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Comparison of Electrophoretic Protein Profiles from Sheep and Goat Parotid Saliva

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Abstract Saliva provides a medium for short-term adaptation to changes in diet composition, namely, the presence of plant secondary metabolites. Salivary proteins have biological functions that have particular influence on oral homeostasis, taste, and digestive function. Some salivary proteins, such as proline-rich proteins, are present in browsers but absent in grazers. Despite the significance of salivary proteins, their expression patterns in many herbivores are unknown. We investigated the sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile of parotid salivary proteins from two domesticated species, one a grazer, the sheep, *Ovis aries*, and the other a mixed feeder, the goat, *Capra hircus*, both fed on the same conventional diet. With 12.5% polyacrylamide linear gels, we observed uniform patterns of salivary proteins within the two species. In the goat profile, 21 major bands were observed, and 19 in the sheep profile. Each band was subjected to peptide mass fingerprinting for purposes of identification, allowing for 16 successful protein identifications. Marked differences were observed between the species in the region of 25–35 kDa molecular weights: one band was present in significantly different intensities; three bands were present only in goats; and one band was present only in sheep. This is the first report of a comparison of the protein salivary composition of sheep and goats and suggests that future research should be conducted to reveal a physiological function for salivary proteins related to the differences in feeding behavior of these species.

Keywords *Capra hircus* · Feeding behavior · MALDI-TOF MS · *Ovis aries* · Parotid saliva · Protein identification · Salivary proteins · SDS-PAGE

Introduction

Salivary function is closely related to oral health and digestion. Humphrey and Williamson (2001) organized the functions of saliva into five major categories: (1) lubrication

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and protection, (2) buffering action and clearance, (3) maintenance of tooth integrity, (4) antibacterial activity, and (5) taste and digestion. Saliva modulates taste perception through the transportation of taste substances and the protection of taste receptors, as well as through the chemical interaction of salivary constituents with taste substances. Salivary flow rate and composition are influenced by the quality of taste stimuli (Spielman 1990), drugs and physiological factors (Aps and Martens 2005), and, at the same time, salivary flow rate and composition affect taste perception (Matsuo 2000). Some salivary proteins are involved in feeding behavior, namely von Ebner's gland salivary protein (Kock et al. 1994), salivary cystatins (Katsukawa et al. 2002), and salivary kallikreins (Yamada et al. 2006). Levels of tannin-binding salivary proteins (TBSPs) in animal saliva are associated with tannin levels in the diet (Mehansho et al. 1983, 1987, 1992; Austin et al. 1989; Hagerman and Robbins 1993; Fickel et al. 1998; Makkar and Becker 1998; Clauss et al. 2003a, b). A recent review by Shimada (2006) stresses the importance of gathering basic information on salivary proteins as a way of understanding the relationships between feeding niches and saliva composition.

Sheep and goats are both generalist herbivores. They have similar body sizes and frequently graze together in major farming systems (Bartolome et al. 1998; El Aich and Waterhouse 1999). Although they are competing species that co-exist in the same niche and have access to the same forage items, they often show different feeding behavior, selecting and ingesting diets that overlap to variable degrees (Ngwa et al. 2000; Pande et al. 2002). In the context of the three feeding types proposed by Hofmann (1989), sheep are considered grazers, whereas goats are viewed as intermediate feeders, capable of dealing with large amounts of browse in their diets. There are several possible explanations for these differences in feeding behavior. According to the detoxification limitation hypothesis (Freeland and Janzen 1974, recently reviewed by Marsh et al. 2006), goats could have a greater ability to eliminate plant secondary metabolites (PSMs), when compared to sheep. A second explanation is based on one of the deductive generalizations of Hofmann's morphophysiological hypothesis, which suggests that goats may have large salivary glands that produce large amounts of fluid, which helps to digest browse and provides a medium of defense against PSMs.

To our knowledge, salivary protein expression patterns have not been reported from sheep and goats, and only a limited number have been identified for these two species. Austin et al. (1989) used electrophoretic approaches to search for TBSPs in the whole saliva of sheep, cattle, and deer but did not characterize the entire protein profile in the saliva. The aim of the present study was to gain a better understanding of the parotid saliva protein composition of the domestic sheep, *Ovis aries*, and of the goat, *Capra hircus*. To this end, we used one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for protein separation and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for protein identification by peptide mass fingerprinting (PMF).

Methods and Materials

Animals To obtain saliva samples, we used adult females that had been reared in separate sheep and goat flocks and had grazed on Mediterranean rangeland. Collections were made during six different periods, over the course of 1 yr. In each period, five Merino sheep, *O. aries*, and five Serpentina goats, *C. hircus*, were used and kept in the same location in separated crates for 15 d preceding the saliva collection. During this period, all animals were fed with vetch-oat hay, *Vicia sativa*×*Avena sativa*, and had water and food available *ad libitum*. The objective of the pre-trial period was to keep sheep and goats in similar conditions so as to minimize diet effects when comparing them. Before each saliva collection period, polyethylene urinary cat stylet catheters (1.0 or 1.3×130 mm) were introduced into one of the parotid ducts of each animal, which had previously been anaesthetized intravenously with Xylazine/Ketamine (0.1/5.0 mg/kg). To facilitate the positioning of the catheter, an intravenous 14G cannula was previously inserted into the masseter muscle from the inside to the outside. The catheters were then introduced into the parotid papilla, from the exterior to the interior of the mouth (Fickel et al. 1998), by using guide wires. The free end of the catheter, which protruded 1 cm, was fixed to the cheek skin by transfixation knots. To avoid any possible effect of the anesthetics on the saliva composition (Edwards and Titchen 2003; Edwards et al. 2003), sample collection was initiated 1 d after surgery. In the morning and before food distribution, samples were collected during each of the following 3 d. At least 2 ml of parotid saliva were collected from each animal by aspiration with a syringe.

Saliva Collection and Preparation for Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis Each saliva sample was collected into capped polypropylene sample tubes. All samples were frozen immediately in liquid nitrogen and stored at −70°C. Prior to protein quantification, samples were centrifuged at 16,000×*g* for 5 min at 4°C to remove particulate matter. Protein concentration of the parotid saliva was determined by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA), in which bovine serum albumin (BSA) is used as the standard. For

the analysis, 10 μl of either BSA (0–2.0 mg/ml) or saliva were mixed with 200 μl of the BCA reagent and incubated for 30 min at 37°C. Absorbance was measured at 565 nm by using a microtiter plate reader (SpectroMAX 340, Molecular Devices, Union City, CA, USA). Before SDS-PAGE separation, salivary proteins were concentrated with a 5-kDa cut-off ultra-filtration microfuge tube (Millipore, Eschborn, Germany; Ref: UFV5BCC00).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis Individual samples of parotid saliva from sheep and goats were run simultaneously in each gel for comparison. Proteins were separated with 12.5% SDS-PAGE (200× 200×1 mm) in a Protean II xi slab gel apparatus (BioRad, CA, USA). Saliva samples with 70 μg of protein were mixed with 4× concentrated SDS sample buffer (0.125 M Tris–HCl pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 20% glycerol with traces of bromophenol blue). The mixture was heated at 90°C for 5 min and immediately cooled on ice until gel application. Electrophoresis was carried out by using a running buffer [0.025 M Tris, 0.192 M glycine, 1% (*w*/*v*) SDS] at pH 8.3, with 100 V constant current. After the sample entered the separation gel, the voltage was changed to 250 V. Molecular mass protein standards (from 15 to 200 kDa; PageRuler Protein Ladder, SM0661, Fermentas, Ontario, CA, USA) were also included in each gel for reference.

Gel Staining and Densitometry Gels were fixed and stained overnight in a solution of 0.1% Coomassie brilliant blue R-250 in 50% (*v*/*v*) methanol and 10% (*v*/*v*) acetic acid and destained with several changes of 10% (*v*/*v*) acetic acid, following the protocol of Beeley et al. (1991) for proline-rich proteins. Digital images of the gels were obtained by using a densitometer (Molecular Dynamics, Amersham Biosciences Europe GmbH, Freiburg, Germany), and the gels were subjected to linescan analysis by using ImageQuant 5.0 software with parameter sensitivity 9.0 and kernel 4.0 (Amersham Biosciences Europe GmbH) to assign the significant bands in the protein profiles.

Protein Identification For protein identification, the PMF approach was used. Stained bands were excised, washed, reduced with dithiothreitol, alkylated with iodoacetamide, and dried in a speedvac. Gel pieces were rehydrated with digestion buffer (50 mM NH_4HCO_3) containing trypsin (Promega, Madison, WI, USA) and incubated overnight at 37°C. The buffered peptides were acidified with formic acid, desalted, and concentrated with C8 microcolumns (POROS R2, Applied Biosystems, Foster City, CA, USA). The peptides were eluted with matrix solution that contained 10 mg/ml α-cyano-4-hydroxycinnamic acid dissolved in 70% (*v*/*v*) acetonitrile/0.1% (*v*/*v*) trifluoroacetic acid. The mixture was allowed to air-dry (dried droplet method). Mass spectra were obtained with a Voyager-DE STR (Applied Biosystems) MALDI-TOF mass spectrometer in the positive ion reflectron mode. External calibration was made by using a mixture of standard peptides (Pepmix 1, LaserBiolabs, Sophia-Antipolis, France). Spectra were processed and analyzed with MoverZ software (Genomic Solutions Bioinformatics, Ann Arbor, MI, USA). Peakerazor software (GPMAW, General Protein/Mass Analysis for Windows, Lighthouse Data, Odense, Denmark; http://www.gpmaw.com) was used to remove contaminant *m*/*z* peaks and for internal calibration. Monoisotopic peptide masses were used to search for protein identification by using Mascot software (Matrix Science, London, UK; http://www.matrixscience.com). Database searches were performed against MSDB (a non-identical protein sequence database maintained by the Proteomics Department at the Hammersmith Campus of Imperial College, London; http://csc-fserve.hh.med.ic.ac.uk/msdb.html) and SwissProt. The following criteria were used to perform the search: (1) mass accuracy of 50–100 ppm; (2) one missed cleavage in peptide masses; and (3) carbamidomethylation of Cys and oxidation of Met as fixed and variable amino acid modifications, respectively. Criteria used for protein identification in the Mascot software were (1) significant homology scores achieved in Mascot; (2) significant sequence coverage values; and (3) similarity between the protein molecular mass calculated from the gel and for the identified protein.

Results

Salivary Protein Profile Salivary protein concentrations in both animals ranged from 30 to 2,000 μg/ml. Twenty-one and 19 protein bands (Fig. 1) were reproducibly displayed in goat and sheep parotid saliva protein profiles, respectively. There was a similar pattern to the parotid saliva profiles for the two species, except for the 25 to 35 kDa molecular mass range. In this range, the parotid saliva electrophoretic profile from sheep had two visible bands (o and s, corresponding to approximately 32 and 26 kDa, respectively), whereas the profile from goat had four bands (p, q, r, and s, corresponding to approximately 30, 28, 27, and 26 kDa, respectively). Band o, which was an intense band in the sheep profile, was not present in goat saliva. Bands p, q, and r, observed in goats, were absent from sheep. Moreover, the protein band s, common to both species, was more intense in sheep. The parotid salivary protein profiles from individual goats (N=4) and sheep (N=5; Fig. 2) revealed patterns that were similar among individuals of each species but different between the species.

Salivary Protein Identification Mass spectra from a total of 21 and 19 peptide digests of well-resolved bands from

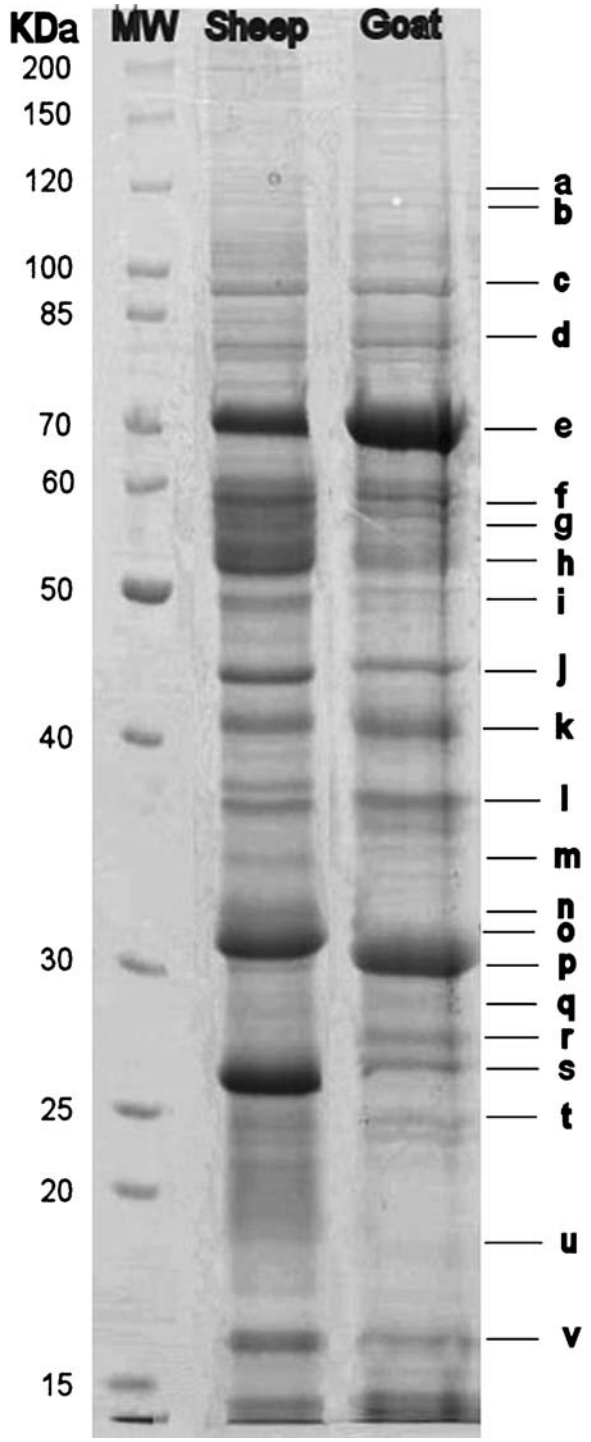

Fig. 1 Typical profiles of sheep and goat parotid salivary proteins in a 12.5% linear gel. Each lane represents the profile from an individual saliva sample. Molecular markers (*MW*) are represented on the *left side* of the figure. The protein bands are identified by letters *a* to *v*

goats and sheep, respectively, were analyzed. The MSDB database was searched by using a taxonomic restriction for "other mammals," and 16 different proteins were identified (Table 1). In most instances, the same proteins were identified in both species. However, the identification of the bands h, n, o, q, r, u, and v were not possible due perhaps to a low amount of protein in some bands or to the existence of several different proteins in the same band or even to a lack of homologous proteins in the searched protein sequence databases. Peptide map comparison for goat and sheep band f shows the presence of some peaks that correspond to the catalase predicted tryptic peptide masses; however, more peaks with relevant intensities were observed. This suggests that catalase is also present in goat f band, probably mixed with other unidentified proteins. Similar results were obtained when peptide maps for band s were compared between goat and sheep. Apolipoprotein A-I was likely present in sheep band s, but the band may also contain other unidentified proteins. This interpretation was supported both by the higher intensity of this band in sheep when compared with the corresponding one from goat and by the presence of mass peaks not observed in goat band s peptide map.

Discussion

Electrophoretic profiles of salivary proteins have been reported for several species, such as rats (Ekström et al. 1996; Williams et al. 1999a), ferrets (Williams et al. 1999b), and cats (Marshall et al. 1993), but the bulk of the studies on salivary profiles have been performed on humans (Ghafouri et al. 2003; Vitorino et al. 2004; Wilmarth et al. 2004; Hardt et al. 2005; Hirtz et al. 2005; Hu et al. 2005; Guo et al. 2006; Walz et al. 2006). According to our knowledge, this is the first study in which the SDS-PAGE electrophoretic profiles of parotid salivary proteins from sheep and goats have been characterized, with MALDI-TOF MS used to identify the more representative proteins.

The proteins identified in the present study (Table 1) can be sorted into three main functional categories. The largest group includes salivary proteins that exhibit immune response or oral protection functions: complement C3 precursor, gelsolin precursor, serotransferrin precursor, catalase, immunoglobulin, annexin A1, cathepsin H precursor, and glutathione S-transferase P. Among these, catalase and glutathione S-transferase P have a more specific role in detoxification. They are associated with feeding behavior because their presence has been associated with plant consumption (Felton and Duffey 1991; Rodman and Miller 1992; Sreerama et al. 1995; Lampe et al. 2000). Annexin A1 has also been related to taste perception (Neyraud et al. 2006).

A second functional category includes proteins involved in protein biosynthesis: elongation factor 2, heat shock protein HSP 90-beta, and protein disulfide-isomerase A3

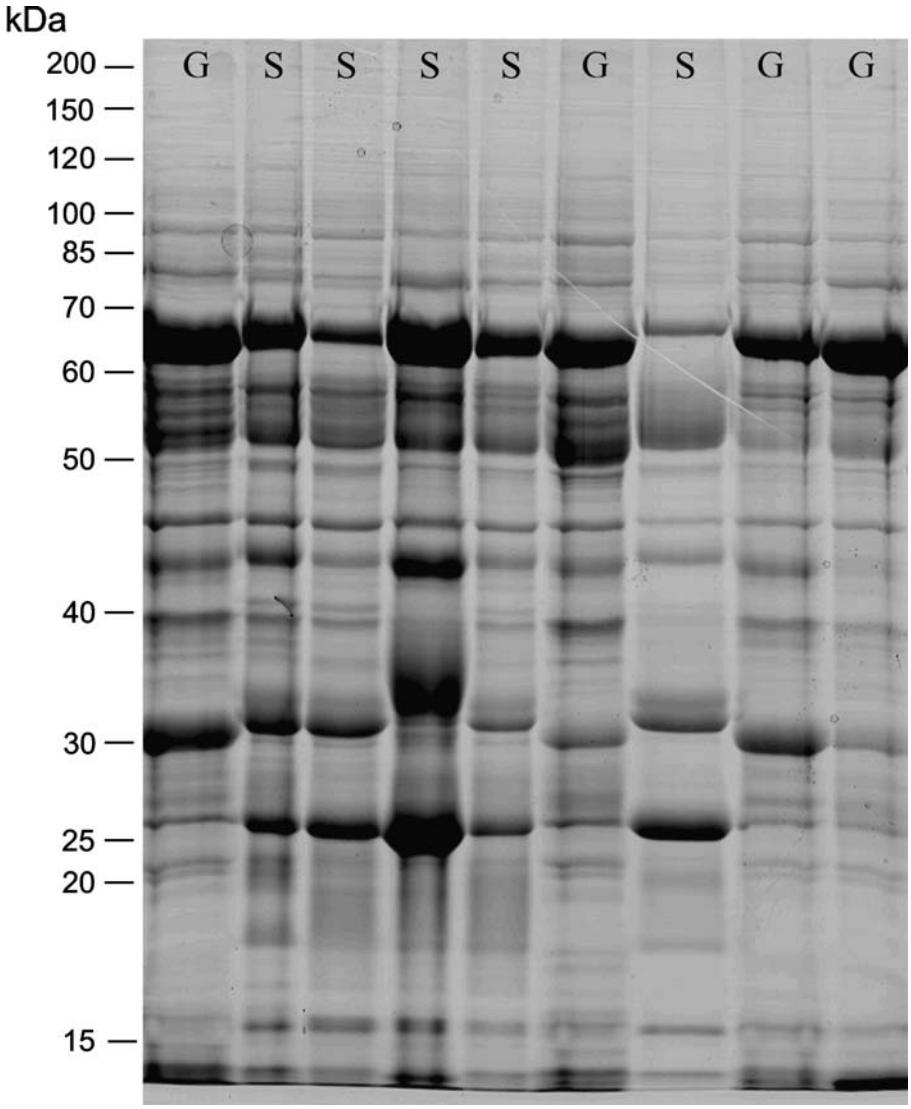

Fig. 2 SDS-PAGE of goat and sheep parotid salivary proteins. Samples were obtained from four different goat (*G*) and five different sheep (*S*) under the same dietary conditions. Similarities among individuals from the same species and differences between species are evident

precursor. The third functional category includes typical serum proteins that, among other functions, are concerned with transport: serotransferrin precursor, serum albumin precursor, and apolipoprotein A-I precursor. The functions of actin and deoxyribonuclease 1 in saliva are not well understood. Some authors have considered deoxyribonuclease 1 as a digestive enzyme (Takeshita et al. 2000), despite others having previously suggested that deoxyribonuclease 1 activity in human parotid saliva is insufficient to fulfill any digestive function (Yaegaki et al. 1982). The presence in saliva of cytoplasmatic proteins, such as actin, may be a consequence of the apocrine-like type of secretion reported for ruminant parotid glands (Stolte and Ito 1996).

Carbonic anhydrase VI is the only protein that has been previously reported from sheep parotid glands, and this is the only sheep salivary protein sequence deposited in databases (Fernley et al. 1988a, b). Carbonic anhydrase VI has a role in electrolytic equilibrium and in the buffer properties of saliva (Kimoto et al. 2006). Its presence in saliva has also been associated with the development of adequate taste function (Henkin et al. 1999).

Our results suggest a strong similarity between the electrophoretic profiles of sheep and goat salivary proteins. From the 16 proteins identified, only one, band p, is not common to both species (Table 1). The similarity likely reflects the phylogenetic proximity of the two species and the consumption of an equal diet during the study. Not surprisingly, we found more pronounced differences when we compared our results with the salivary protein composition of carnivores (Marshall et al. 1993; Williams et al. 1999b) and omnivores (Beeley et al. 1991; Williams et al. 1999a; Hardt et al. 2005). In the dietary habits ranging from carnivores through omnivores to those animals that are exclusively herbivores, plant allelochemical levels increase progressively. It has been suggested (McArthur et al. 1995) that during the evolution from meat to plant eater, selective

Table 1 Proteins identified from SDS-PAGE analysis of parotid saliva of goat, *Capra hircus*, and sheep, *Ovis aries*

Band ID	Animal species	Protein name	Score[a]	Coverage[b] (%)	Pep[c]	MSDB Accession number	MW a[d] (kDa)	MW t[e] (kDa)	Ref.[f]
a	Goat	Complement C3 precursor (fragment)	68	22	9/38	O46544_SHEEP	121	40	Andoh et al. 1997
	Sheep	Complement C3 precursor (fragment)	128	33	12/28				
b	Goat	Elongation factor 2	85	21	13/35	EF2_BOVIN	117	96	Xie et al. 2005
	Sheep	Elongation factor 2	86	24	16/53				
c	Goat	1) Gelsolin precursor +	1) 149	1) 32	1) 20/53				Xie et al. 2005
		2) Heat shock protein HSP 90-beta	2) 60	2) 19	2) 12/53	1)Q3SX14_BOVIN	100	1) 81	
	Sheep	1) Gelsolin precursor +	1) 102	1) 27	1) 16/52	2)HS90B_BOVIN		2) 84	
		2) Heat shock protein HSP 90-beta	2) 109	2) 25	2) 18/52				
d	Goat	Serotransferrin precursor	62	16	10/36	AAA96735	90	80	Xie et al. 2005; Huang 2004;
	Sheep	Serotransferrin precursor	78	17	12/37				Wilmarth et al. 2004
e	Goat	Serum albumin precursor	75	16	7/14	ABSHS	77	71	Xie et al. 2005; Huang 2004;
	Sheep	Serum albumin precursor	81	17	8/13				Wilmarth et al. 2004; Hardt et al. 2005; Ghafouri et al. 2003; Vitorino et al. 2004; Hu et al. 2005
f	Goat	Unidentified	128	32	16/50	CATA_BOVIN	70	60	Xie et al. 2005; Huang 2004
	Sheep	Catalase							
g	Goat	Protein disulfide-isomerase A3 precursor	94	23	13/36	JC2385	67	55	Xie et al. 2005
	Sheep	Protein disulfide-isomerase A3 precursor	73	19	9/32				
h	Goat	Unidentified							
	Sheep	Unidentified							
i	Goat	Ig heavy chain C region	88	39	8/30	C30554	58	52	Xie et al. 2005; Huang 2004;
	Sheep	Ig heavy chain C region	60	31	7/45				Wilmarth et al. 2004
j	Goat	Actin cytoplasmic 1 (Beta-actin)	90	33	10/30	ATBOB	51	42	Hu et al. 2005; Walz et al. 2006
	Sheep	Actin cytoplasmic 1 (Beta-actin)	98	42	14/47				
k	Goat	Carbonic anhydrase VI	63	38	9/41	CAH6_SHEEP	45	36	Xie et al. 2005; Wilmarth et al. 2004;
	Sheep	Carbonic anhydrase VI	133	58	12/32				Hardt et al. 2005; Ghafouri et al. 2003; Vitorino et al. 2004; Hu et al. 2005; Fernley et al. 1988a

Table 1 (continued)

Band ID	Animal species	Protein name	Score[a]	Coverage[b] (%)	Pep[c]	MSDB Accession number	MW a[d] (kDa)	MW t[e] (kDa)	Ref.[f]
l	Goat	Annexin A1	76	36	9/38	S28228	40	40	Xie et al. 2005;
	Sheep	Annexin A1	109	41	11/36				Wilmarth et al. 2004; Hu et al. 2005; Neyraud et al. 2006
m	Goat	Deoxyribonuclease-1	62	30	5/17	B26325	37	29	Tenjo et al. 1993;
	Sheep	Deoxyribonuclease-1	60	38	6/34				Nadano et al. 1993; Williams et al., 1999a
n	Goat	Unidentified							
	Sheep	Unidentified							
o	Goat	Not present							
	Sheep	Unidentified							
p	Goat	Cathepsin H precursor	55[g]	23	7/41	Q3T0I2[h]	30	38	Saliva Proteome Project, http://fields.scripps.edu/public/project/saliva/
	Sheep	Not present							
q	Goat	Unidentified							
	Sheep	Not present							
r	Goat	Unidentified							
	Sheep	Not present							
s	Goat	Apolipoprotein A-I precursor	174	44	17/51	AAI02942	25	30	Xie et al. 2005; Huang 2004; Wilmarth et al. 2004; Ghafouri et al. 2003
	Sheep	Unidentified							
t	Goat	Glutathione S-transferase Pi	96	64	10/47	AF186248	22	24	Xie et al. 2005; Wilmarth et al. 2004; Ghafouri et al. 2003; Vitorino et al. 2004; Hu et al. 2005
	Sheep	Unidentified	100	64	10/43				
u	Goat	Unidentified							
	Sheep	Unidentified							
v	Goat	Unidentified							
	Sheep	Unidentified							

[a] The minimum Mascot score for a probability less than 5% for the match to be a random event is 59.
[b] Percentage of identified protein sequence covered by matched peptides
[c] Number of peptides from experimental PMF whose masses match those from a theoretical PMF determined from a known sequence/Number of peptides from experimental PMF submitted for Mascot search
[d] Molecular weight apparent
[e] Molecular weight theoretical
[f] Articles reporting the presence of identified proteins in saliva
[g] For Swiss-Prot database searches the minimum score for a probability less than 5% for the match to be a random event is 53.
[h] Swiss-Prot accession number

pressure encouraged salivary proteins with defense functions against anti-nutritive and/or toxic substances present in plants. Saliva is one of the behavioral and physiological mechanisms that mammals have evolved for coping with hazards related to feeding. For herbivores, this can mean having to deal with toxic and anti-nutritive substances, whereas for omnivores, the major risk faced is that of foodborne illness. The tradeoff between costs/nutritional benefits could be reflected in the salivary profiles of different trophic groups, with differences in the proportion of proteins. In humans and rodents, the proportion of serum proteins, relative to total salivary proteins, is lower than the proportion observed in the present study for sheep and goats. Saliva with a composition similar to serum can be more useful for ruminants than for humans or rodents. The lack of digestive enzymes in ruminant saliva has been widely reported and probably reflects digestive characteristics, such as the low levels of starch in the diet and the

importance of ruminal fermentation of structural carbohydrates. An adequate digestion is achieved by the rhythms of salivary secretion and by a more marked role of saliva in providing and maintaining a buffered environment for ruminal fermentation, contributing to half of the bicarbonate entering the rumen (Owens et al. 1998). The digestive differences between ruminants and omnivores, such as humans and rodents referred to above, can also explain why the latter possess other salivary proteins, which we have not found in sheep and goats.

Despite the similarities, the differences found between sheep and goats parotid salivary protein profiles are also meaningful. From the bands common to both profiles, differences in intensity were observed only for band s, identified as apolipoprotein A-I, which was more intense in the sheep profile. The large number of peaks in the peptide map for band s suggests additional unidentified proteins of similar mass in the same gel band. A more pronounced difference was observed in the region of 25–35 kDa. Band p, which was only observed in the goat profile, contained cathepsin H. This protein is involved in the degradation of proteins in lysosomes, and no role in digestion has been attributed to it. As previously discussed, the presence of this cytoplasmic protein in saliva may result from the apocrine-like type of secretion characteristic of ruminants (Stolte and Ito 1996). In addition to the cathepsin peaks, a large number of other peaks were present in the peptide map, suggesting the presence of unidentified proteins in this intense gel band as well. It is possible that cathepsin is also present in sheep parotid saliva in low concentrations, which were insufficient to allow a band observation in Coomassie stained gels. For band o, which was only observed in the sheep profile, and bands q and r, only observed in the goat profile, we were unable to obtain identification.

Some authors (Austin et al. 1989; Fickel et al. 1998) refer to the presence of salivary PRPs in browser ruminant species and to their absence in sheep saliva. As sheep are grazers and goats are intermediate feeders, one possibility is that goats could have salivary PRPs. Human basic PRPs, which are the group of PRPs with a higher affinity for tannins, have molecular masses between 14 and 45 kDa (Bedi and Bedi 1995), which correspond to mass values of the unidentified bands. We tested the presence of PRPs by staining the gels with Coomassie brilliant blue R-250, following the protocol of Beeley et al. (1991), but were unable to observe the characteristic pink bands. The absence of salivary PRPs in sheep and goat parotid saliva may reflect the low tannin diet consumed by the two species during the experiment. Further studies with the incorporation of high levels of tannins into the diet may be useful in assessing the induction of this particular group of salivary proteins.

This study provides a first step to the full characterization of the goat and sheep parotid saliva protein profile, and it provides useful information that can be used to study further the immediate oral adaptation to the diet. Based on the differences between the species, even when fed under a similar feeding situation, we suspect that salivary protein composition can play an important role in feeding choices. The complexity of parotid saliva is evident from the great number of protein bands, the lack of identification of some of them, and the large number of tryptic peptides obtained for each one. This highlights the importance of the use of more powerful separation techniques. Moreover, we think that more dynamic information can be obtained by studying these two species subjected to different diets. We intend to use two-dimensional electrophoresis coupled to MS and MS/MS to study potential changes in the parotid saliva proteome caused by the consumption of tannin-enriched diets.

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