Phytochemistry, Vol. 25, No. 7, pp. 1591–1593, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

INTERACTIONS OF CONDENSED TANNINS WITH SELECTED PROTEINS

THOMAS N. ASQUITH* and LARRY G. BUTLER

Department of Biochemistry, Purdue University, West Lafayette, IN 47907, U.S.A.

(*Received* 15 *November* 1985)

Key Word Index—Protein binding; binding specificity; tannins.

Abstract—The relative affinities of condensed tannins purified from sorghum, pinto bean, quebracho and wattle for six dissimilar proteins have been determined by a competitive binding assay. The results indicate that tannin/protein interactions may be specific for different tannins as well as for different proteins. The highly specific interactions suggest that the differences in affinity are functionally significant.

INTRODUCTION

Tannin/protein interactions are potentially important in such diverse phenomena as plant resistance to herbivores [1], herbivore nutrition [2] and fruit or seed maturation [3]. The precise role of tannins in plants, and their mechanism of action in animals, is uncertain. An important clue to the function of condensed tannins was provided by the demonstration that sorghum tannin is highly selective in its binding to proteins [4]. *In vitro* binding specificity implies that tannins interact with specific subsets of proteins or other macromolecules within their particular microenvironments. Tannin-binding salivary proteins [5] share some biochemical characteristics with tannin-associated protein from sorghum grain [6]. Proteins with high affinity for tannin tend to have open, loose conformations, high molecular weights, and high contents of proline and other hydrophobic amino acids [7].

Comparison of the interaction of several tannins with a single standard protein showed that the capacity of procyanidins to precipitate hemoglobin is a function of polymer chain length [8], whereas conformational mobility seems to be the determining factor in the binding of hydrolysable tannins to bovine serum albumin (BSA) [9]. There have, however, been no extensive comparisons of the binding affinities of different tannins for selected proteins. We report here the relative affinities of a set of six dissimilar proteins for condensed tannins purified from four different sources. The results show that tannin/protein interactions are tannin-specific as well as protein-specific.

RESULTS AND DISCUSSION

Table 1 lists the biochemical characteristics of tannins purified from *Sorghum bicolor* Moench, *Phaseolus vulgaris* (pinto bean), *Shinopsis lorentzii* (quebracho) and *Acacia mearnsii* (wattle). Sorghum and pinto bean tannins are procyanidins [10, 11] whereas quebracho and wattle tannins are primarily profisetinidins [12]. The tannins differ up to five-fold in their chemical assay values, but differ only two-fold in their relative chain lengths. Wattle and quebracho tannins have longer relative chain lengths but lower protein precipitation activities than sorghum tannin, whereas pinto bean tannin is shorter and precipitates less BSA than sorghum tannin (Table 1). This indicates that tannin chain length may not be the only criterion associated with protein precipitation.

Differences in the solubilities of the tannin/protein complexes may be due to differences in tannin secondary-tertiary structure. The preferred conformation of proanthocyanidins are compromises between solvent exclusion and steric interactions [13]. Procyanidins are thought to assume fairly rigid, helical conformations [14] while profisetinidins may assume more globular, flexible conformations [15]. Binding of ligands to proteins is known to change the conformation of the protein, and tannins may also undergo conformational changes upon binding to proteins, with resulting diminished solubility.

Application of competitive binding techniques to tannin–protein interactions led to the demonstration that sorghum tannin discriminates between 'high affinity' and 'low affinity' proteins [4]. This approach has been extended to survey the binding affinity of four condensed tannins for selected proteins (Table 2). The relative affinity values (see Experimental) of the tannins for the proteins are in good agreement with those reported by Hagerman and Butler [4]. Quebracho, wattle and pinto bean tannins exhibit the same ability as sorghum tannin to selectively bind gelatin in the presence of excess BSA. They also have little measureable affinity for ovalbumin. Further, all the tannins have high affinity for fetuin and GP-66 sm, a mouse salivary proline-rich protein [16], and very low affinity for soybean trypsin inhibitor or dextrans. However, quebracho tannin has a much higher affinity for GP-66 sm than do any of the other tannins, and only pinto bean tannin has a measurable affinity for soybean trypsin inhibitor.

The data indicate that most, if not all, condensed tannins can distinguish between 'high affinity' and 'low affinity' proteins. All four tannins have the same rank order of affinity for the proteins (i.e. gelatin > BSA > ovalbumin).

*Present address: Procter & Gamble Beverage Division, Winton Hill Technical Center, 6250 Center Hill Road, Cincinnati, OH 45224, U.S.A.

Table 1. Chemical characterization of purified tannins

Tannin	Vanillin* A_{520}/mg	Proantho-cyanidin† A_{550}/mg	Chain length‡	Protein pptn activity§ (μg BSA)
Sorghum	3.05	2.75	4.2	82
Pinto bean	3.79	2.13	2.9	39
Quebracho	0.75	0.88	5.1	22
Wattle	1.13	1.60	5.9	25

*100 μg of tannin assayed as described in ref. [20].
†100 μg of tannin assayed as described in ref. [19].
‡Calculated as described in ref. [21].
§Amount (μg) of [^{14}C]-BSA precipitated by 40 μg of purified tannins when added to 100 μg of [^{14}C]-BSA under the conditions described in Experimental.

Table 2. Relative affinities of proteins for condensed tannins*

Protein	Tannin			
	sorghum	quebracho	pinto bean	wattle
GP-66sm	4.5	12	3.3	3.4
Gelatin	5.0	5.0	4.0	3.0
Fetuin	5.5	7.5	—	2.0
BSA	1.0	1.0	1.0	1.0
Ovalbumin	0.07	0.05	0.10	0.125
Soybean trypsin inhibitor	nd†	nd	0.25	—
Dextran	nd	nd	nd	nd

*Relative affinity values were calculated as described in the text.
†Binding of the competitor to tannin was not detectable by this assay.

All had high affinities for gelatin or GP-66sm and low affinities for ovalbumin or soybean trypsin inhibitor. This implies that the tannins interact with proteins by similar mechanisms.

However, there are significant differences in the affinity of the tannins for two of the proteins. Quebracho tannin has a much higher affinity for GP-66 sm than do the other tannins. Likewise, pinto bean tannin has higher affinity than the other tannins for soybean trypsin inhibitor. This suggests that although condensed tannins are somewhat similar, each seems to be uniquely suited to bind tightly to a limited number of proteins. The unique affinity of GP-66 sm for quebracho tannin is linked to the oligosaccharide moieties of the native protein. The deglycosylated protein has the same affinity for sorghum and quebracho tannins [17]. The relatively small size of pinto bean tannin may allow it to interact strongly with the rigid, β-barrel structure [18] of soybean trypsin inhibitor.

The results presented here strongly suggest that tannins are rather specific even in their interactions with proteins which have high affinity for tannins. Because this specificity is likely to be a reflection of physiological function, interactions of tannins and proteins must be evaluated individually. Tannins are not merely universal protein binding agents.

EXPERIMENTAL

Tannin assays. Purified tannins were dissolved in MeOH (1 mg/ml) and assayed using the direct proanthocyanidin [19] and the vanillin–glacial acetic acid [20] assays. Their chain lengths were calculated as outlined [21]. Protein precipitation values were determined by adding 40 μg of purified tannin to 100 μg of [^{14}C]-BSA in 100 μl of 0.2 M acetate (pH 4.8).

Tannin purification. Sorghum and pinto bean tannins [6] and quebracho and wattle tannins [22] were purified as described.

Competitive binding assays. The method of Hagerman and Butler [4] was modified as follows: increasing amounts of competitor were mixed with a series of 100 μg samples of [^{14}C]-BSA in a total vol. of 640 μl of 0.2 M acetate (pH 4.8). To these were added 160 μl of MeOH containing 20–40 μg of tannin (enough to ppt 75% of the labelled protein in the absence of any competitor). After vortexing and centrifuging (1000 *g*, 5 min), the supernatants were carefully removed and the pellets dissolved in 2 × 100 μl vols of 1% (w/v) sodium dodecyl sulphate and counted in 4.0 ml of scintillation cocktail. Relative affinity is defined as the amount of labelled standard protein in the assay divided by the amount of competitor needed to inhibit the precipitation of the standard protein by 50% [22]. As much as possible, each tannin was assayed against all the competitors on the same day. Each set of assays was normalized to 100% precipitation by including a blank which had no competitor.

Preparation of mouse salivary proline-rich protein. This protein, GP-66 sm, was obtained as outlined [16].

Acknowledgements—Supported in part by USAID/INTSORMIL and USAID/PSTC. Journal of Paper Number 10521 from the Agricultural Experiment Station, Purdue University.

REFERENCES

1. Schultz, J. C. (1983) *Plant Resistance to Insects*, p. 37. Am. Chem. Soc., Washington DC.
2. Lindroth, R. L. and Batzli, G. O. (1983) *Biochem. Syst. Ecol.* **11**, 405.
3. Josyln, M. A. and Goldstein, J. L. (1964) *Adv. Food Res.* **13**, 179.
4. Hagerman, A. E. and Butler, L. G. (1981) *J. Biol. Chem.* **256**, 4494.
5. Mehansho, H., Hagerman, A. E., Clements, S., Butler, L. G., Rogler, J. and Carlson, D. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2948.
6. Hagerman, A. E. and Butler, L. G. (1980) *J. Agric. Food Chem.* **28**, 944.
7. Hagerman, A. E. (1980) Ph.D. Thesis, Purdue University.
8. Porter, L. J. and Woodruffe, J. (1984) *Phytochemistry* **23**, 1255.
9. McManus, J. P., Davis, K. G., Beart, J. E., Gaffney, S. H., Lilley, T. H. and Haslam, E. (1985) *J. Chem. Soc. Perkin Trans.* 2, 1429.
10. Gupta, R. K. and Haslam, E. (1978) *J. Chem. Soc. Perkin Trans.* 1, 892.
11. Asquith, T. N. (1985) Ph.D. Thesis, Purdue University.
12. Roux, D., Ferreira, D. and Botha, J. J. (1980) *J. Agric. Food Chem.* **28**, 216.
13. Foo, L. Y. and Porter, L. J. (1983) *J. Chem. Soc. Perkin Trans.* 1, 1535.
14. Fletcher, A. C., Porter, L. J., Jacques, D., Haslam, E. and Tanner, R. J. N. (1977) *J. Chem. Soc. Perkin Trans.* 1, 1628.
15. Hemingway, R. W., McGraw, G. W., Karachesy, J. J., Foo, L. Y. and Porter, L. J. (1983) *J. Appl. Poly. Sci.* **37**, 967.
16. Mehansho, H., Clements, S., Sheares, B., Smith, S. and Carlson, D. (1985) *J. Biol. Chem.* **260**, 4418.
17. Asquith, T. N., Uhlig, J., Mehansho, H., Putman, L. J., Carlson, D. and Butler, L. G., *J. Biol. Chem.* (submitted).
18. Richardson, J. S. (1981) *Advances in Protein Chemistry*, Vol. 34, p. 168. Academic Press, New York.
19. Watterson, J. J. and Butler, L. G. (1983) *J. Agric. Food Chem.* **31**, 41.
20. Butler, L. G., Price, M. L. and Brotherton, J. E. (1982) *J. Agric. Food Chem.* **30**, 1087.
21. Putman, L. J. and Butler, L. G. (1985) *J. Chromatogr.* **318**, 85.
22. Asquith, T. N. and Butler, L. G. (1985) *J. Chem. Ecol.* **11**, 1235.