Histochemical evaluation of lectin binding in the submandibular glands of *Suncus murinus*

Yoshifumi Tajima, Jun Ohno, Nobuo Utsumi, Masanori Kashimata* and Masahiko Hiramatsu*
Departments of Oral Pathology and * Dental Pharmacology, Josai Dental University, Sakado, Saitama 360-02, Japan

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Introduction

The house musk shrew, *Suncus murinus*, has recently been recognized as a new experimental animal in the biomedical field of laboratory research. The animal, which is distributed mainly in Southeast Asia including the southern-most part of Japan, is classified under the subfamily of *Crocidurinae* categorized in the *Insectivora*.

There is significant sexual dimorphism in morphology and histochemistry between the male and female submandibular glands of this animal, similar to that in mice. The most conspicuous sexual difference is that the male glands has a more well-developed granular duct (GD) cells as compared with those of the female gland1,2). Further, there are striking histochemical differences in the acinar compartment which consist of two types of cells: serous acini in the core region and mucous acini (demilunes) at the periphery. The mucous acinar cells of the male gland were shown to exhibit alcianophilia (pH 2.5), indicating the presence of acidic glycoproteins in this region, whereas the female gland lack this histochemical feature2).

The purpose of this study was to investigate the differences in terminal sugar of glycoconjugates between the male and female *Suncus murinus* submandibular glands by the use of lectin probes.

Materials and Methods

Four male and four female adult *Suncus murinus* were supplied by Dr. Matsuzaki, Central Institute for Experimental Animals (Kanagawa). The animals were anesthetized with diethyl ether, and the submandibular glands were quickly removed and fixed in Bouin's solution for 24 h at room temperature. All tissues were processed through a series of graded alcohols and xylene and infiltrated with paraffin. Serial paraffin sections at 4-5 μm were cut and prepared for lectin histochemistry.

Lectin binding sites were visualized using biotinyl lectins of Con A, WGA, PNA, RCA-I, and DBA, and avidin-horseradish peroxidase conjugates3. The sections were first covered with 1 % bovine serum albumin (BSA) in 10 mM PBS (pH 7.5). After a rinse with PBS, they were next incubated with biotinyl lectins (25 μg/ml, Vector Lab. Inc.) in 0.1 % BSA-PBS for 30 min at room temperature. The sections were rinsed again in PBS and then incubated with avidin-horseradish peroxidase conjugate (12.5 μg/ml, Vector Lab. Inc.) in 0.1 % BSA-PBS for 30 min at room temperature. After being washed with PBS, the sections were immersed in 3,3'-diaminobenzidine-4HCl (DAB; Wako Chemical Co., Japan, 0.2 mg/ml)-H₂O₂ (0.005 %) for 10 min. The sections were rinsed with water, dehydrated, and mounted without counter staining. The lectins used in the present study and the sugars to which they exhibit specific affinity are shown in Table 1.

Results and Discussion

As shown in Table 2, there was a remarkable sexual difference in the lectin binding
patterns in the glands between male and female *Suncus murinus*. Based on the findings of lectin histochemistry with Con A, WGA, PNA, RCA-I, and DBA, two fundamental binding sites were observed, depending on the histological structures and the lectins used: cell outline (bound by WGA) and intracellular secretory components or luminal substances (bound by Con A, PNA, RCA-I). DBA staining was negative in all structures of the glands (data not shown).

In the acinar compartment, moderate PNA binding was restricted in the luminal aspect of serous acini, whereas secretory components or luminal substances of GD cells showed a rather intense reaction in both sexes (Figs. 1 a, 1 b). This finding indicated the presence of \( \beta \)-galactose residues in these regions, and it was generally more abundant in the male than in the female *Suncus murinus*. The lectin binding patterns observed with lectin-HRP conjugates in the mouse submandibular glands\(^6\) showed that all acinar cells contain oligosaccharides with terminal sialic acid and penultimate \( \beta \)-galactose residues, as revealed by positive DBA and SBA reactions. In the present study, *Suncus murinus* showed no identical reaction of DBA in the cells of

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**Legends**

Fig. 1-4 Comparison of lectin binding patterns in male(a) and female(b) *Suncus murinus* submandibular glands.

Fig. 1 PNA binding reaction is intense in the GD cells, and moderate in intensity in the luminal aspect of serous acinar cells (arrows) which occupy the central core region of acinar compartment. \( \times 50 \)

Fig. 2 WGA binding reaction is highly restricted to the cell outline of acinar compartment. Substances in the luminal aspect of male GD cells show moderate staining, while female glands lack this reaction (GD). The striated ducts of female glands also show distinct reaction (arrow heads). \( \times 50 \)

Fig. 3 RCA-I binding reaction in the GD cells is moderate in male, and trace in female glands. \( \times 50 \)

Fig. 4 Con A binding reaction in the GD cells is moderate in male, and negative in female glands. \( \times 50 \)
acinar compartment while positive PNA binding was observed in the luminal aspect of serous acini (Figs. 1a, 1b). Furthermore, the cells of acinar compartment revealed no positive reaction with any of the lectins used in this study with exception of WGA, which showed moderate binding to the cell outline (Figs. 2a, 2b). The specific localization of N-acetyl-D-glucosamine with terminal sialic acid as revealed by the positive WGA reaction in the out line of acinar cells remains to be explained. However, the data suggest that there is no qualitative sexual difference in complex carbohydrates in the acinar compartment of Suncus murinus submandibular glands, and the lectin binding affinities in this region are consistently different from those of the mouse and rat whose secretory forms are "seromucous".

The lectin binding affinities of GD cells of the animal, on the other hand, showed distinct sexual differences: an intensely positive reactions toward PNA (Figs. 1a, 1b) and moderate ones toward WGA (Fig. 2a), RCA (Fig. 3a), and Con A (Fig. 4a) were observed in the male, while the female gland failed to stain with WGA (Fig. 2b) and Con A (Fig. 4b). RCA-I reaction of the female gland was consistently slight (Fig. 3b). These findings indicated that the secretory materials in the GD cells contain high amounts of β-galactose with terminal sialic acid residues in both sexes, while mannose and N-acetyl-D-glucosamine are found particularly in the male gland. The GD cells in the female gland, on the contrary, presumably lack the sugar residues of the core region of oligosaccharide chains. Schulte and co-workers reported that all GD cells showed moderate to strong staining with PNA and RCA-I in the male mouse submandibular gland. Further, Naito et al. also observed positive binding of PNA and RCA-I as galactose-specific lectins in GD cells, and they noted that these reactions were rather conspicuous in the mouse and generally less so in rat GD cells. In the rat submandibular gland, Schulte et al. found an affinity for RCA-I but not for PNA in secretory granules in the apical cytoplasm of striated duct cells, and suggested that the presence of secretory glycoprotein(s) containing N-glycosyl-linked oligosaccharides in this region. Our results of lectin binding in GD cells of Suncus murinus are mostly in accord with the mouse patterns, and indicate apparent sexual dimorphism in the structure of sugar residues in this region. Although Naito et al. reported that the lectin binding in the granules of GD cells in the mouse submandibular gland was higher in females than in males, the present results indicate that the male dominates in lectin binding affinities in Suncus murinus. An accurate explanation for these contradictory findings must await further investigation.

The histochemical observations described here provide new information concerning sexual differences in glycoconjugates in Suncus murinus submandibular glands. Although the significance of a sexual difference in lectin binding affinities remains a question for further investigation, it is hoped that further studies using additional lectins and histochemical examination of other species will presumably give a clue to the structural and functional significance carbohydrate-containing glycoproteins in this new experimental animal.

References


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