acid and galloylglucose derivatives (Haslam and Lilley, 1988). Moreover, recent studies demonstrate the influence of amino acid composition and the conformation of the proteins on the binding ability of polyphenols. In particular, the proline-rich proteins (PRPs) found in saliva, which are reported as strongly associating with tannins, cannot be conformationaly compared with BSA (Williamson, 1994). The open conformation of PRP allows the formation of hydrophobic interactions and hydrogen bonds with tannins (Hagerman and Robbins, 1993; Haslam, 1996). The larger and more hydrophobic polyphenols have been reported to bind more strongly to the proline-rich peptide, and some of the binding interactions for the larger polyphenols may arise from multidentate association (Murray et al., 1994; Baxter et al., 1997). The aim of our work was to study the

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10.1021/jf9805146 CCC: $18.00
Published on Web 12/08/1998

nature of condensed tannins involved in the precipitation of salivary proteins as a first step to approach the solution of the astringency problem.

MATERIALS AND METHODS

Materials. *Reagents.* All solvents and acids used were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) was supplied by Sigma Chemical Co. (Poole, Dorset, U.K.).

Grape (Vitis vinifera var. Alicante bouschet) Seed Proanthocyanidin Fractions. Grape seeds were ground under liquid nitrogen. The resulting powder was extracted with acetone/water (60:40 v/v) and centrifuged (5000*g*, 15 min) to eliminate particle residues. The supernatant was recovered, filtered on GF/C Whatman (Maidstone, Kent, U.K.), and evaporated under vacuum before dissolution in methanol. The proanthocyanidin fraction used was obtained by chromatography on Fractogel (Prieur-Delorme, 1994) and characterized by HPLC analysis after thiolysis (Rigaud et al., 1991).

Saliva. Saliva was collected from a single individual (P.S.-M.) by retaining saliva in the mouth and then expectorating into an ice-cooled tube. Saliva was centrifuged at 10000*g* for 10 min to remove any insoluble material, and the supernatant is referred to as whole saliva (Madapallimattam and Bennick, 1990).

Protein concentrations were determined by BCA protein assay using BSA (Pierce Chemical Co., Rockford, IL) as standard.

Binding Assay. Interaction mixtures (80 μL final volume) contained 40 μL of freshly collected crude saliva (proteins = 1.3 mg mL^{-1}) and different amounts of tannins, solubilized in 40 μL of 10% ethanol (v/v). Binding assays were performed in tubes maintained at 25 °C for 5 min. The mixture was then centrifuged for 10 min at 10000*g*. The analyses were performed on the resulting supernatant and pellet.

Electrophoresis. Samples mixed with an equal volume of 2× electrophoresis sample buffer (0.125 M Tris-HCl, 4% SDS; 20% v/v glycerol, 0.2 M DTT, 0.02% bromophenol blue, pH 6.8) and heated at 100 °C for 5 min were analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) according to the method of Laemmli (1970) using 12.5% acrylamide separating gels. The stacking gels were 4% acrylamide; the acrylamide *N*,*N*′-methylenebis(acrylamide) ratio was 30:08 (Bioprobe, Montreuil-sous-bois, France). Electrophoreses were performed on a Bio-Rad Protean IIxiCell electrophoresis apparatus (Hercules, CA) using a Gen-Apex power supply set at 25 mA/gel for stacking gel and 35 mA/gel for separating gel. The gels were fixed with a mixture of ethanol, acetic acid, and deionized water (40:10:50) for 1 h. After a water wash of 5 min, the gels were soaked in a 10% gluteraldehyde solution for 30 min, followed by extensive deionized water washes. The gluteraldehyde fixation step is essential for retaining small proteins in the gel throughout the staining procedure (Heukeshoven and Dernick, 1988; Austin et al., 1989). The proteins were then stained with silver nitrate (Hochstrasser et al., 1988) or with Coomassie Brillant Blue R250 (0.1% in 25% methanol, 10% acetic acid). The Coomassie Blue destaining step by incubation in 10% acetic acid (methanol or ethanol was omitted) distinguishes PRP, which stain pink-violet, from other proteins, which stain blue.

Molecular weights were estimated by comparison with the migration rates of standard proteins (low molecular weight protein kit, Pharmacia LKB Biotechnology AB, Uppsala, Sweden).

Thioacidolysis. The samples (20 μL of the supernatants or total mixtures added to 80 μL of methanol, pellets redissolved in 100 μL of methanol containing 0.2% of SDS) were introduced into a glass ampule together with an equal volume of a solution of toluene-α-thiol (5%) in methanol containing HCl (0.2 M). After sealing, the mixture was shaken and heated at 90 °C for 2 min. The hydrolyzed solution was then analyzed directly by HPLC under the following conditions on a Nucleosil C18 3 μm (125 × 4 mm) (Macherey-Nagel, Düren, Germany): flow rate, 0.8 mL min^{-1}; temperature, 30 °C; solvent A, HCOOH 2% in H_2O; solvent B, CH_3CN/H_2O/HCOOH (80:18:2). A linear gradient performed from 85 to 35% of A during 15 min allowed elution of the reaction products followed by column washing and reconditionning steps.

Calibration curves (based on peak areas at 280 nm) were established using flavan-3-ol and benzyl thioether standards. The latter were prepared by semipreparative HPLC after thiolysis of grape seed and skin extracts (Prieur et al., 1994; Souquet et al., 1996).

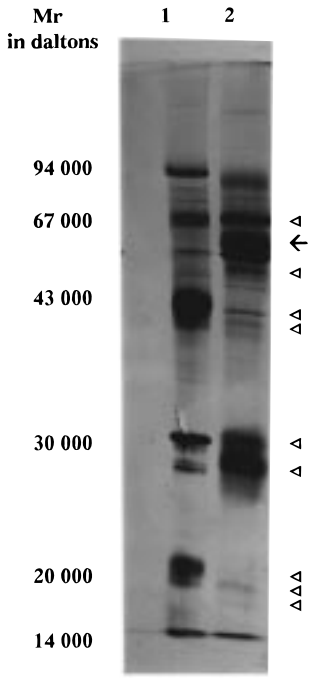

Figure 1. SDS−PAGE of human salivary proteins (PS): lane 1, molecular weight markers; lane 2, saliva sample (19.5 μg); triangles point to proteins indicated. PRP stained violet pink, and the arrow indicates α-amylase.

RESULTS AND DISCUSSION

Characterization of the Salivary Proteins. SDS−PAGE is widely used for the analysis of protein-containing fluids and is well suited for the study of human salivary secretions (Beeley et al., 1991). Saliva from many species, including human, contains a family of so-called proline-rich proteins (PRP). They are reported to account for ∼70% of total proteins secreted in human parotid saliva. However, whole saliva has yielded variable results with reduced or absent levels of PRP.

Thus, 12% acrylamide gel SDS−PAGE electrophoresis followed by Coomassie Blue staining or silver staining was carried out. In agreement with Beeley et al. (1991), there was substantial variation among individuals in the band patterns which for each individual tested were independent of the time of day and were constant over several months. Thus, the whole saliva from the volunteer presenting the richest protein pattern detected by electrophoresis was selected for these experiments and was freshly collected on the day of experimentation (to avoid degradation during storage).

The electrophoregram (Figure 1, lane 2) showed a band pattern similar to those reported by Beeley (1993). The apparent molecular weight (MW) estimated by migration in SDS−polyacrylamide gels varied from 16 000 to 90 000 as shown by Kauffman and Keller (1983). The major signal around 67 kDa corresponds to the major glycosylated PRP and α-amylase, which

Table 1. Characteristics of Grape Seed Condensed Tannins Determined by Thiolysis in Methanol

monomer	abbrev	terminal units (%)	extension units (%)
(+)-catechin	c	5.35	8.50
(−)-epicatechin	Ec	3.40	58.00
(−)-epicatechin 3-*O*-gallate	EcG	8.80	15.80

stained blue, can be resolved into two bands that correspond to the glycosylated and the nonglycosylated forms (62 and 57 kDa, respectively). The other bands, which stained pink-violet, correspond to PRP (52, 42, 37, 30, 28, 20, 19, and 16 kDa, arrows in Figure 1).

Characterization of Grape Seed Proanthocyanidin Fractions. The preparative Fractogel chromatography is convenient to isolate oligomeric and polymeric tannins from the crude seed acetonic/water extract. The structures of the condensed tannins (i.e., proanthocyanidins) are characterized by the nature of their constitutive extension and terminal units and their degree of polymerization (DP, number of units in the polymer). Thiolysis reaction (acid-catalyzed degradation in the presence of toluene-α-thiol) allows distinction between extension units (released as benzyl thioethers) and terminal units (released as flavan-3-ols), which can be separated by HPLC (Rigaud et al., 1991). Moreoever, it preserves gallic ester moieties of proanthocyanidins, giving access to their average composition and mean DP (mDP, ratio between the summed concentrations of all released units and the summed concentrations of terminal units after thiolysis). The fraction obtained was monomer free and stable for 6 months as checked by thiolysis analyses. The average characteristics of the grape seed proanthocyanidin fraction used in these experiments are shown in Table 1. (+)-Catechin (c), (−)-epicatechin (Ec), and epicatechin 3-*O*-gallate (EcG) were found as both extension and terminal units, indicating that the analyzed condensed tannins were partly galloylated procyanidins. Epicatechin predominated in the extended chains and was the major component of these procyanidins. The mDP of the grape seed fraction determined by means of thiolysis was 5.7, and the average proportion of galloylated units was 24.6, in agreement with previous data (Prieur et al., 1994).

Binding Assay. The ability of tannins to interact with proteins was investigated by use of a model system containing grape seed condensed tannins and crude saliva from the volunteer. Saliva samples were used without purification to represent buccal physiological conditions. Likewise, tannins were dissolved in a 10% ethanol solution to mimic hydroalcoholic medium close to wine. As tannin−protein interactions are reported to be pH dependent, the pH value of the binding mixture was measured and found to be 6.5, indicating that saliva buffered the mixture.

In all cases, the amounts of tannins in the supernatant and in the pellet must account for the initial amount added to the binding mixture. Preliminary experiments directly on supernatants and pellets showed that the procyanidins in the pellets were not accessible under the classical thiolysis conditions because of their inability to solubilize the pellet and certainly to dissociate complexes. Thus, thiolysis of pellets was carried out in the presence of SDS. Grape seed proanthocyanidins were totally recovered in the fractions analyzed after binding experiments (Table 2), suggesting that the compositions of supernatants, pellets, and initial mixtures can be determined and compared.

Table 2. Procyanidin Content (Micrograms) and Recovery in the Supernatant and in the Pellet after Binding with Salivary Proteins

initial amount	5	6.25	7.5	12.5	18.75	25
pellet amount (P)	2.89	2.98	4.58	10.7	12.87	16.38
supernatant amount (S)	1.97	2.63	3.2	1.3	5.5	6.74
P + S	4.86	5.6	7.7	12	18.4	23.12
% of recovery	97.2	89.7	104	96.8	98	92.5

The SDS treatment allows the dissociation of the insoluble tannin−protein complexes and provides a simple method to give access to tannins and proteins by means of thiolysis and SDS−PAGE, respectively.

Characterization of Tannin-Binding Proteins. Proteins are the only components of the model system that are detected on gel electrophoregram. The Coomassie Blue and silver nitrate staining were directly related to the amount of proteins in the mixture. Even though tannins induced a higher background for silver nitrate staining, this procedure is a more sensitive method that allows detection of proteins in nanogram quantities. Thus, both procedures were applied in parallel.

The analyses of the total protein pattern of binding mixture (before centrifugation) presented in Figure 2A, lanes 3−7, showed the major proteins of whole saliva (Figure 2A, lane 2, and Figure 2B, lane 8), suggesting that the tannin−protein complexes were dissociated under these denaturing conditions. Moreover, the similar intensity of the bands showing that the amounts of proteins were quite equal in all of the samples demonstrated that the method led to total recovery of proteins. Thus, this staining procedure can be used to estimate differences between samples. No precipitation occurred in the control mixture in which tannins were omitted (Figure 2A, lane 8, and Figure 2B, lane 2). The supernatant patterns (Figure 2A, lanes 8−12) showed a decrease of salivary protein content as the amount of tannins was increased in the binding mixtures. Moreover, some proteins were totally excluded from the supernatant after tannin treatment (42, 30, 28, 20, 19, and 16 kDa). The protein removals from the supernatants were concomitant with their recoveries in the pellet (Figure 2B, lanes 3−6). As previously observed by Austin (1989), very small amounts of tannins were required to precipitate proteins and detect them in the pellet. The lowest molecular weight PRPs were removed from the supernatant and recovered in the pellet with an intensity similar for each tested tannin amount, indicating that they were preferentially precipitated by tannins, whereas the α-amylase (57 kDa), the glycosylated α-amylase (62 kDa), and the glycosylated PRP (67 kDa) were removed more gradually. Moreover, the analyses of the supernatant allowed the detection of very low molecular weight proteins running at the dye front of the gel. These data are in agreement with previous work of Yan and Bennick (1995), which showed the precipitation of low molecular weight proteins running at the dye front of the gel, especially when small amounts of tannins were present. These proteins were identified as histatins. The pattern obtained with grape seed tannins was similar to that observed by Yan and Bennick (1995) in which the PRP precipitation also occurred at higher levels of tannins. The low molecular weight proteins and the PRPs were shown to be predominantly precipitated by grape seed condensed tannins. The specificity of interaction is a function of the size, conformation, and charge of the protein molecule. The high affinities of PRPs for tannin are at least partially due to their open conformations and their

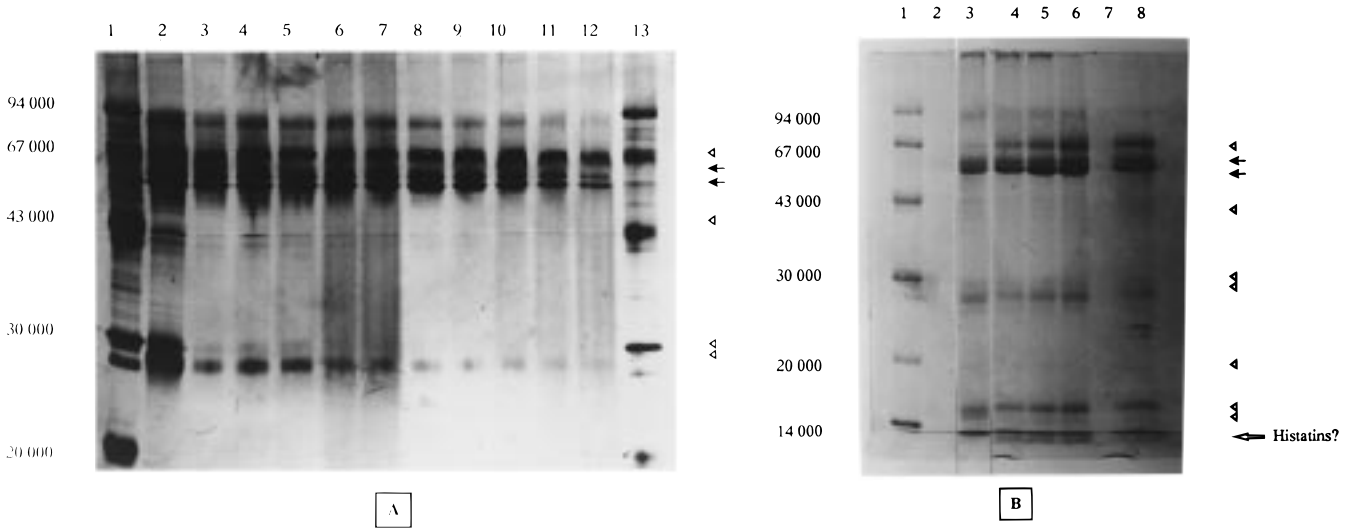

Figure 2. SDS−PAGE of binding mixture obtained with human salivary proteins and grape seed condensed tannins [saliva samples (40 μL) were mixed with different amounts of condensed tannins for 5 min at 25 °C]. (A) Silver nitrate staining of lanes 1 and 13, molecular weight markers (protein load = 17 μg); lane 2, saliva (protein load = 19.5 μg); lanes 3−7, total binding mixture prior to centrifugation containing, respectively, 0, 6.25, 12.5, 18.75, and 25 μg of condensed tannins (protein load = 6.5 μg); lanes 8−12, supernatant obtained after binding mixture centrifugation (theoretical protein load = 6.5 μg). (B) Coomassie blue staining of lane 1, molecular weight markers (protein load = 17 μg); lane 8, saliva (protein load = 39 μg); lanes 2−6, pellet obtained after binding mixture centrifugation containing, respectively, 0, 6.25, 12.5, 18.75, and 25 μg of condensed tannins (maximum protein load = 39 μg). Triangles point to proteins indicated. PRP stained violet pink, and the arrow indicates α-amylase.

capacities to form hydrophobic interactions and strong hydrogen bonds with tannins (Hagerman and Robbins, 1993; Haslam, 1996). The different behaviors of high molecular weight and/or glycosylated proteins suggest that the size and the presence of sugar moieties tend to impede binding to tannins or to favor the formation of soluble complexes which precipitate only when the amount of tannins increases. Hagerman (1989) noticed that larger proteins tend to bind tannin more tightly, although small proline-rich polymers are preferred over large proline-poor molecules. Turbidimetry experiments which detected both soluble and insoluble complexes (Asquith et al., 1987) have shown that the solubility of tannin−peptide complexes is greater with glycosylated peptides, but competitive binding experiments (Hagerman and Klucher, 1986) have suggested that glycosylation may protect proteins from interacting with tannins. In our work, we showed that large and/or glycosylated proteins were precipitated with even small amounts of condensed tannins but that a higher concentration of tannins was needed to precipitate a larger amount of proteins, suggesting that glycosylation did not impede tannin affinity. The soluble glycosylated protein−tannin complexes might be present in the supernatant, but SDS electrophoresis was unable to discriminate between free proteins and proteins involved in the soluble complexes. Their gradual decrease in the supernatant suggests that the increase of tannins led to insolubilization of these complexes. Their precipitation, when tannins were in excess, may be due to the formation of hydrophobic tannin-coated proteins and cross-linked tannin−protein complexes (Spencer et al., 1988).

Moreover, our results suggest that the ratio between protein and tannins may influence the rate and the nature of the precipitated proteins as noticed for haze formation by Siebert et al. (1996).

Characterization of Protein-Binding Tannins. The abilities of grape seed procyanidins to precipitate with saliva have been quantitatively studied (Figure 3). As

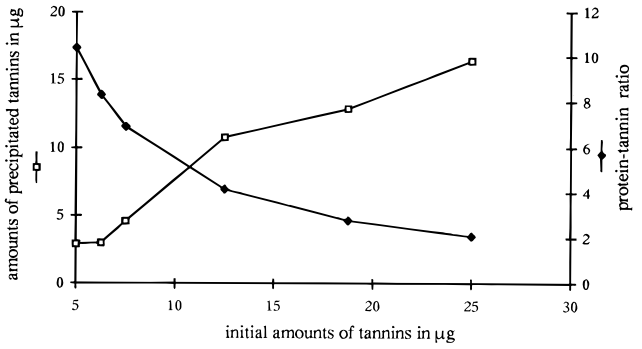

Figure 3. Precipitation of grape seed tannins in the tannin−salivary protein binding assays. Saliva samples (40 μL) were mixed with different amounts of condensed tannins for 5 min at 25 °C, final volume 80 μL: □, amount of precipitated tannins; ◆, ratio between initial protein amount and initial tannin amount.

Table 3. Average DP of the Initial Grape Seed Tannins, Precipitated and Nonprecipitated Fractions Obtained after Binding with Salivary Proteins[a]

initial tannin amount	5	6.25	7.5	12.5	18.75	25
protein/tannin ratio	10.4	8.32	6.93	4.16	2.77	2.08
mDP in the pellet	5.66[a]	6.58[a]	6.30[a]	6.75[a]	7.17[b]	7.43[b]
mDP in the supernatant	2.25[b]	2.62[b]	2.89[b]	2.63[b]	3.19[c]	3.61[c]
mDP of initial condensed tannins	5.74[a]	5.74[a]	5.74[a]	5.74[a]	5.74[a]	5.74[a]

[a] Means with different letters in a column differ ($p < 0.05$)

in the protein control (without tannins), the omission of proteins in the tannin control did not allow the formation of a precipitate. The amount of precipitated tannins increased as their initial amount increased, indicating an increase in the number of tannin molecules bound to salivary proteins.

The average procyanidin DP in the pellet and supernatant (Table 3) showed that the largest procyanidins were recovered in the pellet while the mDP in the supernatant corresponded to low molecular weight tannins. At low tannin concentrations, the procyanidins

recovered in the pellets were not significantly different from those of the initial mixture and only dimers and trimers remained in solution, meaning that at this tannin−protein ratio (4.16), the precipitation affected all procyanidins except the lower molecular weight molecules, as reported by Cheynier et al. (1997). When the amount of added tannins was increased (to >13 μg of tannins, ratio 4.16), selective precipitation of the higher molecular weight procyanidins was observed. The increase of mDP in the supernatant suggests that the precipitation of the large molecular weight procyandins protects dimers and trimers from precipitation. As well, recent studies on red wine fining with gelatins (Sarni-Manchado et al., 1998), which are archetypal PRP, have demonstrated that wine condensed tannins recovered in the pellet presented higher mDP than the initial wine, meaning that the largest procyanidins were removed predominantly from wine by interacting with gelatins at low protein−tannin ratio (0.12). In that case, the level of precipitation was higher. These data were in agreement with previous works (Haslam, 1974; Artz et al., 1987) which suggested that the most polymerized proanthocyanidins (i.e., trimer) bind more tightly to proteins than the lower DP tannins, suggesting that lower amounts of larger tannins are required to precipitate the same amounts of proteins. The precipitation of large procyanidins, while the dimeric and trimeric structures are still free in the supernatant, suggests that the affinity of procyanidins for salivary PRPs may be related to the number of monomeric units as it has been demonstrated for galloyl esters by several methods including precipitation of haemoglobin from aqueous solution (Bate-Smith, 1973). Use of NMR experiments (Murray et al., 1994; Baxter et al., 1997) has shown that the larger and more hydrophobic polyphenols bind more strongly to PRP and that some of the binding interactions for the larger polyphenols may arise from multidentate association. The interaction has been interpreted as hydrophobic association between phenolic rings and proline residues stabilized by hydrogen bonds. The number of active sites (phenolic and *o*-diphenolic groups) increases with the DP and can help to form complexes. The long-chain polyphenol can act as potential multivalent adaptaters able to cross-link both proteins and other polyphenol molecules as suggested by Baxter et al. (1997).

Our data confirmed the impact of the protein−tannin ratio on both the amount and the nature of proteins and tannins precipitated. The lower the ratio (i.e., more tannin, less protein), the higher the mDP of the precipitated tannins, the larger the yield of protein precipitated, but with a lower selectivity for proteins.

CONCLUSION

The purpose of our work was to evaluate the precipitation of tannins and salivary proteins, and it was therefore necessary to develop suitable methods. Protein−tannin interactions were approached by using precipitation models between salivary proteins and grape seed condensed tannins. The extent of interaction was estimated by assaying tannins remaining after the removal of the insoluble protein−tannin complexes but also in the insoluble complexes. Proteins were determined by means of electrophoresis and condensed tannins by thiolysis. We demonstrated that the use of SDS and thiolysis methods allowed sensitive qualitative and quantitative measurements of precipitated and nonprecipitated condensed tannins after reactions with proteins. Thus, this approach is helpful for the study of the precipitated and nonprecipitated tannins and salivary proteins.

The high molecular weight procyanidins (7.43) were selectively precipitated by the salivary proteins, whereas the dimeric and trimeric oligomers remained in solution. The larger molecular weight tannins interacted more readily with proteins, thus protecting dimers and trimers. When small amounts of procyanidins were present, the lower molecular weight PRP and other low molecular weight proteins (probably histatins) had the highest ability to precipitate with tannins. The human PRP's ability to complex tannins may thereby minimize their detrimental biological effects and influence astringency. Moreover, recent studies established that PRP expression, which is enhanced by tannin exposure, is under regulatory elements, among which an isoprenaline/tannins-dependent regulatory region was found (Ann et al., 1997). Therefore, tannin stimulation of PRP synthesis may decrease astringent sensation by both increasing the flow rate of saliva and trapping polyphenols. Tannins trapped by PRPs were not free to interact readily with the glycosylated proteins (67 kDa), which reportedly contribute greatly to the lubricating power of saliva (Hatton et al., 1985). Increasing the amounts of tannins leads to larger precipitation of proteins, in particular that of glycosylated PRP. In that way, the presence of a large amount of tannins in a wine sensed as astringent, corresponding to a low salivary protein−tannin ratio in mouth, could favor the precipitation of the glycosylated PRP.

As protein−tannin interactions and probably those related to astringency do not only involve insoluble complexes, our further objective is to develop methodology to evaluate and characterize soluble complexes and consequently to achieve better understanding of the mechanisms responsible for the astringent character of tannins.

ABBREVIATIONS USED

DP, degree of polymerization; DTT, dithiothreitol; PRP, proline-rich protein; HPLC, high-performance liquid chromatography; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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Received for review May 15, 1998. Revised manuscript received October 20, 1998. Accepted October 21, 1998.

JF9805146