

Full Paper

HISTOCHEMICAL STUDY OF MUCUS LECTIN DISTRIBUTION ON SEVERAL TISSUES OF TILAPIA (*Oreochromis mossambicus*)

STUDI HISTOKIMIA PENYEBARAN LEKTIN MUKUS PADA BERBAGAI JARINGAN TILAPIA (*Oreochromis mossambicus*)

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Abstract

The aim of this research was to know the distribution pattern of mucus lectin from the different tissue of tilapia (*Oreochromis mossambicus*). The samples were taken from the epithelium of buccal cavity, gills, esophagus and skin of tilapia with standard body length (SL) 15.2-25.0 cm and body weight (BW) 145-250 g. Histochemical slides were observed under fluorescent microscope. The results showed that tilapia had four kinds of different lectin, such as wheat germ agglutinin (WGA), peanut agglutinin (PNA) and dolichos biflorus (DBA). Wheat germ agglutinin lectin was present in the epithelium of buccal cavity, gill, esophagus and skin. Staining intensity of WGA mucus lectin was strong in the epithelium of buccal cavity, gill, esophagus and skin. While the PNA was present in the epithelium of buccal cavity and esophagus, and DBA lectin was only present in the esophagus. The intensity of staining of PNA was weak, both in the epithelium of buccal cavity and esophagus, same with DBA lectin which was found in the esophagus.

Key words: histochemical, mucus lectin, tilapia

Introduction

Body surface of fish is entirely covered with mucus. Mucus cells are distributed over the skin, the gills and lining the digestive tract of teleostean fish. Some of these studies have been reported histological and histochemical of that mucus (Fletcher *et al.*, 1976). The mucus coat is in constant contact with the external aqueous environment. It has been recognized that the mucus played a variety of important physical and physiological functions. Beside that, some defense substances such as immunoglobulin (Itami *et al.*, 1988; Rombout *et al.*, 1995) and lectin (Suzuki, 1995) have been identified in the secreted mucus.

Lectins are protein which recognize as a specific carbohydrate structures, agglutinate various types of animal cells by binding cell-surface glycoconjugate. However, the biological function of that mucus lectin has not yet been clarified, although it has been suggested that this serves as part of the defensive role against bacterial colonization on the skin mucus. They are now known as sugar binding proteins (or glycoprotein) that agglutinate cell or precipitate glycoconjugates. Precipitation of glycoproteins (and polysaccharides) by lectins is similar in many respect to the well know precipitation reaction between antibody and antigen (Sharon, 1983).

In vertebrates including fish, it has been suggested that differences in reactivity of

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the goblet cells to the lectins may depend upon their position in its differences on tissues location (Madrid *et al.*, 1989). There are several kinds of glycoconjugated of lectin in which are present at different tissues side. The Wheat Germ Agglutinin (WGA) lectin binds to acetylglucosamine and sialic acid, which are involved in sperm function. Changes in the molecular composition of sperm membrane, can at least be monitored by studying WGA lectin (Fouri *et al.*, 1996). In the different lymphoid organ of lizard expressed that Peanut Agglutinin (PNA)-binding glycoproteins are present on the lymphocytes by studying the reactivity of Fluorescence Immunocytochemistry (FITC)-PNA. Additionally, direct immunofluorescence assays have demonstrated that the majority of lizard thymocytes (70%) and only a fraction of lymphocytes in the spleen, peripheral blood and bone marrow were PNA-positive (Brown & William, 1982). While the WGA and Dolichos Biflorus Agglutinin (DBA) lectin are present in the small intestine of *Testudo graeca*. That WGA and DBA lectin staining showed a strong affinity towards to the whole population of goblet cells. Positive reactivity also occurred in the small intestine of *Rana perezii* (lake frog) that all the goblet cells are stained with WGA and DBA (Madrid *et al.*, 1989).

Materials and Methods

Experimental fish

The fish used in the present study was tilapia (*Oreochromis mossambicus*). Tilapia were collected from Okukubi River, Kin, Okinawa, Japan by casting net and maintained in freshwater tanks (one metric ton capacity), with a filtered and recirculating system. The body length (SL= standard length) and body weight (BW) of tilapia ranged 15,2-25,0 cm and 145-250 g, respectively. After anaesthetizing the fish in

cal Tokyo, Japan) small pieces of the surface epithelium of the buccal cavity, the primary lamella of the gill arch, the esophagus and the skin were taken from tilapia.

Histochemical procedures

Each tissues from the tilapia was fixed in 4% paraformaldehyde in 0,1 M phosphate buffer, pH 7,2 and stored for several days at 4°C. They were dehydrated with serial concentrations of ethanol and embedded in histoparaffin (m.p. 56-58°C, Merck, Darmstadt, Germany). The embedded tissues were serially sectioned at 5 µm. Lectins conjugated with FITC (Honen Corporation, Tokyo, Japan) were used for histochemical staining. The present staining procedures were as follows:

- 1) After deparaffinization with xylene and serial ethanol concentrations (100 to 70%), slides were washed three times with phosphate-buffered saline (PBS) pH 7,4.
- 2) The slides were incubated with FITC-lectins which were diluted with PBS (1:1000) at room temperature for 1 hour under dark condition.
- 3) They were washed three times with PBS.
- 4) They were mounted using 1,4-Diazabicyclo (2-2-2) octane solution (Sigma, St. Louis, MO) mixture with glycerol (1:1).
- 5) Observation was done under a fluorescent microscope (Nikon, Microphot FXA, Japan).

Control slides were incubated with PBS without FITC-lectin at room temperature for 1 hour under dark condition.

Result and Discussion

The epithelium of four different tissues of tilapia were stained with FITC-lectin. Table 1 summarizes the results of reaction by the FITC-lectin. Of 8 lectin tested in the present study; WGA, PNA and DBA, showed positive reactions in the goblet

cells of some tissues. WGA had positive reactions in the goblet cell of the surface epithelium of the buccal cavity, the primary lamella of the gill arch, the esophagus and the skin of tilapia. PNA reaction appeared in the epithelium of buccal cavity and esophagus. Reaction with DBA were only observed in the esophagus. Other lectins; LCA, RCA, PHA, Con A and UEA, did not show any positive reaction in all epithelium of tissues. The goblet cells in the control section were not stained because that control slides were incubated without using FITC-lectin, therefore there was not any reactions. Staining patterns were observed in the four distinct tissues using 8 kinds different lectin and showed variation intensity from faint to strong. The strong and weak reaction occurred in the tissue by the WGA-lectin. PNA-lectin had weak and faint reactivity. While the DBA-lectin occurred faint (reactivity) against esophagus. The staining pattern of WGA were widely more reactive against several tissues and then followed by the PNA and DBA, respectively. The staining pattern of WGA represented as a strong reactivity showed with the goblet cells in the epithelium of buccal cavity, and almost all of the goblet cells was stained with this lectin. The other pattern also showed that the difference of staining intensity between WGA and PNA occurred in the some goblet cells of the surface epithelium of buccal cavity. Staining intensity of WGA was stronger than that by PNA in epithelium of buccal cavity. Almost all of these goblet cells reacted with WGA and approximately one-third of the goblet cells reacted with PNA (Table 2 and Figure 1).

Lectins are proteins which recognized as a specific carbohydrate structures and agglutinate various type of animal cell by binding cell-surface glycoconjugated. In the present study, eight lectins, WGA, PNA, Lentil Agglutinin (LCA), *Ricinus communis* Agglutinin (RCA), *Phaseolus vul-*

garis Agglutinin (PHA), *Canavalia ensiformis* Agglutinin (Con-A), *Ulex ueropeus* (UAE) and DBA were used as a tool for detection of glycoproteins in the mucus of tilapia. Out of eight lectins tested in this present study, WGA, PNA, and DBA showed positive reactions. Reaction with WGA appeared in the surface epithelium all of tissues. As WGA lectin is specific for N-acetylglucosamine and/or siliac acid, this result suggested that such carbohydrate residues binding glycoprotein exist commonly in the mucus of tilapia. Gona (1979), observed that all the functions of mucus layer are closely related to the kind of glycoprotein produced by mucus cells. It has been recorded that WGA bound to the goblet cells of the small and large intestine in rat and monkey, small intestine in human, and large intestine in rat and guinea pig (Madrid *et al.*, 1989). A histological and histochemical analyses were carried out on the entire alimentary canal of the rainbow trout *Oncorhynchus mykiss*, that in the enterocytes of the entire intestine, lectin-terminal of WGA N-acetylglucosamine (GlcNAc) were found (Menghi *et al.*, 2006). Additionally, Scocco *et al.* (2005) reported, the stomach of adult shi drum *Umbrina cirrosa* was investigated using a battery of nine horseradish peroxidase.conjugated lectins combined with enzymatic treatment, in order to distinguish glycoconjugate sugar residues. In the epithelial cells showed the presence of galactosyl ($\beta 1 \rightarrow 4$) N-acetylglucosamine. While in the gastric glands were characterized by the presence of glycoconjugates containing N-acetylglucosamine, galactosyl ($\beta 1 \rightarrow 4$) N-acetylglucosamine, and a small amount of sialic acid linked to N-acetylgalactosamine. Furthermore, WGA was the lectin that showed the strongest affinity toward the intestinal microvilli in the above mentioned animals. These finding indicated wide distribution of GlcNAc and/or siliac acid residues in glycoconjugated of animals.

Table 1. Screening of FITC-lectin staining on several tissue of teleost fishes

	Epithelium of buccal cavity	Gill	Esophagus	Skin
WGA	++	++	++	+
PNA	+	-	±	-
LCA	-	-	-	-
RCA	-	-	-	-
PHA	-	-	-	-
Con-A	-	-	-	-
UEA	-	-	-	-
DBA	-	-	±	-

Intensity of staining : ++: strong; +: weak;; ±: faint; and - : negative

Table 2. Patterns of WGA, PNA and DBA-lectin staining in tilapia

Staining	Tissue of Fish	Location	Stain-Intensity	Part of Goblet Cell
WGA-lectin	Epithelium of buccal cavity	Epidermis	Strong	Almost all
	Gill	Apical region	Strong	Almost all
	Esophagus	Epidermis	Strong	Almost all
	Skin	Epidermis	Strong	One-third
PNA-lectin	Epithelium of buccal cavity	Epidermis	Weak	One-third
	Esophagus	Epidermis	Faint	One-second
DBA-lectin	Esophagus	Epidermis	Faint	One second

In case of PNA positive reaction appeared in the mucus cell of the surface epithelium of buccal cavity and esophagus of tilapia, Menghi *et al.* (1996), reported that in the empty stomach of tilapia, PNA positive sites were present in some gastric pits and on the cell coat. These result suggested that glycoproteins secreted from these sites have terminal residues of β -galactose and α -N-acetylgalactosamine. Scocco *et al.* (2004), the saccharide composition of surface and secretion glycoconjugates in the oesophagus of *Umbrina cirrosa* was examined by means of lectin histochemistry. PNA positive were present in the mucus cells that showed the presence of N-acetylgalactosamine, and sialic acid linked to the dimer galactosyl (β 1 \rightarrow 3) N-acetylgalactosamine. Columnar epithelial cells had a positive reaction with almost all the lectins employed, located in

the supranuclear region and in the cell coat. The presence of abundant and various glycoconjugates in the secretions of shi drum oesophagus was correlated to the absence of salivary glands in fishes in general. The carbohydrate chains containing these sugars were considered to contribute viscoelastic barrier formation which protected the mucosa from the acid environment and proteolysis (Pajak & Duguay, 1993). On the other hand, DBA had only faint reaction in the esophagus of tilapia. This lectin binded with the epithelium and gastric glands of the stomach of tilapia (Menghi *et al.*, 1996) and the goblet cells of the small intestine of lake frog, *Rana perezi*, and Greek tortois, *Testudo graeca* (Madrid *et al.*, 1989). In the human gastric mucosal cells (Hirano *et al.*, 2005), revealed specific binding patterns for each lectin by light microscopy.

Among the lectins tested, in particular, DBA gave a characteristic pattern. It specifically stained the supranuclear region of surface epithelial cells and the perinuclear region of parietal cells along line at at the mucosal. Kilariski & Fiertak (2004) reported, that in the goblet cells of the intestines of four cyprinids, the presence of neutral and acidic glycoconjugates was confirmed. The smallest amount of acidic glycoconjugates was present in the second region of sneep intestine. Sulphated glycoconjugates were absent in the third and fourth region of chub intestine. Lectin histochemistry provided

evidence for the presence of D-galactose, N-acetylgalactosamine, N-acetylglucosamine and sialic acids. The present histochemical observations revealed that distribution of the mucus cells reacting with these two lectins (PNA and DBA) were different from that of WGA. In the present study, it was not confirmed whether same mucus cells produced two distinct glycoproteins, WGA and PNA/DBA reacted ones, or not. However, it may be considered that the glycoproteins binding with these two lectins, PNA and DBA, are related to specific functions of each site.

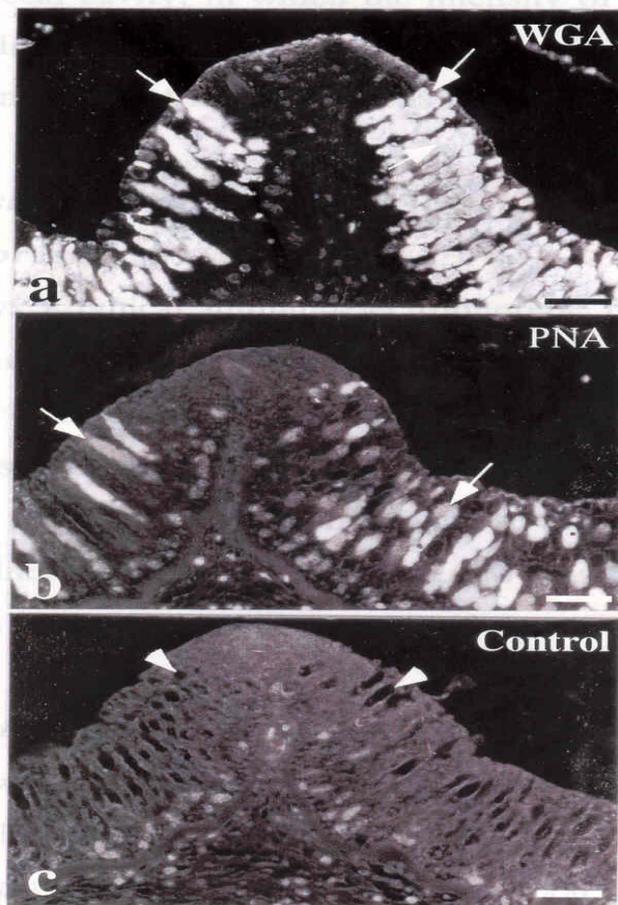


Figura 1. FITC-lectin staining on epithelium of buccal cavity of tilapia. a, epithelium buccal cavity stained with WGA. Goblet cell were strongly stained in epidermis (arrow); b, epithelium of buccal cavity stained with PNA. The stain intensity of the goblet cells was less intense than that WGA (arrow); c, epithelium of buccal cavity used as control. No reactions appeared in the goblet cells (arrowhead). Scale bars = 100 μ m.

Conclusion

There are three kinds of mucus lectin present in the tissues of tilapia; WGA, PNA, and DBA lectin. N-acetyl glucosamine binding proteins of WGA lectin is the most common carbohydrate residues present in the mucus cells of tilapia, and then followed by PNA and DBA. PNA is present in the epithelium of buccal cavity and esophagus, and DBA lectin is only present in the esophagus. Staining intensity of the WGA lectin is higher than that PNA and DBA. The intensity of staining of PNA is weak, both in the epithelium of buccal cavity and esophagus, same with DBA lectin which found in the esophagus.

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