Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: https://www.tandfonline.com/loi/taar20

Glycoconjugates in Sheep Buccal Glands Investigated by Conventional and Lectin Histochemistry

V. Pedini , C. Dall'Aglio , F. Mercati , L. Pascucci & P. Scocco

To cite this article: V. Pedini , C. Dall'Aglio , F. Mercati , L. Pascucci & P. Scocco (2008) Glycoconjugates in Sheep Buccal Glands Investigated by Conventional and Lectin Histochemistry, Journal of Applied Animal Research, 34:1, 49-54, DOI: 10.1080/09712119.2008.9706939

To link to this article: https://doi.org/10.1080/09712119.2008.9706939

J. Appl. Anim. Res. 34 (2008) : 49-54

Glycoconjugates in Sheep Buccal Glands Investigated by Conventional and Lectin Histochemistry

V. Pedini[1]*, C. Dall'Aglio[1], F. Mercati[1], L. Pascucci[1], P. Scocco[2]

[1]Department of Biopathological Sciences and Hygiene of Animal and Food Production
Via San Costanzo 4, 06126 Perugia, Italy

[2]Department of Environmental Sciences
Via Circonvallazione 93-95, 62024 Matelica (MC), Italy

(Received April 15, 2008; accepted August 21, 2008)

Abstract

Pedini, V., Dall'Aglio, C., Mercati, F., Pascucci, L. and Scocco, P. 2008. Glycoconjugates in sheep buccal glands investigated by conventional and lectin histochemistry. J. Appl. Anim. Res., 34: 49-54.

Sheep buccal glands consist of mucous acini capped by demilunes. Information on the chemical structure of their secretory glycoconjugates were obtained by means of a battery of peroxidase conjugated lectins with affinity for specific terminal and/or internal sugars. Neuraminidase procedures followed by lectin staining were also used to visualize the carbohydrate sequence. Stored secretions in mucous acinar cells contained glycoconjugates with N-acetylglucosamine and sialic acid linked to αN-acetylgalactosamine and galactosyl (β1→3) N-acetylgalactosamine. Demilunar cells displayed fucose, mannose, N-acetylglucosamine and α,N-acetylgalactosamine residues. Cells lining duct system showed a very strong staining at the apical surface and in the cytoplasm with UEA I, LTA and ConA.

Keywords: Sheep, lectin histochemistry, buccal glands, glycoconjugates.

Introduction

Salivary mucins have a number of functions: they hydrate and lubricate the oral structures, facilitate the oral phase of swallowing by enhancing the formation of a slippery food bolus, regulate the oral microbial flora, protect oral surfaces against chemical and mechanical damage (de Almeida *et al.*, 2008). Salivary mucins are synthesized by the secretory cells of major salivary glands, as well as by minor salivary glands distributed throughout the palatal and buccal mucosa and in the tongue (Hand *et al.*, 1999).

*For correspondence: Tel: +39 75 585 7632;
Fax: +39 75 585 7631; E-mail: vera.pedini@unipg.it

Buccal glands are located in the submucosa and arranged among striated muscle bundles of the cheeks. In ruminants, they are made of strings of lobules that extend parallelly to molar arches and consist of three masses: dorsal, ventral and intermediate. Dorsal buccal glands are the most voluminous group: they are situated along the dorsal border of the buccinator muscle. They are made of different lobules grouped together in their caudal portion. Ventral buccal glands are less compact and abundant and they are located along the ventral border of the buccinator muscle. Intermediate buccal glands are specific of ruminants and, actually, these are part of the ventral ones. Buccal glands are compound tubuloacinar glands composed of acini with

J. Appl. Anim. Res. 0971-2119/2008/$10.00

mucus secreting cells, which border the lumen and thin demilunes at the periphery (Dyce *et al.*, 2002).

The aim of this study was to investigate the glycoconjugate composition of sheep buccal gland secretion by conventional histochemistry and using a panel of ten peroxidase-labelled lectins combined with enzymatic digestion.

Materials and Methods

Ten clinically healthy sheep, 5 of each sex, killed at the slaughterhouse of Perugia, were used. Samples of dorsal and ventral buccal glands were fixed at room temperature in Carnoy's fluid for 24 h and postfixed in 2% calcium acetate – 4% paraformaldehyde solution (1:1) for 3 h. Specimens were then dehydrated through a series of graded ethanols, cleared in xylene, embedded in paraffin wax and cut in 5 µm thick serial sections.

Carbohydrate characterization was obtained on sections with the following stainings: Periodic acid-Schiff (PAS), Alcian-blue (AB) pH 2.5, Neuraminidase (Neu)-AB, KOH-Neu-AB, AB-PAS, AB pH 1, AB pH 0.5, low iron diamine (LID), high iron diamine (HID) (Pearse, 1985).

Sections were immersed in a solution of 3% hydrogen peroxide in absolute methanol for 10 min at room temperature to inhibit endogenous peroxidase activity, which was followed by washing in distilled water and 0.1 M Na^+ and K^+ phosphate-buffer (PBS) pH 7.2. Then sections were incubated in a solution of 10-40 µg ml^{-1} lectin-horseradish peroxidase conjugate (Sigma, St. Louis, MO, USA) in PBS, containing 0.1 mM $CaCl_2$, $MnCl_2$ and $MgCl_2$ for 1 h at room temperature. Thereafter, lectin binding sites were revealed using an incubation medium containing diaminobenzidine-hydrogen peroxide for 15 min at room temperature (Schulte *et al.*, 1985). The horseradish peroxidase-lectin conjugates were purchased from Sigma, St. Louis, MO, USA and were: *Triticum vulgare* (WGA) specific for N-acetylglucosamine (GlcNAc), *Griffonia simplicifolia* (GSA II) specific for N-acetylglucosamine (GlcNAc), *Griffonia simplicifolia* (GSA IB_4) specific for α galactose (Gal), *Ricinus communis* (RCA I) specific for galactosyl (β1→4) N-acetylglucosamine (Galβl→4GlcNAc), *Dolichos biflorus* (DBA) specific for α, N-acetylgalactosamine (αGalNAc), *Glycine max* (SBA) specific for α, βN-acetylgalactosamine (α, βGalNAc), *Arachis hypogea* (PNA) specific for galactosyl (β1→3)N-acetylgalactosamine (Galβl→3GalNAc), *Ulex europaeus* (UEA I) specific for α fucose (Fuc), *Lotus tetragonolobus* (LTA) specific for α fucose (Fuc), *Canavalia ensiformis* (ConA) specific for D-mannose > D-glucose (Man). As negative controls, sections were incubated in lectin solutions to which 0.2 M hapten sugars had been added.

Enzymatic treatment was carried out on adjacent sections. Prior to lectin histochemistry, sections were incubated at 37C for 16 h in a 0.8 IU ml^{-1} solution of neuraminidase (Neu) (sialidase) from *Clostridium perfringens* (Sigma, St. Louis, MO, U.S.A.) in 0.1 M sodium acetate buffer, pH 5.5, containing 10 mM $CaCl_2$. The controls for enzyme digestion were exposed to neuraminidase-free buffer under the same experimental conditions. Sialic acid residues with O-acetyl substituents at C-4, that resisted sialidase treatment (Moschera and Pigman, 1975), were cleaved after removal of the acetyl groups by saponification. This was performed by immersing the sections in a 1% solution of potassium hydroxide in 70% ethanol for 15 min at room temperature prior to enzymatic digestion.

Results and Discussion

Sheep dorsal and ventral buccal salivary glands showed the same results with conventional histochemistry and the same staining patterns with lectins.

Acinar cells revealed an intense staining with PAS, AB pH 2.5, LID, demonstrating the

presence of neutral and acidic glycoconjugates. Acidic materials present in the secretory endpieces consisted of glycoconjugates containing prevalently carboxyl groups, as demonstrated by the loss of alcianophilia after neuraminidase digestion. HID and AB pH 1.0 reactivities demonstrated the presence of carbohydrates with sulphate groups. Demilunar and ductal cells, stained only by PAS, secreted neutral mucins.

For all the lectins employed, staining was completely inhibited when the lectin-horseradish peroxidase conjugates were incubated with the appropriate specific sugar.

Lectin histochemistry allowed a further characterization of glycoconjugates. WGA binds to both sialic acid and N-acetylglucosamine (Pedini *et al.*, 2000) but sialidase treatment did not affect the positive staining observed with WGA (Fig. 1) in some acinar cells, suggesting the presence of GlcNAc residues inside them. GSA II negativity, also after neuraminidase treatment, and WGA positivity demonstrated that these residues were not localized in terminal or subterminal position of glycoconjugates (Pedini *et al.*, 2005). Furthermore, GlcNAc was probably not linked to galactose, as no reactivity was observed with RCA I, a lectin with nominal specificity for galactosyl(β1→4)N-acetylglucosamine.

Acinar cells showed particularly the presence of sialoglycoconjugates with sialic acid linked to αN-acetylgalactosamine and galactosyl (β1→3)N-acetylgalactosamine, testified by the intensification of DBA (Figs. 2, 3) and PNA (Fig. 4) staining after neuraminidase digestion. The failure of saponification to alter reaction intensity of DBA and PNA staining after sialidase digestion indicated the absence, or the presence at only low levels, of sialic acid residues containing O-acetyl substituents at C_4 (Accili *et al.*, 2008). The terminal disaccaride NeuNAc-αGalNAc and the terminal sequence NeuNAc-(α2→3,6)-Galβ1→3GalNAc were observed by Schulte *et al.* (1985) and by Menghi *et al.* (1992) in the mucous cells of ovine and bovine submandibular glands respectively. According to Schulte *et al.* (1985), DBA's high specificity for the alpha anomer of GalNAc confirmed the understanding that GalNAc was linked to serine or threonine residues in the alpha configuration. Furthermore DBA and SBA overlapped stainings indicated the absence of the beta anomer of GalNAc.

Complete ConA (Fig. 5) negativity of acinar cells evidenced the absence in such cells of glycoproteins containing N-linked oligosaccharides, in fact, according to Spicer and Schulte (1992), "O-linked chains contain less than 1% Man and lack the Man and branches and bisection at Man on which the lectin affinity depends". Therefore N-acetylglucosamine visualized by WGA in some acinar cells was not linked to asparagine, but it was localized in O-linked class of glycoproteins.

On the other hand, N-glycosylation was present in demilunar cells, testified by the very strong staining obtained with ConA (Fig. 5). This result, associated with weak WGA reactivity (Fig. 1), allowed also to hypothesize that N-linked chains belong to the subgroups containing high mannose residues (Spicer and Schulte, 1992). O-glycosidically bound chains containing GalNAc were detected by DBA (Fig. 2) in demilunar cells. Enzyme digestion failed to increase DBA staining (Fig. 3), therefore, GalNAc was not linked to sialic acid. Moreover, the same staining intensity obtained with DBA and SBA demonstrated that the alpha anomer of GalNAc was the only one present. A notable amount of glycoconjugates with terminal agalactose were visualized in demilunar cells by GSA I and the very strong stainings caused by UEA I and LTA revealed a remarkable presence of fucose residues. Fucose in N-linked glycoproteins was preferably marked by LTA, whereas, UEA I reacted with Fuc residues in O-linked ones (Pedini *et al.*, 2005), therefore, the results obtained with these two lectins confirmed the simultaneous presence of O and

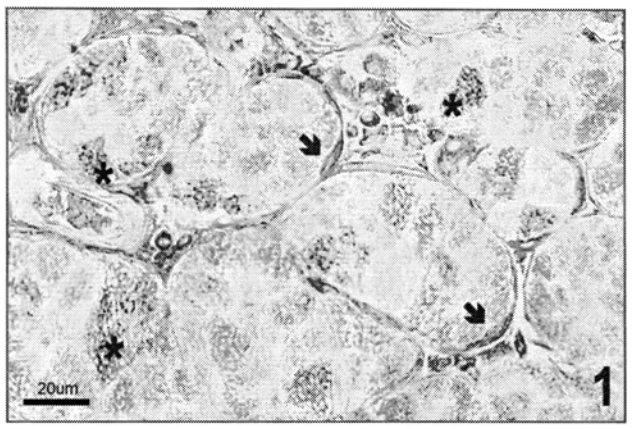

Fig. 1. WGA-HRP staining. This lectin moderately labelled some acinar cells (asterisks) and demilunes (arrows).

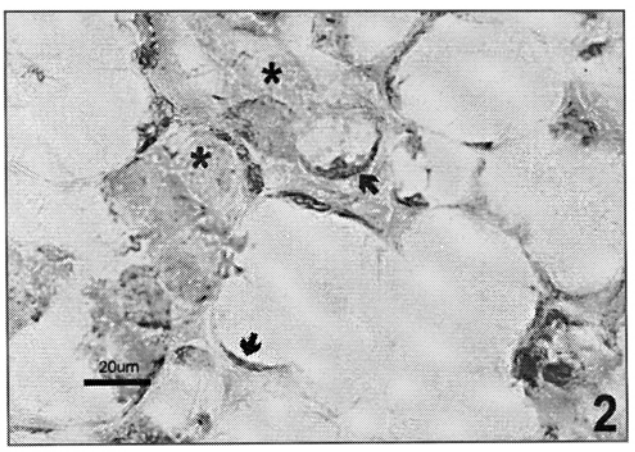

Fig. 2. DBA-HRP staining. Demilunar cells showed a strong reaction (arrows), while a moderate staining was localized in some acinar cells (asterisks).

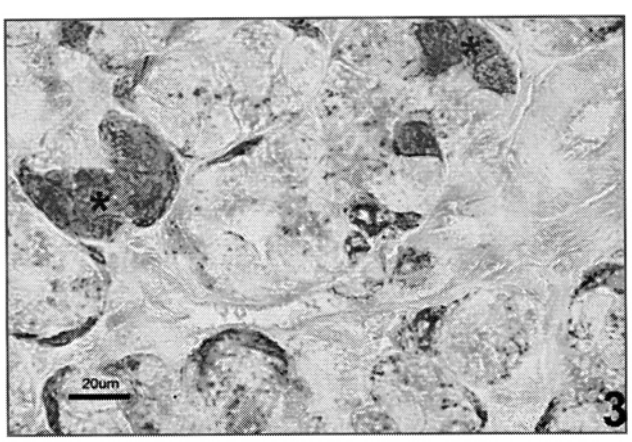

Fig. 3. Neu-DBA-HRP staining. Acinar cells enhanced their reactivity toward this lectin after enzymatic digestion (asterisks).

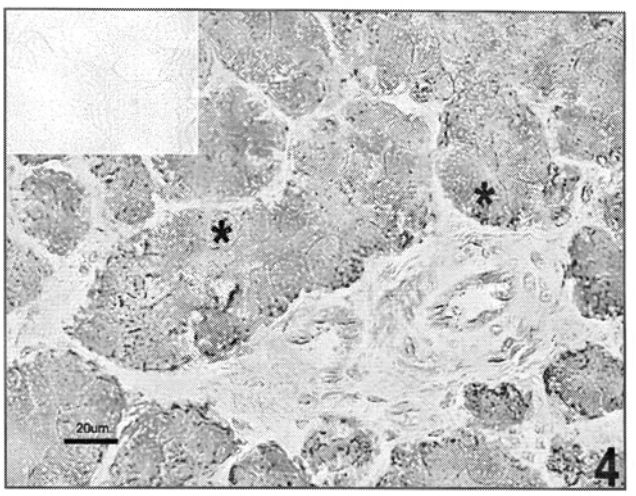

Fig. 4. Neu-PNA-HRP staining. Neuraminidase treatment caused a strong staining with this lectin (asterisks). Insert. PNA-HRP staining. A complete negativity was observed with the use of PNA only.

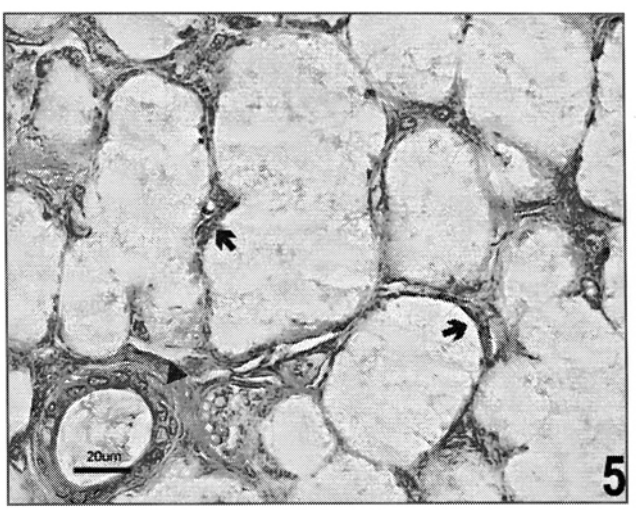

Fig. 5. ConA-HRP staining. Demilunar cells showed a very strong reaction (arrows) and ductal cells a little less staining (headarrows).

N-glycosylation in demilunar cells. Schulte *et al.* (1985) did not find carbohydrate-rich macromolecules other than glycogen in ovine mandibular demilunes, but these authors also couldn't explain this result which was very unusual. In fact studies performed on salivary glands of different mammals always revealed the presence of glycoconjugates in demilunar cells (Pedini *et al.*, 1997; 2000; Triantafyllou *et al.*, 2004; Scocco and Pedini, 2006).

Cytoplasm of cells lining ducts was stained intensely with UEA I, LTA and ConA (Fig. 5): these findings may be attributable to the presence of fucose and mannose or glucose containing glycoconjugates. A similar staining pattern was reported in mandibular gland duct system of fallow deer (Pedini *et al.*, 1997), pig (Pedini *et al.*, 2000) and horse (Scocco and Pedini, 2006). The relative hydrophobicity of fucose perhaps contributes in some way to transport functions of duct cells (Spicer and Schulte, 1992). ConA staining was probably due to the glucose residues in the glycogen present at these sites. Glycogen represents a reserve of energy that is used by ductal cells to produce hypotonic saliva (Pedini *et al.*, 1997; 2000).

In conclusion, glycoconjugates secreted by sheep buccal glands showed a great variety in the structure of carbohydrate chains, like those produced in other sheep salivary glands (Schulte *et al.*, 1985). The structural diversity of salivary mucins may represent a mosaic of potential binding sites for the attachment of exogenous macromolecules such as those of bacterial or viral origin, allowing microorganisms trapping in the mucus. In addition, the diversity of glycoconjugates could permit to the host to mask potential receptor sites for noxious microorganisms (Yasui *et al.*, 2005; Scocco and Pedini, 2006).

Acknowledgements

The authors wish to thank G. Mancini and P. Coliolo for their excellent technical assistance.

References

Accili, D., Menghi, G. and Gabrielli, M.G. 2008. Lectin histochemistry for *in situ* profiling of rat colon sialoglycoconjugates. Histol. Histopathol., 23: 863-875.

de Almeida Pdel, V., Gregio, A.M., Machado, M.A., de Lima, A.A. and Azevedo, L.R. 2008. Saliva composition and functions: a comprehensive review. J. Contemp. Dent. Pract., 9: 72-80.

Dyce, K.M., Sack, W.O. and Wensing, C.J.G. 2002. *Textbook of Veterinary Anatomy*. W.B. Saunders Company, Philadelphia.

Hand, A.R., Pathmanathan, D. and Fielf, R.B. 1999. Morphological features of the minor salivary glands. Arch. Oral Biol., 44: S3-S10.

Menghi, G., Accili, D., Scocco, P. and Materazzi, G. 1992. Sialoglycoderivatives of bovine submandibular gland identified *in situ* by histochemical techniques combined with lectins. Histochem., 97: 397-403.

Moschera, J. and Pigman, W. 1975. The isolation and characterization of rat sublingual mucus glycoprotein. Carbohydrate Res., 40: 53-67.

Pearse, A.G.E. 1985. *Histochemistry. Theoretical and Applied*. Churchill Livingstone, Edinburgh, pp. 735-753.

Pedini, V., Ceccarelli, P., Gargiulo, A.M. and Dall'Aglio, C. 1997. Histochemical study of lectin binding in the major salivary glands of adult fallow-deer (*Dama dama* L.). Acta Histochem., 99: 81-89.

Pedini, V., Dall'Aglio, C., Parillo, F. and Scocco, P. 2005. Glycoconjugate distribution in gastric fundic mucosa of *Umbrina cirrosa* L. revealed by lectin histochemistry. J. Fish Biol., 66: 222-229.

Pedini, V., Scocco, P., Dall'Aglio, C., Ceccarelli, P. and Gargiulo, A.M. 2000 Characterisation of sugar residues in glycoconjugates of pig mandibular gland by traditional and lectin histochemistry. Res. Vet. Sci., 69: 159-163.

Schulte, B.A., Spicer, S. and Miller, R.L. 1985. Lectin histochemistry of secretory and cell-surface glycoconjugates in the ovine submandibular gland. Cell Tissue Res., 240: 57-66.

Scocco, P. and Pedini, V. 2006. Equine mandibular gland: *in situ* characterisation of sialoderivatives. Equine Vet. J., 38: 410-415.

Spicer, S.S. and Schulte, B.A. 1992. Diversity of cell glycoconjugates shown histochemically: a perspective. J. Histochem. Cytochem., 40: 1-38.

Triantafyllou, A., Fletcher, D. and Scott, J. 2004. Glycosylations in demilunar and central acinar cells of the submandibular salivary gland of ferret investigated by lectin histochemistry. Arch. Oral Biol., 49: 697-703.

Yasui, T., Tsukise, A., Fukui, K., Kuwahara, Y. and Meyer, W. 2005. Histochemical localization of complex carbohydrates in the nasolabial glands of the Japanese deer (*Cervus nippon yakushimae*). Acta histochem., 107: 187-198.

वी. पेदिनी, सी. दत्ल एग्लियो, एफ. मर्केती, एल. पास्कुस्सी, पी. स्कोक्को। परंपरागत और लेक्टिन ऊतकी रसायन विधि से अन्वेषण से भेड़ों की मुखीय ग्रंथियों में ग्लाइको संयुग्मी।

भेड़ों की मुखीय ग्रंथियां अर्द्धचन्द्र से मुंडाच्छादित श्लेष्मल कोष्टकों की होती है। उनके स्रावक ग्लाइको संयुग्मियों की रसायनिक संरचना की जानकारी

परआक्सिडेज संयुग्मित लेक्टिनों की विभिन्न अंतस्थ और/अथवा आंतरिक शर्कराओं से लगाव से ज्ञात की गयी। कार्बोहाइड्रेटों के क्रम को ज्ञात करने के लिए न्यूराएमिनीडेज क्रिया के बाद लेक्टिन रंजन का उपयोग किया गया। श्लेष्मल कोष्टकों में संचित स्रावों में एन-एसिटाइलग्लुकोसामिन और अल्फा एन एसिटाइल गैलेक्टोसामिन युग्मित सियालिक अम्ल और गैलेक्टोसील (बीटा 1 → 3) एन एसिटाइललैक्टोसामिन था। अर्द्धचन्द्र कोशिकाओं में फुकोस, मैनोज, एन एसिटाइलग्लुकोसामीन और अल्फा एन एसिटाइल गैलेक्टोसामिन अवशेष थे। वाहिनी भित्ति स्तर कोशिकाओं की शीर्ष सतह और कोशिका द्रव में यूईए 1, एलटीए और कोन ए प्रचुर रंजन पाया गया।