J Chem Ecol (2006) 32: 1149–1163
DOI 10.1007/s10886-006-9077-0

REVIEW ARTICLE

Salivary Proteins as a Defense Against Dietary Tannins

Takuya Shimada

Received: 27 December 2005 / Revised: 18 February 2006 /
Accepted: 2 March 2006 / Published online: 23 May 2006

Abstract Tannins, a diverse group of water-soluble phenolics with high affinity to proteins, are widely distributed in various parts of plants, and have negative effects in herbivores after ingestion. Some mammalian species are thought to counteract tannins by secreting tannin-binding salivary proteins (TBSPs). Several types of TBSPs are found in the saliva of laboratory animals, livestock, and wildlife. Among them, proline-rich proteins (PRPs) and histatins are effective precipitators of tannins. It is widely accepted that, at the least, PRPs act as a first line of defense against tannins. Many observations support this idea: *in vitro* affinity of PRPs to tannins is far higher than that of other proteins such as bovine serum albumin; complexes formed between PRPs and tannins are stable even under the conditions in the stomach and intestine; and PRP production is induced by ingesting tannins. It is believed that species that usually ingest tannins as part of their natural diets produce high levels of PRPs, whereas species not exposed to tannins produce little or no PRPs. This hypothesis is generally supported, although studies on TBSPs in wildlife are limited. This work stresses the importance of gathering basic information on such items as the characteristics of unidentified TBSPs, and seasonal and geographical variations in PRP production.

Keywords Affinity to tannins · Defense mechanisms against tannins · Feeding niche · Histatins · Nitrogen costs · Proline-rich proteins (PRPs)

Introduction: Tannins and Mammalian Countermeasures

Tannins, which are among the most widespread plant secondary metabolites (PSMs), are a diverse group of water-soluble phenolic compounds with high affinity for proteins. They are broadly distributed in various parts of plants (Blytt et al., 1988; Bernays et al., 1989; Waterman and Mole, 1994). On the basis of their chemical structures, tannins can be divided into two major groups: condensed and hydrolyzable (Zucker, 1983). Condensed tannins are oligomers and polymers of

T. Shimada (✉)
Kansai Research Center, Forestry and Forest Products Research Institute,
Momoyama, Kyoto 612-0855, Japan
e-mail: tshmd@affrc.go.jp

flavan-3-ols that are linked by C–C bonds and thereby difficult to hydrolyze. In contrast, hydrolyzable tannins are esters of polyols (usually glucose) with simple phenolic acids, such as gallic acid or hexahydroxydiphenic acid, and are readily hydrolyzed by acidic or basic conditions as well as by esterase enzymes.

Ingestion of tannins is known to cause various types of negative effects in mammalian herbivores, such as reduction in digestibility (Robbins et al., 1987; Chung-MacCoubrey et al., 1997; Shimada and Saitoh, 2003), damage to the gastrointestinal mucosa and epithelium (Blytt et al., 1988), kidney or liver failure (Fowler and Richards, 1965), and endogenous nitrogen loss (Blytt et al., 1988; Shimada and Saitoh, 2003). It has long been believed that tannins primarily act as protein digestion inhibitors by binding dietary proteins and digestive enzymes (see review by Zucker, 1983). However, recent studies have revealed that tannins do not bind to digestive enzymes in *in vivo* situations, because digestive enzymes are usually protected from tannins by occurring in a particulate membrane-bound form (Blytt et al., 1988; Bernays et al., 1989; Skopec et al., 2004). Instead, ingested tannins probably bind salivary proteins and the mucosa and epithelium of the gut. This may cause endogenous nitrogen loss, thereby reducing protein digestibility. Moreover, phenolic acid released by hydrolysis of tannins can be absorbed from the intestine and cause acute toxicity, such as necrosis and kidney and liver failure (Fowler and Richards, 1965; Niho et al., 2001). For these reasons, tannins should now be regarded not as digestion inhibitors that act relatively mildly, but as toxins that have acute negative effects on herbivores.

Animals can use both preingestive and postingestive countermeasures against dietary tannins. Avoiding highly tanniferous species or parts of plants is the simplest preingestive countermeasure. This behavior has been frequently reported in various taxa, such as ungulates (Cooper and Owen-Smith, 1985), rodents (Shimada and Saitoh, 2003), lagomorphs (Clausen et al., 1990; Dearing, 1997), and primates (Takemoto, 2003). Some herbivores have been shown to regulate their rates of intakes of tannins or other PSMs in order to circumvent overloading of one detoxification pathway (Dearing and Cork, 1999; Burritt and Provenza, 2000; Foley and Moore, 2005). Furthermore, reduction of tannin levels in plants through leaching during the hoarding period can be considered as a preingestive countermeasure (Dearing, 1997). Postingestive countermeasures, which mammalian herbivores are known to use, include the secretion of tannin-binding proteins in saliva, increased gastrointestinal mucus production, degradation of tannins by microorganisms in the gut, activation of detoxifying enzymes, and increased capacity of intestinal permeability glycoprotein (see reviews by McArthur et al., 1991; Dearing et al., 2005). These are thought to be key elements in overcoming, or at least mitigating, the negative effects caused by tannins.

This review concerns tannin-binding salivary proteins (TBSPs) as a defense against dietary tannins. TBSPs, which are defined as proteins secreted in saliva that have high affinity for tannins, can bind readily to dietary tannins in the oral cavity and are thought to prevent the tannins from interacting with other proteins (McArthur et al., 1995; Bennick, 2002). TBSPs interact with ingested tannins in the first stage of digestion. This feature stresses the importance of TBSPs in counteracting tannins, because the behavior of ingested tannins in the gastrointestinal tract may be inevitably regulated by these early encounters with TBSPs. In the last two decades, a considerable number of studies have been undertaken on TBSPs (Ann and Carlson, 1985; Asquith et al., 1985; Austin et al., 1989; Robbins et al., 1991;

Glendinning, 1992; Carlson, 1993; Hagerman and Robbins, 1993; Bacon and Rhodes, 2000; Gehrke, 2001; Isemura, 2003). However, most have been conducted on humans and laboratory animals. This is partly because TBSPs originally attracted attention in the field of dental science and nutrition. As a consequence, information on TBSPs in wildlife is still limited and scattered. In addition, studies on TBSPs have been relatively biased toward *in vitro* data, such as genetics, amino acid compositions, protein structures, and *in vitro* affinity to tannins. Thus, the straightforward question—to what extent TBSPs would be effective in overcoming the potential negative effects of tannins in herbivores—is still open, even in laboratory animals.

The aims of this review are to summarize the information on TBSPs (obtained mostly in laboratory animals) and to discuss the significance to wildlife of producing TBSPs.

General Characteristics of Tannin-Binding Salivary Proteins

Two families of salivary proteins, proline-rich proteins (PRPs) and histatins, are generally recognized as TBSPs. However, there is evidence for the presence of other, different types of proteins. These are characterized by their high affinity to tannins, but they differ greatly in terms of molecular size, amino acid composition, structure, and taxonomic distribution in mammals.

Proline-Rich Proteins

PRPs were first detected in human saliva (Mandel et al., 1965). They have also been found in the saliva or salivary glands of several laboratory animals and wildlife (Table 1). In these mammals, PRPs are thought to be the most prevalent group of proteins in the saliva; PRPs account for 70% of all salivary proteins in humans (Mehansho et al., 1987b) and 74% of those in the Japanese wood mouse *Apodemus speciosus* (T. Shimada, unpublished data).

The molecular weights of PRPs are estimated to range from a low of 5000 to over 25,000 (McArthur et al., 1995; Bennick, 2002). As their name suggests, the amino acid composition of PRPs is unique in that the content of proline is markedly high. Proline comprises at least 20% of total amino acid content of PRPs (Kauffman and Keller, 1979; Mole et al., 1990). Some species, such as humans, rats, and mice, produce PRPs containing about 40% proline (Mehansho et al., 1983, 1985; Mole et al., 1990). In light of the fact that most types of proteins contain, at most, 5% proline (Schulz and Schirmer, 1979), PRPs have a markedly characteristic amino acid composition. They are also rich in glycine, glutamine, and glutamic acid. Mole et al. (1990) compared the amino acid compositions of PRPs from several species and found that in all cases the sum of the quantities of these four amino acids accounted for 70–90% of the total protein. These four amino acids, especially proline, are the so-called alpha-helix breakers, which prevent proteins from forming secondary structures. As a consequence, PRPs do not have any recognizable conformational structures, thereby existing in solution as extended random coils (Hagerman and Butler, 1981; Murray and Williamson, 1994). This feature may enable PRPs to universally bind various types of tannins that have a variety of shapes and sizes.

PRPs are encoded by tissue-specific multigene families. The nucleotide sequences of PRP cDNAs from mouse (Clements et al., 1985), rat (Ziemer et al., 1984), human

Table 1 List of mammalian species, in which the presence of tannin-binding salivary proteins (TBSPs) has been examined, and the summary of the results

Order	Species		Feeding niches	Types of TBSPs	Induced secretion[a]		Reference
	Common name	Scientific name			By tannins	By β-agonist	
Rodentia	Mouse	*Mus musculus*	Omnivore	PRPs[b]	yes	yes	Mehansho et al., 1985; Glendinning, 1992
	Rat	*Rattus norvegicus*	Omnivore	PRPs	yes	yes	Mehansho et al., 1983; Mehansho and Carlson, 1983; Mole et al., 1990
	Japanese wood mouse	*Apodemus speciosus*	Omnivore	PRPs	yes	yes	Shimada et al., 2004
	Root vole	*Microtus oeconomus*	Intermediate	PRPs	yes		Juntheikki et al., 1996
	Meadow vole	*Microtus pennsylvanicus*	Grazer	Not detected[c]		no	Dietz et al., 1994
	Hamster	*Mesocricetus auratus*	Omnivore	PRPs	no	yes	Mehansho et al., 1987a
	Beaver	*Castor canadensis*	Bark eater	Unidentified[d]			Hagerman and Robbins, 1993
Lagomorpha	Rabbit	*Oryctolagus cuniculus*	Intermediate	PRPs			Mole et al., 1990; Ferreira et al., 1992
	Mountain hare	*Lepus timidus*	Intermediate	PRPs			Mole et al., 1990
	North American pika	*Ochotona princeps*	Intermediate	Unidentified			Dearing, 1997
Artiodactyla	Sheep	*Ovis aries*	Grazer	Not detected Unidentified	no		Austin et al., 1989; Mole et al., 1990 Vaithiyanathan et al., 2001
	Musk ox	*Ovibos moschatus*	Intermediate	PRPs			Gehrke, 2001
	Moufflon	*Ovis orientalis*	Grazer	Unidentified			Gehrke, 2001
	Goat	*Capra hircus*	Intermediate	Unidentified			Vaithiyanathan et al., 2001
	Cattle	*Bos taurus*	Grazer	Not detected Other type[e]	no no		Austin et al., 1989; Mole et al., 1990 Makkar and Becker, 1998; Gehrke, 2001
	Fallow deer	*Dama dama*	Intermediate	Other type			Gehrke, 2001
	Mule deer	*Odocoileus hemionus*	Intermediate	PRPs	no		Austin et al., 1989; Hagerman and Robbins, 1993

	White-tailed deer	*Odocoileus virginianus*	Grazer	Other type		Mole et al., 1990
	Roe deer	*Capreolus capreolus*	Browser	PRPs		Fickel et al., 1998; Gehrke, 2001
	Moose	*Alces alces*	Intermediate	PRPs		Hagerman and Robbins, 1993; Juntheikki, 1996; Gehrke, 2001
	Reindeer	*Rangifer tarandus*	Intermediate	Unidentified		Gehrke, 2001
	Pig	*Sus sucrofa*	Omnivore	Not detected		Mole et al., 1990
Perissodactyla	White rhino	*Ceratotherum simum*	Grazer	Unidentified		Clauss et al., 2005
	Indian rhino	*Rhinoceros unicornis*	Intermediate	Unidentified		Clauss et al., 2005
	Black rhino	*Diceros bicornis*	Browser	Unidentified	yes	Clauss et al., 2005
Carnivora	Cat	*Felis catus*	Carnivore	Not detected		Mole et al., 1990
	Dog	*Canis familiaris*	Carnivore	Not detected		Mole et al., 1990
	American black bear	*Ursus americanus*	Omnivore	Unidentified		Hagerman and Robbins, 1993
Primates	Human	*Homo sapiens*	Omnivore	PRPs	no	Oppenheim, et al. 1971; Mole et al., 1990
				Histatins		Yan and Bennick, 1995
	Crab-eating macaque	*Macaca fascicularis*	Omnivore	PRPs		Oppenheim et al., 1979
				Histatins		Sabatini et al., 1989; Bennick, 2002
Marsupialia	Common brushtail possum	*Trichosurus vulpecula*	Browser	Not detected		Mole et al., 1990
	Common ringtail possum	*Pseudocheirus peregrinus*	Browser	Not detected		Mole et al., 1990
	Koala	*Phascolarctos cinereus*	Browser	Not detected		Mole et al., 1990

[a] These columns indicate the presence of inducibilty of TBSP synthesis by intake of tannins or by injection of a β-agonist.

[b] Proline-rich proteins (PRPs).

[c] Any types of TBSPs have not been found.

[d] The presence of TBSPs has been confirmed, but the detail characteristics have not been investigated.

[e] The presence of TBSPs, which belong neither to PRPs nor to histatins, has been confirmed.

(Maeda et al., 1985), and crab-eating macaque (Oppenheim et al., 1987), and PRP genes from mouse (Ann and Carlson, 1985), hamster (Ann et al., 1987), and human (Kim and Maeda, 1986) have been reported. All proteins encoded by cDNAs and genes share a common structure consisting of a signal peptide, a transition region, a repeat region, and a carboxyl terminal region. The single peptides and repeated regions exhibit marked homologies among these species, whereas the transition regions and carboxyl terminal regions vary largely (Ann and Carlson, 1985; Clements et al., 1985; Ann et al., 1987). In mice, the PRP gene family is located on chromosome 8 (Azen et al., 1984). In humans, the PRP family is encoded by only six genes located on chromosome 12 (Maeda, 1985; Maeda et al., 1985); two encoding acidic PRPs, and four encoding basic and glycosylated PRPs (Kim and Maeda, 1986). Many of the proproteins encoded by these genes are subsequently cleaved before secretion, giving rise to a large number of secreted PRPs (Lyons et al., 1988). Consequently, more than 20 PRPs are present in human saliva (Bennick, 2002).

PRPs are generally classified as basic (BPRPs) or acidic (APRPs) (McArthur et al., 1995; Bennick, 2002). APRPs are composed of two major regions: the core *C*-terminal region, comprising 70–80% of the molecule, and the highly acidic *N*-terminal region. BPRPs are structurally equivalent to the *C*-terminal region of APRPs. The *N*-terminal region of APRPs contains little proline, and thereby the proline level of the whole molecule is higher in BPRPs than in APRPs. In humans, BPRPs are secreted only from the parotid glands, whereas APRPs are secreted from all salivary glands, including the parotid, submandibular, and sublingual glands (Kauffman and Keller, 1979). A similar observation has been made in rabbits (Ferreira et al., 1992). This phenomenon may, therefore, be common in mammals that produce salivary PRPs.

BPRPs, in general, have a much higher affinity to tannins than APRPs (Lu and Bennick, 1998), and the main function of BPRPs is regarded as counteracting dietary tannins (Bacon and Rhodes, 2000; Chan and Bennick, 2001). In contrast, APRPs act primarily in maintaining oral homeostasis. An extensive argument for the evolution of the different types of PRPs can be found in the review by McArthur et al. (1995); they hypothesized that the ancestral type is APRPs whose main function is maintaining oral homeostasis, and that BPRPs evolved later when mammals began to consume a diet with a high content of tannins. Interestingly, it has been proved that variation in the molecular structures within BPRPs and APRPs does not largely influence the affinity of PRPs to tannins (Lu and Bennick, 1998).

The affinity of PRPs to tannins differs among mammalian species. It is estimated as 5–80 times higher than that of bovine serum albumin (BSA) and 1000 times higher than that of lysozyme (Asquith et al., 1987; Austin et al., 1989; Mole et al., 1990). This high affinity enables PRPs to act as a first line of defense. Because tannins bind preferentially to PRPs, even in the presence of excessive amounts of other proteins of lower affinity (Asquith and Butler, 1985), PRPs can form stable complexes with tannins in the oral cavity. They thereby prevent tannins from interacting with other proteins, such as enzymes or those in the mucous membranes and epithelia, and then degrading into noxious phenolic compounds (Robbins et al., 1991; Hagerman and Robbins, 1993). The tannin–PRP complexes are also stable in the gastrointestinal tract, and most of the complexes formed are excreted in the feces (Mitaru et al., 1984; Skopec et al., 2004). These reactions of PRPs may present both quantitative and qualitative nitrogen savings to animals. The amount of protein

needed to bind all the tannins can be reduced. In addition, the four major amino acids of PRPs are all nonessential in mammals. This means that nonessential amino acids from PRPs are excreted instead of the dietary or endogenous essential amino acids (McArthur et al., 1995).

Interestingly, some species produce salivary proteins, which are rich in proline, but do not show the high affinity to tannins (Mole et al., 1990). For instance, salivary proteins in some marsupials, such as koalas and common ringtail possums, contain about 30% of proline, but their affinity to tannins is a tenth of that of BSA. Similar observations were also obtained in cattle and pigs (Mole et al., 1990). Some modifications in these proteins, such as extensive glycosylation, might reduce the affinity to tannins. If so, these nontannin-binding proline-rich proteins might include an important clue for studies on the evolutionary process of tannin-binding PRPs. These nontannin-binding proline-rich proteins cannot be regarded as TBSPs because of the lack of high affinity to tannins. Thus, in this review, this type of "proline-rich proteins" is not regarded as PRPs in the following discussion.

Another notable feature of PRPs is that their synthesis is induced by the presence of β-agonists and tannins, but this response varies among species (Table 1). PRPs are constitutively produced at high levels in humans and mule deer (Oppenheim et al., 1971; Austin et al., 1989). In contrast, in rats, mice, and hamsters, synthesis of PRPs is induced by injection of the β-agonist isoproterenol (Mehansho and Carlson, 1983; Mehansho et al., 1985, 1987a). This reaction is also found in the Japanese wood mouse (T. Shimada, unpublished data). PRP synthesis can also be induced by ingesting tannins. This response has so far been confirmed exclusively in rodent species, such as rats (Mehansho et al., 1983), mice (Mehansho et al., 1985), root voles (Juntheikki et al., 1996), and Japanese wood mice (Shimada et al., 2004). In black rhinos, the induction of TBSP synthesis by tannins has also been demonstrated (Clauss et al., 2005). Their TBSPs may also be PRPs, although this has not yet been examined. Interestingly, in hamsters, PRP synthesis is induced by β-agonist injection, but not by ingestion of tannins (Mehansho et al., 1987a). This induction mechanism supports the importance of PRPs as a defense against dietary tannins.

Histatins

Histatins are a group of relatively small proteins with high affinity to tannins; their molecular weight is, in general, less than 5000 (Yan and Bennick, 1995). Histatins are found only in the saliva of humans and some primates (Sabatini et al., 1989; Bennick, 2002). Histatins are characterized by a high content of histidine, which accounts for about 25% of all amino acids present (Yan and Bennick, 1995).

Unexpectedly, histatins in human saliva account for only 2.6% of all salivary proteins (Sugiyama and Ogata, 1993). They readily bind both hydrolyzable and condensed tannins (Yan and Bennick, 1995). The affinity of histatins to tannic acid is estimated to be twice that of gelatin, which is an effective binder of tannic acid. Furthermore, the complexes with tannins are stable in the gut. These findings indicate that histatins may act as defenses against dietary tannins, as do PRPs. However, PRPs are likely to be more important than histatins in defense against tannins if both of them are secreted coincidentally in an animal, because their levels of secretion in saliva are very different.

Histidine is regarded as an essential amino acid for most mammals, but its essentiality in adult humans is supposed to be relatively low (Schmidt-Nielsen,

1997), and it is regarded as semiessential. Assuming that other primates have similar requirements for histidines, this low essentiality of histidine might have enabled the evolution of histatin production in primates, although there is a possibility that histatins may be found in other taxonomic groups.

Other Types of TBSPs

PRPs and histatins are two major groups of TBSPs, but some researchers have found other types of salivary proteins with high affinity to tannins. First, Mole et al. (1990) investigated the presence of TBSPs in several mammals, including laboratory animals, livestock, and wildlife. In the saliva of white-tailed deer, they found TBSPs whose affinity to tannins was six times that of BSA. These salivary proteins contained only 7% proline, and the sum of the four amino acids (proline, glycine, glutamine, and glutamic acid) accounted for only 29% of the total. The molecular weight of these proteins was estimated to be over 10,000. These findings suggest that the salivary proteins from white-tailed deer are neither PRPs nor histatins.

In cattle saliva, Makkar and Becker (1998) found salivary proteins with high affinity to tannins: about six times that of BSA. Their proline content was only 6.5%, and the sum of the four amino acids accounted for about 31% of the total. Ingesting tannins did not alter the amino acid compositions or the affinity of these TBSPs to tannins. These authors speculated that the primary role of these proteins might not be to defend the animals against dietary tannins. Interestingly, Austin et al. (1989) and Mole et al. (1990) did not detect TBSPs in cattle saliva, even with the same isolation methods used by Makkar and Becker (1998).

Gehrke (2001) found TBSPs in the saliva of the fallow deer whose affinities to tannic acid and quebracho tannins (condensed tannins) were almost twice those of cattle saliva. These proteins contained 13% of proline, and the sum of the four amino acids accounted for 35.3% of the total. This proline content is lower than typical PRPs but relatively high as a usual protein. The author concluded that these TBSPs were not PRPs.

It is unknown whether such salivary proteins possess any evolutionary relationship to PRPs or histatins. However, the presence of several types of TBSPs suggests that parallel evolution may have occurred at least several times in herbivorous mammals, that some salivary proteins that originally had other functions may have incidentally acquired a high affinity to tannins and thus consequently have come to act as a defense mechanism against dietary tannins.

TBSPs in Relation to Feeding Niches

The possibility that TBSPs act as defense products against dietary tannins has promoted surveys of the presence of these proteins in various species of mammals. Some of these have tried to link the presence or affinity of TBSPs to the feeding niches of those animals. Table 1 shows the summary of previous studies that examined the presence of TBSPs in various mammalian species. Among 33 species examined, TBSPs are found in 26 species, including two controversial ones—cattle and sheep. The wide prevalence of TBSPs in various taxa implies a staple role in counteracting dietary tannins. Characterizations of TBSPs have been carried in 16

species, but include only 8 species of wild mammals. Thus, our knowledge of TBSPs is quite limited for wildlife.

It is hypothesized that animals with high tannin contents in their natural diets have developed high levels of TBSPs, but that those with low tannin contents produce little or no TBSPs (McArthur et al., 1995; Fickel et al., 1998; Clauss et al., 2005). In general, the leaves of monocots and forbs contain less tannin compared to fruits, seeds, and the leaves of shrubs and trees (Waterman and Mole, 1994). Thus, the above hypothesis implies that browsers, frugivores, and omnivores produce TBSPs at high levels, but grazers should have only small amounts.

Among rodents, rats, mice, and hamsters, which are all omnivores, are confirmed as secreting PRPs (Table 1). Furthermore, Shimada et al. (2004) found PRPs in the saliva of the Japanese wood mouse (an omnivore that is close to being frugivorous). Root voles, which are supposed to be intermediate between grazers and browsers, also produce PRPs in their saliva (Juntheikki et al., 1996). In contrast, meadow voles (grazers) have neither PRPs nor other types of TBSPs (Dietz et al., 1994).

Among ungulates, Austin et al. (1989) found TBSPs in the saliva of mule deer (intermediate), but not in cattle (grazers) or sheep (grazers). Mole et al. (1990) also reported similar results: white-tailed deer (intermediate) produce TBSPs, but sheep and cattle do not. Furthermore, Gehrke (2001) examined the affinity of TBSPs in six species of ungulates and found that a browser (roe deer) and intermediates (reindeer, fallow deer, and musk ox) produce TBSPs with higher affinity to tannins than grazers (cattle and mufflon). Similarly, the recent study by Clauss et al. (2005) also supports this hypothesis by comparing the affinity of TBSPs in three species of rhinos with different feeding niches: Indian rhinos (intermediate) and black rhinos (browsers) produce TBSPs with higher affinity than do white rhinos (grazers).

Hagerman and Robbins (1993) examined the affinity between various types of tannins and crude saliva from the moose, beaver, mule deer, and American black bear by using an electrophoretic tannin-binding assay. Interestingly, the black bear (omnivore) produced the most effective TBSPs, which showed high affinity to all types of tannins: linear and branched condensed tannins and two types of hydrolyzable tannins (gallotannin and ellagitannin). TBSPs from the mule deer (intermediate) were more specific than those from the black bear; they could bind to all types of tannins other than ellagitannin. The moose (intermediate) produced TBSPs that bound only to linear condensed tannins. They also found that TBSPs from the beaver also bound only to linear condensed tannins, but their affinity was much lower than those from the mule deer. The main food of beavers is the bark and sapwood of the aspen, birch, willow, and alder, which are tannin-free or contain relatively low levels of condensed tannins.

In this regard, the above hypothesis is mostly supported. However, as already noted, some contradictory studies have found unclassified TBSPs in the saliva of cattle and sheep, which are typical grazers (cattle, Makkar and Becker, 1998; Gehrke, 2001; sheep, Vaithiyanathan et al., 2001).

We note, however, that there are some obstacles that may complicate such comparative studies. The first of such barriers are methodological differences found in various studies—methods for extracting salivary proteins, examining the presence of TBSPs, and quantifying the affinity. Each method likely has a different sensitivity and applicability. Thus, comparisons between different studies require care. Second, these previous comparative studies did not necessarily deal with the same types of TBSPs. In other words, the presence or affinity of TBSPs that may have originated

from different evolutionary processes may complicate the examination of the hypothesis. For instance, among the four species examined in the study of Hagerman and Robbins (1993), the characteristics of TBSPs were determined only in the mule deer and moose, which have been confirmed to have PRPs (mule deer, Austin et al., 1989; moose, Gehrke, 2001). It is possible that TBSPs from the other two species may not have been PRPs. To investigate the evolutionary relationships among feeding niches and salivary proteins as defenses against dietary tannins, we need to characterize the TBSPs in each species.

In Vivo Effectiveness of TBSPs

There are sufficient data to show that TBSPs, and especially PRPs, bind effectively to both condensed and hydrolyzable tannins *in vitro* (Mehansho et al., 1987b; Bennick, 2002). *In vitro* evidence cannot always be applied *in vivo*, because some factors that differ between *in vitro* and *in vivo* situations—such as the state of tannins in the diet and the presence of other dietary chemicals—may interfere with the *in vivo* reaction between tannins and TBSPs. Furthermore, the production of TBSPs may inevitably involve costs *in vivo* that are not considered in *in vitro* conditions (McArthur et al., 1995; Clauss et al., 2003). Thus, to evaluate the significance of TBSPs in counteracting dietary tannins, we need to examine their *in vivo* effects. However, the evidence that TBSPs are effective *in vivo* is limited, partly because it is difficult to measure levels of TBSPs nondestructively. Hereafter, I focused on PRPs, because data on this topic are lacking in other TBSPs.

Glendinning (1992) first demonstrated the significance of PRPs in dealing with tannins *in vivo* by using mice injected with isoproterenol (a β-agonist) to enhance PRP secretion. The isoproterenol-injected mice exhibited a greater preference for a tannin-rich diet compared to controls. Mole et al. (1993) found that injection of a β-antagonist (propranolol), which prevents PRP synthesis, amplified the negative effects of tannins in rats. Recently, Skopec et al. (2004), using fecal proline level as an index of secreted PRPs, demonstrated clearly that induction of PRP synthesis in rats reduces the negative effects of dietary tannins by preventing tannins from breaking up into small phenolics and being absorbed. In a similar way, Jansman et al. (1994), also using rats and fecal proline level as an index, revealed that PRPs are effective as defenses against tannins, but that this defense is incomplete when dietary tannins are not readily extracted from the diet during chewing. In addition, Shimada and coworkers (2004, 2006) suggested the significance of PRPs in counteracting tannins in terms of interindividual differences in secretion levels of PRPs in the Japanese wood mouse: individuals with high levels of PRPs were less sensitive to tannins in acorns.

Until now, *in vivo* evidence noted above has been obtained only from rodents. It is noteworthy that all of these rodents can induce PRP synthesis by ingesting tannins. This inducibility is presumably an adaptive mechanism that reduces the costs arising from PRP production in animals whose natural diet varies in tannin content. In other words, the *in vivo* effectiveness of PRPs as defenses against tannins is mostly untested in animals that, unlike rats and mice, do not have this feedback mechanism to reduce the costs involved in PRP synthesis. This topic is further discussed in the next section.

Cost and Inducibility of PRP Synthesis

In this section, strategies for producing PRPs are argued in the light of the costs and inducibility of PRP synthesis. Production of PRPs may involve some costs in terms of metabolism and nitrogen balance. PRPs are poorly digested, because many peptide bonds involving proline are difficult to cleave (Muenzer et al., 1979; McArthur et al., 1995). Thus, production of excess PRPs may have a nitrogen cost, even if they consist mostly of nonessential amino acids.

There is some evidence for costs arising from PRP production. In rats, Haghighat et al. (1996) found that injection of a β-agonist induced PRP synthesis, but by 15 d after the end of injections the production of PRPs had ceased. My colleagues and I have obtained similar results in the Japanese wood mouse (Shimada et al., unpublished data). The rapid termination of PRP synthesis observed in these studies implies that it is costly to keep producing them. Furthermore, Skopec et al. (2004) have demonstrated nitrogen costs involved in producing PRPs: the apparent nitrogen digestibility of a diet containing 3% pentagalloyl glucose (a hydrolyzable tannin) in PRP synthesis-induced rats was over 20% lower than in control rats. This reduction in nitrogen digestibility may be mostly attributable to excretion of the PRP–tannin complexes.

The costs of producing PRPs may be overcome by the benefits of PRPs when animals consume diets containing tannins. However, if the diet is tannin-free or includes little tannin, animals may still incur the costs involved in PRP production. Induction of PRP synthesis in response to tannin injection may reduce the costs of production. Animals whose natural diets vary in tannin content may benefit from such a feedback mechanism (McArthur et al., 1995; Bennick, 2002). The inducibility of PRP synthesis by tannins seems to have been reported only in rats, mice, root voles, and Japanese wood mice. As expected, the natural diet of the two wild rodents (excluding rats and mice) varies seasonally (Juntheikki et al., 1996). For instance, the Japanese wood mouse varies its primary food items seasonally; in summer these are soft plant parts or small invertebrates, and in autumn and winter these are seeds or acorns, which generally contain considerable amounts of tannins (Tatsukawa and Murakami, 1976; Shimada, 2001).

In contrast, some mammalian species lack this feedback mechanism, thereby secreting PRPs constitutively at relatively high levels (Austin et al., 1989). It has been hypothesized that the feedback mechanism may not have evolved or may have become lost in those animals whose diets constantly contain high tannin contents (Bennick, 2002; Clauss et al., 2005). Unfortunately, only two species have been reported to use this latter strategy—humans and mule deer—and there is, therefore, much to examine in regard to the above hypothesis. The tannin content of the natural diet of mule deer is likely to vary among seasons, because their diet varies seasonally (Sowell et al., 1985). If mule deer cannot adjust their levels of secretion of PRPs in accordance with their intake levels of tannins, they may suffer the costs of unnecessary PRP synthesis. Nonetheless, do they keep producing PRPs at a high level in any season? The inducibility of PRP synthesis in mule deer has been examined in captive animals by the semiquantitative method (Austin et al., 1989). Thus, the above question is still open, and it may be too early to conclude that such a wasteful strategy is adaptive for animals.

To understand the strategies of PRP production in accordance with tannin intake, it should be helpful to examine the variations in PRP production among populations

as well as among seasons, because levels of tannins in the natural diets will vary among populations even within a species. However, only a few researchers have addressed this topic; Juntheikki (1996) found that the binding affinity of TBSPs to tannins of Scandinavian moose was higher than that of North American moose.

Conclusions

TBSPs, and especially PRPs, probably act as a first line of defense against dietary tannins in far more mammalian species than have already been reported. However, our knowledge of TBSPs in wild animals is limited and is also biased toward *in vitro* data. Detailed characterizations of TBSPs in each species and further comparative studies among species, populations, or seasons will reveal the relationships between feeding niches and production of TBSPs and shed insight on the process of evolution of different types of TBSPs.

Acknowledgments I am grateful to T. Saitoh, T. Matsui, and R. Osawa for helpful discussions, two anonymous reviewers for useful comments, and Bill Foley for offering me the chance to write this review. This study was supported in part by Grants-in-Aid (no. 17570027) from the Ministry of Education, Science and Culture of Japan.

References

ANN, D. K. and CARLSON, D. M. 1985. The structure of organization of a proline-rich protein gene of a mouse multigene family. *J. Biol. Chem.* 260:15863–15872.

ANN, D. K., GADBOIS, D., and CARLSON, D. M. 1987. Structure, organization, and regulation of a hamster proline-rich protein gene—a multigene family. *J. Biol. Chem.* 262:3958–3963.

ASQUITH, T. N. and BUTLER, L. G. 1985. Use of dye-labeled protein as spectrophotometric assay for protein precipitants such as tannin. *J. Chem. Ecol.* 11:1535–1544.

ASQUITH, T., MEHANSHO, H., ROGLER, J., BUTLER, L., and CARLSON, D. M. 1985. Induction of proline-rich protein-biosynthesis in salivary-glands by tannins. *FASEB J.* 44:1097.

ASQUITH, T. N., UHLIG, J., MEHANSHO, H., PUTMAN, L., CARLSON, D. M., and BUTLER, L. 1987. Binding of condensed tannins to salivary proline-rich glycoproteins—the role of carbohydrate. *J. Agric. Food. Chem.* 35:331–334.

AUSTIN, P. J., SUCHAR, L. A., ROBBINS, C. T., and HAGERMAN, A. E. 1989. Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. *J. Chem. Ecol.* 15:1335–1347.

AZEN, E. A., CARLSON, D. M., CLEMENTS, S., LALLEY, P. A., and VANIN, E. 1984. Salivary proline-rich protein genes on chromosomes-8 of mouse. *Science* 226:967–969.

BACON, J. R. and RHODES, M. J. C. 2000. Binding affinity of hydrolyzable tannins to parotid saliva and to proline-rich proteins derived from it. *J. Agric. Food. Chem.* 48:838–843.

BENNICK, A. 2002. Interaction of plant polyphenols with salivary proteins. *Crit. Rev. Oral Biol. Med.* 13:184–196.

BERNAYS, E. A., DRIVER, G. C., and BILGENER, M. 1989. Herbivores and plant tannins. *Adv. Ecol. Res.* 19:263–302.

BLYTT, H. J., GUSCAR, T. K., and BUTLER, L. G. 1988. Antinutritional effects and ecological significance of dietary condensed tannins may not be due to binding and inhibiting digestive enzymes. *J. Chem. Ecol.* 14:1455–1465.

BURRITT, E. A. and PROVENZA, F. D. 2000. Role of toxins in intake of varied diets by sheep. *J. Chem. Ecol.* 26:1991–2005.

CARLSON, D. M. 1993. Salivary proline-rich proteins—biochemistry, molecular biology, and regulation of expression. *Crit. Rev. Oral Biol. Med.* 4:495–502.

CHAN, M. and BENNICK, A. 2001. Proteolytic processing of a human salivary proline-rich protein precursor by proprotein convertases. *Eur. J. Biochem.* 268:3423–3431.

CHUNG-MACCOUBREY, A. L., HAGERMAN, A. E., and KIRKPATRICK, R. L. 1997. Effect of tannins on digestion and detoxification activity in gray squirrels (*Sciurus carolinensis*). *Physiol. Zool.* 70:270–277.

CLAUSEN, T. P., PROVENZA, F. D., BURRITT, E. A., REICHARDT, P. B., and BRYANT, J. P. 1990. Ecological implications of condensed tannin structure: a case study. *J. Chem. Ecol.* 16:2381–2392.

CLAUSS, M., LASON, K., GEHRKE, J., LECHNER-DOLL, M., FICKEL, J., GRUNE, T., and STREICH, W. J. 2003. Captive roe deer (*Capreolus capreolus*) select for low amounts of tannic acid but not quebracho: fluctuation of preferences and potential benefits. *Comp. Biochem. Physiol. B* 136:369–382.

CLAUSS, M., GEHRKE, J., HATT, J.-M., DIERENFELD, E. S., FLACH, E. J., HERMES, R., CASTELL, J., STREICH, W. J., and FICKEL, J. 2005. Tannin-binding salivary proteins in three captive rhinoceros species. *Comp. Biochem. Physiol. A* 140:67–72.

CLEMENTS, S., MEHANSHO, H., and CARLSON, D. M. 1985. Novel multigene families encoding highly repetitive peptide sequences—sequence analyses of rat and mouse proline-rich protein cDNAs. *J. Biol. Chem.* 260:3471–3477.

COOPER, S. M. and OWEN-SMITH, N. 1985. Condensed tannins deter feeding by browsing ruminants in a South African savanna. *Oecologia* (Berlin) 67:142–146.

DEARING, M. D. 1997. The manipulation of plant toxins by a food-hoarding herbivore, *Ochotona princeps*. *Ecology* 78:774–781.

DEARING, M. D. and CORK, S. 1999. Role of detoxification of plant secondary compounds on diet breadth in a mammalian herbivore, *Trichosurus vulpecula*. *J. Chem. Ecol.* 25:1205–1220.

DEARING, M. D., FOLEY, W. J., and MCLEAN, S. 2005. The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. *Annu. Rev. Ecol. Evol. Syst.* 36:169–189.

DIETZ, B. A., HAGERMAN, A. E., and BARRETT, G. W. 1994. Role of condensed tannin on salivary tannin-binding proteins, bioenergetics, and nitrogen digestibility in *Microtus pennsylvanicus*. *J. Mammal.* 75:880–889.

FERREIRA, F. D., ROBINSON, R., HAND, A. R., and BENNICK, A. 1992. Differential expression of proline-rich proteins in rabbit salivary glands. *J. Histochem. Cytochem.* 40:1393–1404.

FICKEL, J., GORITZ, F., JOEST, B. A., HILDEBRANDT, T., HOFMANN, R. R., and BREVES, G. 1998. Analysis of parotid and mixed saliva in roe deer (*Capreolus capreolus* L.). *J. Comp. Physiol. B* 168:257–264.

FOLEY, W. J. and MOORE, B. D. 2005. Plant secondary metabolites and vertebrate herbivores—from physiological regulation to ecosystem function. *Curr. Opin. Plant Biol.* 8:430–435.

FOWLER, M. E. and RICHARDS, W. P. C. 1965. Acorn poisoning in a cow and a sheep. *J. Am. Vet. Med.* 147:1215–1220.

GEHRKE, J. 2001. Investigations of tannin-binding salivary proteins of roe deer and other ruminants. PhD dissertation, University of Potsdam, Potsdam (in German with English summary).

GLENDINNING, J. I. 1992. Effect of salivary proline-rich proteins on ingestive responses to tannic acid in mice. *Chem. Senses* 17:1–12.

HAGERMAN, A. E. and BUTLER, L. G. 1981. The specificity of proanthocyanidin–protein interactions. *J. Biol. Chem.* 256:4494–4497.

HAGERMAN, A. E. and ROBBINS, C. T. 1993. Specificity of tannin-binding salivary proteins relative to diet selection by mammals. *Can. J. Zool.* 71:628–633.

HAGHIGHAT, M., MOETAMED, A., VASEGHI, T., and AMINLARI, M. 1996. Isoprenaline induces biosynthesis of proline-rich proteins in the salivary glands of rat but not in sheep. *Comp. Biochem. Physiol. C* 115:165–168.

ISEMURA, S. 2003. Salivary proline-rich proteins: protein structure, gene structure, and function. *Curr. Top. Biochem. Res.* 5:141–147.

JANSMAN, A. J. M., FROHLICH, A. A., and MARQUARDT, R. R. 1994. Production of proline-rich proteins by the parotid glands of rats is enhanced by feeding diets containing tannins from Faba beans (*Vicia faba* L). *J. Nutr.* 124:249–258.

JUNTHEIKKI, M.-R. 1996. Comparison of tannin-binding proteins in saliva of Scandinavian and North American moose (*Alces alces*). *Biochem. Syst. Ecol.* 24:595–601.

JUNTHEIKKI, M.-R., JULKUNEN-TIITTO, R., and HAGERMAN, A. E. 1996. Salivary tannin-binding proteins in root vole (*Microtus oeconomus* Pallas). *Biochem. Syst. Ecol.* 24:25–35.

KAUFFMAN, D. L. and KELLER, P. J. 1979. The basic proline-rich proteins from a single subject. *Arch. Oral Biol.* 24:249–256.

KIM, H. S. and MAEDA, N. 1986. Structures of two HAEIII-type genes in the human salivary proline-rich protein multigene family. *J. Biol. Chem.* 261:6712–6718.

LU, Y. and BENNICK, A. 1998. Interaction of tannin with human salivary proline-rich proteins. *Arch. Oral Biol.* 43:717–728.

LYONS, K. M., AZEN, E. A., GOODMAN, P. A., and SMITHIES, O. 1988. Many protein products from a few loci: assignment of human salivary proline-rich proteins to specific loci. *Genetics* 120:255–265.

MAEDA, N. 1985. Inheritance of the human salivary proline-rich proteins—a reinterpretation in terms of 6 loci forming 2 subfamilies. *Biochem. Genet.* 23:455–464.

MAEDA, N., KIM, H. S., AZEN, E. A., and SMITHIES, O. 1985. Differential RNA splicing and post-translational cleavages in the human salivary proline-rich protein gene system. *J. Biol. Chem.* 260:1123–1130.

MAKKAR, H. P. S. and BECKER, K. 1998. Adaptation of cattle to tannins: role of proline-rich proteins in oak-fed cattle. *Anim. Sci.* 67:277–281.

MANDEL, I. D., THOMPSON, R. H. J., and ELLISON, S. A. 1965. Studies on the mucoproteins of human parotid saliva. *Arch. Oral Biol.* 10:499–507.

MCARTHUR, C., HAGERMAN, A., and ROBBINS, C. T. 1991. Physiological strategies of mammalian herbivores against plant defenses, pp. 103–114, *in* R. T. Palo and C. T. Robbins (eds.). Plant Defences Against Mammalian Herbivory. CRC Press, Florida.

MCARTHUR, C., SANSON, G. D., and BEAL, A. M. 1995. Salivary proline-rich proteins in mammals—roles in oral homeostasis and counteracting dietary tannin. *J. Chem. Ecol.* 21:663–691.

MEHANSHO, H. and CARLSON, D. M. 1983. Induction of protein and glycoprotein synthesis in rat submandibular glands by isoproterenol. *J. Biol. Chem.* 258:6616–6620.

MEHANSHO, H., HAGERMAN, A., CLEMENTS, S., BUTLER, L., ROGLER, J., and CARLSON, D. M. 1983. Modulation of proline-rich protein biosynthesis in rat parotid glands by sorghums with high tannin levels. *Proc. Natl. Acad. Sci. USA* 80:3948–3952.

MEHANSHO, H., CLEMENTS, S., SHEARES, B. T., SMITH, S., and CARLSON, D. M. 1985. Induction of proline-rich glycoprotein synthesis in mouse salivary glands by isoproterenol and by tannins. *J. Biol. Chem.* 260:4418–4423.

MEHANSHO, H., ANN, D. K., BUTLER, L. G., ROGLER, J., and CARLSON, D. M. 1987a. Induction of proline-rich proteins in hamster salivary glands by isoproterenol treatment and an unusual growth inhibition by tannins. *J. Biol. Chem.* 262:12344–12350.

MEHANSHO, H., BUTLER, L. G., and CARLSON, D. M. 1987b. Dietary tannins and salivary proline-rich proteins—interactions, induction, and defense mechanisms. *Annu. Rev. Nutr.* 7:423–440.

MITARU, B. N., REICHERT, R. D., and BLAIR, R. 1984. The binding of dietary protein by sorghum tannins in the digestive tract of pigs. *J. Nutr.* 114:1787–1796.

MOLE, S., BUTLER, L. G., and IASON, G. 1990. Defense against dietary tannin in herbivores—a survey for proline-rich salivary proteins in mammals. *Biochem. Syst. Ecol.* 18:287–293.

MOLE, S., ROGLER, J. C., and BUTLER, L. G. 1993. Growth reduction by dietary tannins—different effects due to different tannins. *Biochem. Syst. Ecol.* 21:667–677.

MUENZER, J., BILDSTEIN, C., GLEASON, M., and CARLSON, D. M. 1979. Properties of proline-rich proteins from parotid glands of isoproterenol-treated rats. *J. Biol. Chem.* 254:5629–5634.

MURRAY, N. J. and WILLIAMSON, M. P. 1994. Conformational study of a salivary proline-rich protein repeat sequence. *Eur. J. Biochem.* 219:915–921.

NIHO, N., SHIBUTANI, M., TAMURA, T., TOYODA, K., UNEYAMA, C., TAKAHASHI, N., and HIROSE, M. 2001. Subchronic toxicity study of gallic acid by oral administration in F344 rats. *Food Chem. Toxicol.* 39:1063–1070.

OPPENHEIM, F. G., HAY, D. I., and FRANZBLAU, C. 1971. Proline-rich proteins from human parotid saliva. I. Isolation and partial characterization. *Biochemistry.* 10:4233–4238.

OPPENHEIM, F. G., KOUSVELARI, E. E., and TROXLER, R. F. 1979. Immunological cross-reactivity and sequence homology between salivary proline-rich proteins in human and macaque monkey (*Macaca fascicularis*) parotid saliva. *Arch. Oral Biol.* 24:595–599.

OPPENHEIM, F. G., HAY, D. I., SMITH, D. J., OFFNER, G. D., and TROXLER, R. F. 1987. Molecular basis of salivary proline-rich protein and peptide synthesis—cell-free translations and processing of human and macaque statherin messenger-RNAs and partial amino-acid-sequence of their signal peptides. *J. Dent. Res.* 66:462–466.

ROBBINS, C. T., HANLEY, T. A., HAGERMAN, A. E., HJELJORD, O., BAKER, D. L., SCHWARTZ, C. C., and MAUTZ, W. W. 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. *Ecology* 68:98–107.

ROBBINS, C. T., HAGERMAN, A. E., AUSTIN, P. J., MCARTHUR, C., and HANLEY, T. A. 1991. Variation in mammalian physiological responses to a condensed tannin and its ecological implications. *J. Mammal.* 72:480–486.

SABATINI, L. M., WARNER, T. F., SAITOH, E., and AZEN, E. A. 1989. Tissue distribution of RNAs for cystatins, histatins, statherin, and proline-rich salivary proteins in human and macaques. *J. Dent. Res.* 68:1138–1145.

SCHMIDT-NIELSEN, K. 1997. Animal Physiology. Cambridge University Press, Cambridge.

SCHULZ, G. E. and SCHIRMER, R. H. 1979. Principles of Protein Structure. Springer, Berlin.

SHIMADA, T. 2001. Hoarding behaviors of two wood mouse species: different preference for acorns of two Fagaceae species. *Ecol. Res.* 16:127–133.

SHIMADA, T. and SAITOH, T. 2003. Negative effects of acorns on the wood mouse *Apodemus speciosus*. *Pop. Ecol.* 45:7–17.

SHIMADA, T., SAITOH, T., and MATSUI, T. 2004. Does acclimation reduce the negative effects of acorn tannins in the wood mouse *Apodemus speciosus*? *Acta Theriol.* 49:203–214.

SHIMADA, T., SAITOH, T., SASAKI, E., NISHITANI, Y., and OSAWA, R. 2006. The role of tannin-binding salivary proteins and tannase-producing bacteria in the acclimation of the japanese wood mouse to accorn tannins. *J. Chem. Ecol.* (in press).

SKOPEC, M. M., HAGERMAN, A. E., and KARASOV, W. H. 2004. Do salivary proline-rich proteins counteract dietary hydrolyzable tannin in laboratory rats? *J. Chem. Ecol.* 30:1679–1692.

SOWELL, B. F., KOERTH, B. H., and BRYANT, F. C. 1985. Seasonal nutrient estimates of mule deer diets in the Texas Panhandle. *J. Range Manage.* 38:163–167.

SUGIYAMA, K. and OGATA, K. 1993. High performance liquid chromatographic determination of histatins in human saliva. *J. Chromatogr.* 619:306–309.

TAKEMOTO, H. 2003. Phytochemical determination for leaf food choice by wild chimpanzees in Guinea, Bossou. *J. Chem. Ecol.* 29:2551–2573.

TATSUKAWA, K. and MURAKAMI, O. 1976. On the food utilization of the Japanese wood mouse *Apodemus speciosus* (Mammalia: Muridae). *Physiol. Ecol. Jpn.* 17:133–144.

VAITHIYANATHAN, S., MISHRA, J. P., SHEIKH, Q., and KUMAR, R. 2001. Salivary gland tannins binding proteins of sheep and goat. *Ind. J. Anim. Sci.* 71:1131–1134.

WATERMAN, P. G. and MOLE, S. 1994. Analysis of Phenolic Plant Metabolites. Blackwell, Oxford.

YAN, Q. Y. and BENNICK, A. 1995. Identification of histatins as tannin-binding proteins in human saliva. *Biochem. J.* 311:341–347.

ZIEMER, M. A., SWAIN, W. F., RUTTER, W. J., CLEMENTS, S., ANN, D. K., and CARLSON, D. M. 1984. Nucleotide-sequence analysis of a proline-rich protein cDNA and peptide homologies of rat and human proline-rich proteins. *J. Biol. Chem.* 259:475–480.

ZUCKER, W. V. 1983. Tannins: does structure determine function? An ecological perspective. *Am. Nat.* 121:335–365.