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Ultracytochemistry of glycoproteins in the eccrine nasolabial glands of the North American raccoon (*Procyon lotor*)

By T. Yasui, A. Tsukise and W. Meyer

Department of Veterinary Anatomy, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa, Japan; Institute of Anatomy, University of Veterinary Medicine Hannover, Hannover, Germany

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Abstract

The distribution and quality of glycoproteins was studied by means of electron microscopic cytochemical methods, particularly lectin cytochemistry, in the secretory cells of the eccrine nasolabial glands of the North American raccoon (*Procyon lotor*). In the dark and clear glandular cells, complex glycoconjugates were demonstrable, predominantly, in secretory granules, the cisternae of the Golgi apparatus, the surface coat of the plasma membrane, and as glycogen particles. Secretory granules found in the dark cells contained a variety of saccharide residues, such as α-D-mannose, β-D-galactose, β-*N*-acetyl-D-glucosamine and sialic acid. Several sugars were also detectable in the surface coat of the plasma membrane and the Golgi apparatus.
The results obtained may be helpful to understand the specific functions of the glandular secretions of the raccoon nasolabial glands. These could be, particularly, binding of water on the snout surface and protection against microbial hazards, to maintain the structural and functional integrity of the relatively thin snout epidermis in carnivores.

Key words: *Procyon lotor*, nasolabial glands, glycoproteins, cytochemistry

Introduction

Regional variations in the structure of the mammalian integument are normally connected with manifold functional properties. These modifications may ultimately result in the development of a sense organ, such as the snout with its numerous sensory peculiarities (Halata 1993; Schwarz and Meyer 1994). Apart from this aspect, secretions produced by eccrine nasolabial glands and containing abundantly complex carbohydrates with different saccharide residues, may be concerned with various functions especially on the surface of the snout or the muzzle, respectively (Tsukise et al. 1983, 1988a, b; Meyer and Tsukise 1989; Yasui et al. 2003, 2005c). In contrast to the conditions in the Suidae and the Bovidae, in the Carnivora this body region was normally considered to be free of any gland type (e.g., Schwarz and Meyer 1994; Meyer and Tsukise 1995; Bacha and Bacha 2000). However, we previously reported on the presence of eccrine glands in

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the skin of the raccoon snout, and on the nature of the ingredients of the secretions elaborated (Yasui et al. 2005b). However, precise analyses at the cytological level of complex glycoconjugates in these glands of the raccoon snout skin have not been available until now.

In the present study, therefore, the glandular acini of the eccrine nasolabial glands in the North American raccoon (*Procyon lotor*) were subjected to cytochemical analyses of glycoproteins. The ultrastructural data achieved may be indispensable to understand the general biological significance of such gland type in the snout skin of carnivores.

Material and methods

Experiments were performed following the protocols of the guidelines for the care and use of laboratory animals at the Institute of Experimental Animal Science, College of Bioresource Sciences, Nihon University. Four adult North American raccoons (*P. lotor*; males, weight 8–12 kg) were deeply anesthetized and then exsanguinated from the common carotid arteries. After bloodletting, samples were obtained from the snout skin.

For general ultrastructural observation, the skin samples were fixed in a mixture of 4% paraformaldehyde (PFA) and 2% glutaraldehyde (GA) in 0.1 M phosphate-buffered saline (PBS) (pH 7.4) for 2 h at 4 °C. These tissue pieces were post-fixed in 2% osmium tetroxide solution for 2 h and embedded in Epon 812 in accordance with routine techniques (Luft 1961). From these tissue blocks, ultrathin sections were cut on an ultramicrotome, mounted on copper grids and stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963). For the cytochemical demonstration of glycoconjugates, specimens were fixed in 4% PFA and 0.5% GA solution in 0.1 M PBS (pH 7.4) for 2 h at 4 °C and embedded in LR-White resin (Newman et al. 1983). Ultrathin sections were cut as described above and placed on nylon or nickel grids.

The nylon grid-mounted sections were subjected to the periodic acid–thiocarbohydrazide–silver proteinate-physical development procedure (PA–TCH–SP-PD) for the demonstration of vicinal diols of carbohydrates (Yamada 1993), followed by counterstaining with uranyl acetate and lead citrate. For the cytochemical identification of glycogen in the cytoplasm, enzyme digestion with α-amylase (from *Bacillus subtilis*, Seikagaku Kogyo Co., Tokyo, Japan) (Casselman 1959) was conducted before the PA–TCH–SP-PD staining procedure. Additionally, a specificity control for this enzyme digestion technique was carried out; some sections were incubated in buffer solutions without the enzyme under conditions of the same duration and temperature.

The sections on the nickel grids were incubated with different biotinylated lectins (Seikagaku Kogyo Co.) at concentrations of 5–10 μg/ml in 0.05 M PBS (pH 7.2) for 12 h at 4 °C (Roth 1983, 1996). The lectins used, their specific sugar residues and inhibitory sugars are listed in Table 1 (for lectin specificities see Debray et al. 1981; Spicer and Schulte 1992; Danguy 1995). After rinsing with PBS, the sections were incubated with colloidal gold-labeled streptavidin (British Biocell International, Cardiff, UK) diluted

Table 1. The lectins used and their specific sugar residues

	Lectins	Sugar binding specificity	Inhibitory sugar
Con A	Concanavalin A	α-D-Man, α-D-Glc	α-D-Man, α-methyl-D-glucoside
RCA-120	*Ricinus communis* agglutinin	Galβ1–4GlcNAc	β-D-Gal
WGA	Wheat germ agglutinin	β-D-GlcNAc, Sia	β-D-GlcNAc
SSA	*Sambucus sieboldiana* agglutinin	Siaα2–6Gal/GalNAc	α2-6sialyllactose
MAM (MAL)	*Maackia amurensis* agglutinin	Siaα2–3Galβ1–4GlcNAc	α2-3sialyllactose