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Characterization of Glycoconjugates in the Sublingual Salivary Gand of Malayan Pangolin *(Manis javanica)*

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ABSTRACT

The sublingual salivary gland of Malayan pangolin*(Manis javanica)* was investigated by using conventional and lectin histochemical methods in combination with enzyme digestion procedures. The results showed both mucous and serous cells in secretory endpieces of sublingual salivary gland. Mucous cells showed a large number of vicinal diol groups with acid and neutral glycoconjugates. Lectin histochemistry gave evidence of the presence of mannose, α N - acetylgalactosamine, N- acetylglucosamine and sialic acid linked to Galactosyl (β1→3) N – acetylgalactosamine residues.

Serous cells had positive reaction with almost all the conventional employed, with the exception of acid glycoconjugates which were less diffused. Serous cells secreted predominantly neutral glycoconjugates with mannose, Galactosyl (β1→4) N- acetylglucosamine, N- acetylglucosamine and fucose residues.

Key words: sublingual salivary gland, glycoconjugates, histochemistry, Malayan pangolin, lectin

INTRODUCTION

The Malayan pangolin *(Manis javanica)* or scaly anteater is a unique and interesting mammal found in Thailand and other countries in Southeast Asia. Populations of most pangolin species are somehow threatened. *Manis javanica* is listed by IUCN as Red list or Lower risk, meaning that it is nearly threatened (Nowak,1999). Pangolin has an enormous salivary gland in its chest to lubricate the tongue with sticky, ant catching saliva. Therefore, they are important to the environment because they can control insect and termite populations. By constructing burrows and digging a bit to get at ants and termites, these animals also aid in soil aeration.

Glycoconjugates in salivary glands of mammalian species have been studies previously (Shackleford and Klapper,1962; Spicer and Duvenci,1964; Harrison,1974). From these studies, the cyto – architecture and histochemical reactivities of the salivary gland are known to vary widely, between glands for a single species and between species for a given gland. Lectins are currently valuable histochemical probes for detecting specific carbohydrate residues due to their high affinity and specificity. (Pedini *et al.,* 2002). Nevertheless, glycoconjugates histochemical data on the sublingual salivary gland of Malayan pangolin *(Manis javanica)* is not available. Therefore, this study was performed to detect glycoconjugates in sublingual salivary gland by using conventional

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and lectin staining procedures.

METERIALS AND METHODS

Tissue preparation

The Malayan pangolin *(Manis javanica)* carcasses were obtained from Khao Prathup Chang Wildlife Breeding Center in Ratchaburi Province Thailand. Ratchaburi province, Thailand. Samples of the sublingual salivary gland were fixed in buffered formalin. Tissues were dehydrated through a series of graded ethanols, clearing in xylene and embedded in paraffin wax. Sections were cut at thickness of 3-4 μm, deparaffinized in xylene, hydrated in graded ethanols. They were then subjected to staining with conventional and lectin staining procedures.

Conventional staining procedures

1. Hematoxylin and Eosin (H&E) for the general observation of histological structures.

2. Acian Blue pH 2.5 (AB pH 2.5) for detection of acidic glycoconjugates (Spicer *et al.,* 1967).

3. Periodic acid - Schiff (PAS) for detection of vicinal – diol groups of glycoconjugates (Pearse,1968).

4. AB pH 2.5 - PAS for demonstrating acidic and neutral glycoconjugates (Mowry,1963).

Lectin staining procedures

1. Glycine max (SBA) for β N - acetylgalactosamine residues.

2. *Ulex europaeus agglutinin -I* (UEA-I) for fucose residues (Goldstein and Hayes, 1978).

3. Concanavalin A (Con A) for mannose residues of glycoconjugates (Yamada and Shimizu,1976).

4. *Dolichos biflorus agglutinin* (DBA) for a N - acetylgalactosamine residues.

5. Wheat germ agglutinin (WGA) for N-acetylglucosamine residues (Goldstein and Hayes,1978).

6. *Ricinus communis agglutinin –I* (RCA-I) for Galactosyl (β1 →4) N-acetylglucosamine (Yamada and Shimizu,1977).

7. Peanut agglutinin (PNA) for Galactosyl (β1 →3) N – acetylgalactosamine residues (Stoward, 1980).

Enzyme digestion

Enzymatic digestion with neuraminidase (from *Vibrio cholerae*) was performed. Prior to staining with AB pH 2.5 and lectin PNA, sections were incubated in 0.1 M sodium acetate buffer (pH 5.3) containing 1 unit / ml of the enzyme and 0.04 M $CaCl_2$ at 39 – 41 °C for 12 - 16 h (Spicer *et al.,* 1967). Enzyme digestion was controlled by being exposed to neuraminidase free buffer under the same experimental conditions. (Pedini *et al.*, 2004).

RESULTS

The sublingual salivary gland of Malayan pangolin *(Manis javanica)* showed tubuloacinar gland. It was characterized by the presence of mucous cells and serous cells in secretory endpieces. The staining results in the mucous cells and serous cells are listed in the Table 1.

The mucous cells stained strongly positive with AB pH 2.5 (Figure 9) and PAS (Figure 1). In combined staining, the mucous cells were colored deep blue and some other were bluish - purple with AB pH 2.5 - PAS (Figure 2). The AB pH 2.5 (Figure 10) reaction decreased in intensity after digested with neuraminidase. Mucous cells presented positive staining with all lectins, except SBA (Figure 3) and UEA - I (Figure 4). Labeling was strong with Con A (Figure 5). DBA (Figure 6) weakly to moderately stained mucous cells. Mucous cells were stained by WGA (Figure 7), RCA - I (Figure 8) and PNA (Figure 11). After neuraminidase digestion, the mucous cells stained moderately to strongly with PNA (Figure 12).

In the striated duct cells, the supranuclear region of mucous cells localized at

the apex of the folds showed moderate to strong reactivity with UEA - I, Con A, DBA, WGA, RCA - I and PNA and were weakly stained with AB pH 2.5 - PAS. In contrast, they did not stain with AB pH 2.5 , PAS and SBA.

The serous cells exhibited very strong magenta staining with PAS (Figure 1). In contrast, they stained weakly with AB pH 2.5 (Figure 9) and negative stain or weakly stain after treated with neuraminidase (Figure 10). The serous cells were stained moderately to strongly with UEA – I (Figure 4) and RCA – I (Figure 8) respectively. The labeling was also moderate to strong with Con A (Figure 5) and WGA (Figure 7). The serous cells showed weak staining with DBA (Figure 6), whereas negative stain was evidenced by SBA (Figure 3). Labeling was strong to very strong with PNA (Figure 11). However, digestion with neuraminidase did not cause an increment in PNA (Figure 12) staining.

Table 1 Histochemical reaction of glycoconjugates in sublingual salivary gland of the Malayan pangolin *(Manis javanica)*.

Staining methods	Mucous cells	Serous cells
AB pH 2.5	3	1
Neu - AB pH 2.5	0 - 1	0 - 1
PAS	2 - 3	4
AB pH 2.5 - PAS	2 - 3	3 - 4
SBA	0	0
UEA-I	0	3 - 4
Con A	3	2 - 3
DBA	1 - 2	1
WGA	1	2
RCA-I	1	4
PNA	1	3 - 4
Neu - PNA	2 - 3	3 - 4

Numbers indicate staining intensity on a subjective scale :
0 = unstained, 1= weak, 2= moderate, 3= strong , 4 = very strong , Neu = Neuraminidase

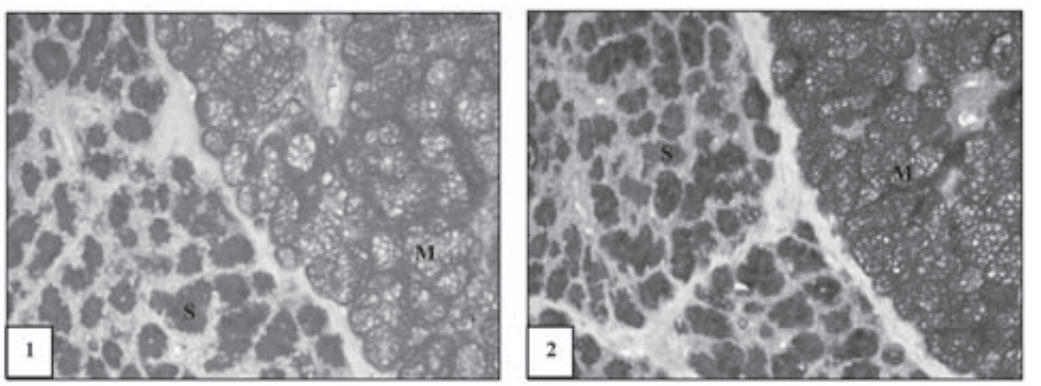

Figure 1 The mucous cells (M) stained strongly positive with PAS. The serous cells (S) exhibit very strong magenta staining with PAS (200X).

Figure 2 The dual staining with AB pH 2.5 – PAS resulted in deep blue with mucous cells (M) and strong red with serous cells (S) (200X).

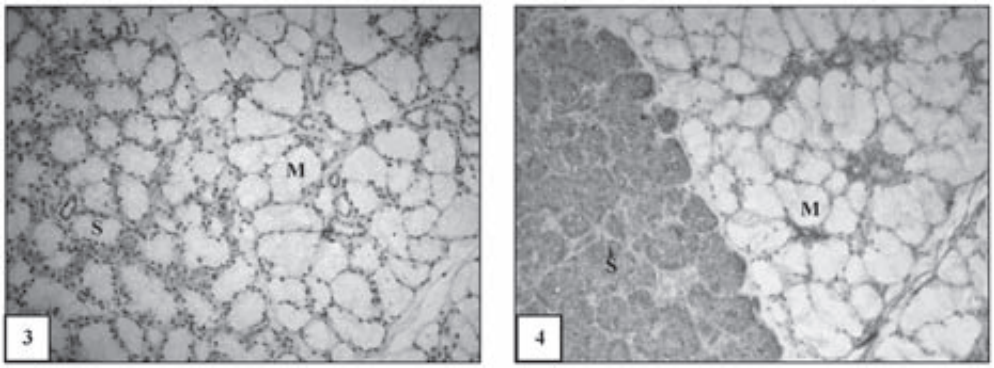

Figure 3 The lectins SBA did not label mucous cells (M) and serous cells (S) (200X).

Figure 4 The mucous cells (M) were unreactive toward UEA-I. In contrast, the serous cells (S) stained positively after UEA-I (200X).

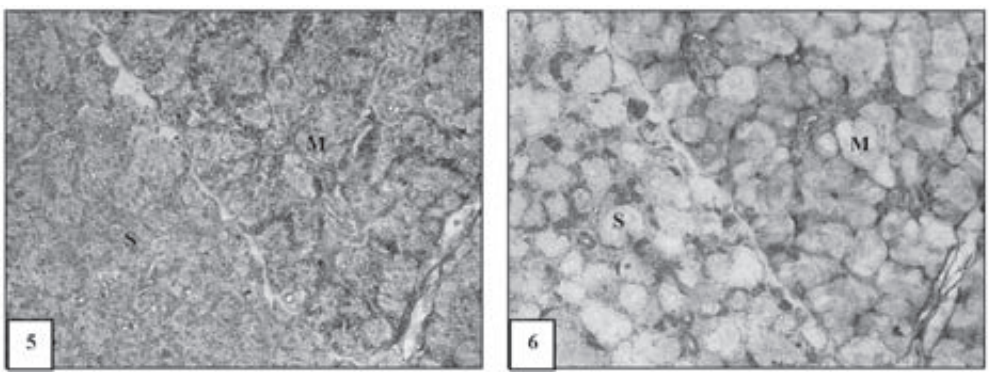

Figure 5 The lectins Con A strongly stained the mucous cells (M) and a moderate to strong reaction in serous cells (S) (200X).

Figure 6 The lectins DBA weakly to moderately marked mucous cells (M), while it only weakly stained serous cells (S) (200X).

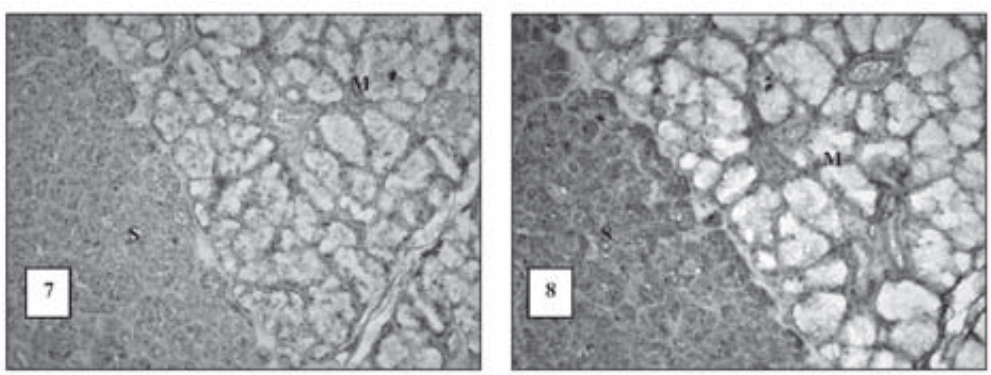

Figure 7 The lectins WGA caused weak staining in mucous cells (M) and the labeling was moderate with serous cells (S) (200X).

Figure 8 The mucous cells (M) show a weak reaction witn RCA - I. In contrast, the serous cells (S) stained very strongly with RCA – I (200X).

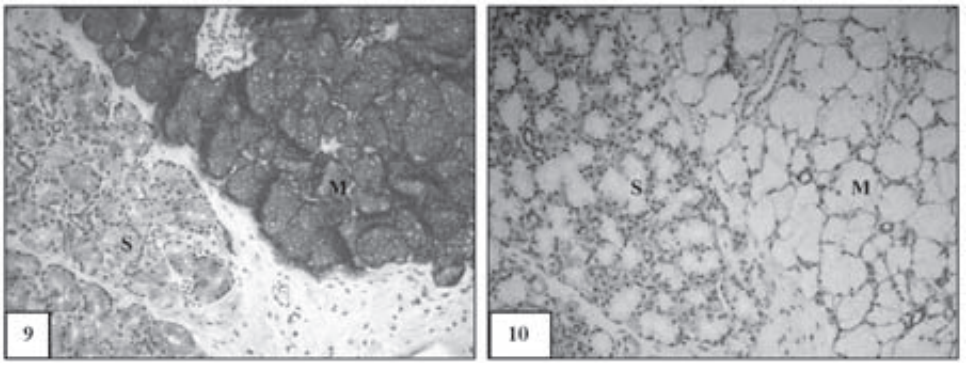

Figure 9 The mucous cells (M) stain intensely with AB pH 2.5 but the labeling was weak in serous cells (S) (200X).

Figure 10 Digestion with neuraminidase greatly diminished the intensity of AB pH 2.5 reaction of mucous cells (M) and serous cells (S) (200X).

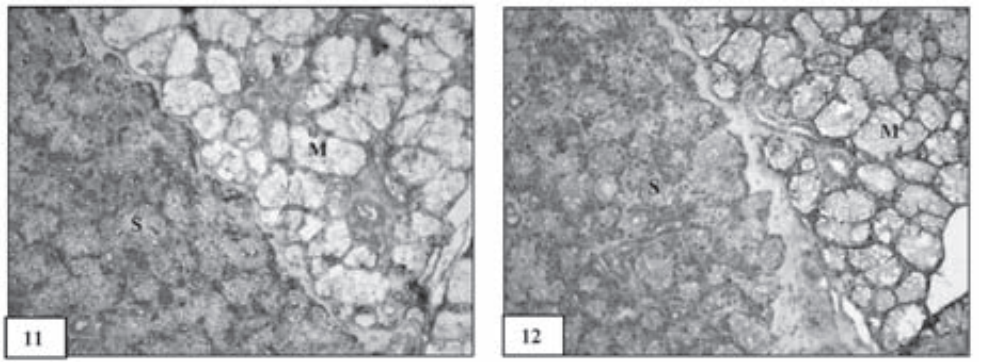

Figure 11 The reaction with the lectins PNA was weak in serous cells (S) but an intense staining was evident after lectins PNA in mucous cells (M) (200X).

Figure 12 After neuraminidase treatment caused an enhancement of lectins PNA reactivity in mucous cells(M) but did not modifly serous cells (S) after treatment (200X).

In the striated duct cells, the supranuclear region of serous cells were stained positively with UEA -I, Con A, DBA, WGA, RCA-I and PNA but the supranuclear region of serous cells were unreactive with AB pH 2.5, PAS, AB pH 2.5 - PAS and SBA.

DISCUSSION

Histochemical methods indicate a precise localization of individual glycoconjugates to specific intra and extracellular sites. More recently, lectin histochemistry demonstrated a remarkable variety saccharides residues of glycoconjugates in the tissue. It is well known that lectin histochemistry represents a more sensitive method than conventional histochemistry for detecting glycoconjugates (Spicer and Schulte,1992; Danguy *et al.*,1998).

In this study, glycoconjugates were formed in both mucous cells and serous cells in secretory endpieces of sublingual salivary gland of Malayan Pangolin *(Manis javanica)*.

The mucous cells reacted strongly with AB pH 2.5 (Spicer *et al.,*1967), PAS (Pearse, 1968) and AB pH 2.5 – PAS (Mowry,1963), revealing the presence of vicinal diol groups with acid and neutral glycoconjugates. Sialic acid residues were furthemore confirmed by AB pH 2.5 staining procedure after enzyme digestion with neuraminidase. Lectin histochemistry revealed the presence of glycoconjugates containing mannose (Con A labeling), α N - acetylgalactosamine (DBA labeling), N - acetylglucosamine (WGA labeling) and Galactosyl (β1 →4) N - acetylglucosamine (RCA - I labeling) residues in mucous cells. Conversely, the negative results obtained in mucous cells after lectins SBA and UEA – I incubation indicated the absence of βN - acetylgalactosamine and fucose residues. In addition, the intensification of PNA staining after neuraminidase digestion confirmed highly presence of sialic acid residues linked to Galactosyl (β1 →3) N – acetylgalactosamine in the terminal position.

The serous cells presented uniform and intense staining with PAS and AB pH 2.5 – PAS demonstrating the presence of primarily neutral glycoconjugates and some acid glycoconjugates. Furthemore, the presence effects of digestion of this enzyme neuraminidase upon the AB pH 2.5 reaction of the serous cells are taken to indicate the existence of the sialic acid residues. In the present study, the lectins Con A, WGA, RCA - I and UEA - I were also positive in the serous cells, indicated the presence of glycoconjugates with mannose, N - acetylglucosamine, Galactosyl (β1 →4) N - acetylglucosamine and fucose residues.

In this study, it could be demonstrated the variety of glycoconjugates in secretory mucins in the sublingual salivary gland of Malayan Pangolin *(Manis javanica).* This general data of glycoconjugates was intended to illustrate the importance of glycoconjugates and to explain the necessity of localizing carbohydrate moieties at cellular levels. Predominant glycoconjugates with terminal sialic acid in mucous cells were believed to coat the mucosal surface so as to provide an environment designed to preserve hydration and to protect the cell from pathogenic organisms (Schulte *et al.,*1984). In addition, predominant neutral glycoconjugates with fucose residues in serous cells were also thought to guard against digestive tract infections (Mondoa *et al.,* 2001; Elkins, 2003). Fucose and another sugar residue, mannose, have the ability to kill bacteria and to fortify resistance to infections. Moreover, both neutral and acid glycoconjugates were important components of saliva. These glycoconjugates were extremely effective in binding masticated food into a slippery bolus and slides easily through digestive tract without inflicting damage to the mucosa.

In conclusion, these data define the normal picture of conventional and lectin labeling in sublingual salivary gland of Malayan pangolin *(Manis javanica)* for the first time. More data is needed in order to better define the significance of these findings.

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