

Lectin Histochemistry of Glycoconjugates in Mandibular Gland of Chicken

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ABSTRACT

The distribution of glycoconjugates in the chicken mandibular gland was studied by means of light microscopic histochemical methods. The staining procedures employed were horseradish peroxidase conjugated lectin, Alcian blue pH 2.5-periodic acid-Schiff (AB pH 2.5-PAS), and high iron diamine-alcian blue pH 2.5 (HID-AB pH 2.5). The lectins used in the present study were Concanavalin A (Con A), *Ricinus communis* agglutinin-I (RCA-I), wheat germ agglutinin (WGA), *Dolichos biflorus* agglutinin (DBA), *Ulex europeus* agglutinin - I (UEA-I), *Limax flavus* agglutinin (LFA), *Lotus tetragonolobus* agglutinin (LTA) and peanut agglutinin (PNA). According to the results obtained, acidic and neutral glycoconjugates with α -D-mannose, α -D-glucose, β -D-galactose, N-acetyl-D-glucosamine, α -L-fucose and sialic acid residues were visualized in the secretory cells of chicken mandibular gland.

Key words : lectin, glycoconjugates, mandibular gland, chicken

INTRODUCTION

A number of glycoconjugates are present in animal tissue, particularly on the secretory granules and cell surface. These molecules contain carbohydrate chains with a specific sequence. In the submandibular gland of different mammals, numerous histochemical studies have been made on glycoconjugates elaborated by their secretory cells (Shackleford and Wilborn 1968; Laden *et al.*, 1984). However, little information is available as to the detail histochemical properties of glycoconjugates in the comparable mandibular gland of chicken. Recently, various kinds of lectins were employed to detect sugar residues in glycoconjugates (Goldstein and Hayes, 1978; Roth 1978; Schulte *et al.*, 1984). In view of the

circumstance mentioned above, attempts were made to analyze glycoconjugates involved in the mandibular gland of chicken, employing the currently available light microscopic methods of peroxidase - conjugated lectins.

MATERIALS AND METHODS

Mandibular glands from both male and female adult Brown Leghorn chicken were fixed by immersion with 10% formalin containing 2% calcium acetate for 12 h at 4°C. or in Carnoy's fluid for 6 hours at room temperature. The tissue specimens were then processed to embed in paraplast. Sections were made at 3 μ m thick and stained with the following histological and histochemical procedures. Hematoxylin and Eosin

(H&E), alcian blue pH 2.5-periodic acid-Schiff (AB pH 2.5-PAS), and high iron diamine alcian blue pH 2.5 (HID-AB pH 2.5). To access the saccharide residues further, the peroxidase-conjugated lectin-diaminobenzidine procedure was performed to the paraplasm sections. Following lectins were employed : Concanavalin A (Con A), *Ricinus communis* agglutinin-I (RCA-I), wheat germ agglutinin (WGA), *Dolichos biflorus* agglutinin (DBA), *Ulex europaeus* agglutinin-I (UEA-I), *Limax flavus* agglutinin (LFA), *Lotus tetragonolobus* agglutinin (LTA) and peanut agglutinin (PNA). All these lectin preparations conjugated with peroxidase were purchased from E.Y. Laboratory (San Mateo, California, U.S.A.). To detect sialic acid residues, sections were treated with neuraminidase (from *Arthrobacter ureafaciens* Marukinshoyu Co. Ltd., Japan) 1 unit/ml in acetate buffer pH 5.3 containing CaCl_2 at 39-41°C for 12-

16 hours prior to staining with LFA or PNA, or AB pH 2.5-PAS.

RESULTS AND DISCUSSION

The secretory epithelium of the chicken mandibular gland was found to consist exclusively of mucous cells (Figure 1). Which agreed with the previous report (Suprasert *et al.*, 1986). The staining results of the mucous cells are listed in Table 1. In the mucous cells, the AB pH 2.5-PAS stained cells were reddish blue (Figure 2). However, they changed into red with AB pH 2.5-PAS after enzyme digestion with neuraminidase. In the mucous cells of mandibular gland, furthermore, the dual staining with HID-AB pH 2.5 resulted in blue coloration together with some pin point staining of black color around certain area (Figure 3). The binding patterns of Con A (Figure 4), RCA-I, PNA (Figure 5), LFA

Table 1 Histochemical staining of mucous cells in mandibular gland of the chicken.

Lectins	References	Intensity ¹	Binding specificities
Con A	Kiernan 1975	2 Br	α -D-glucose, α -D-mannose
RCA-I	Yamada and Shimizu, 1977	1-2 Br	β -D-galactose
WGA	Goldstein and Hayes, 1978	1 Br	β -D-Glc NAC
DBA	Goldstein and Hayes, 1978	O	α -D-Gal NAC
UEA-I	Goldstein and Hayes, 1978	O	α -L-fucose
LFA	Schulte <i>et al.</i> , 1984	2 Br	Neu AC.
LTA	Goldstein and Hayes, 1978	2 Br	α -L-fucose
PNA	Stoward <i>et al.</i> , 1980	1-3 Br	Gal β 1-3-Gal NAC
N ² -LFA	Schulte <i>et al.</i> , 1984	O	Neu AC lost to stain
AB pH 2.5-PAS	Spicer <i>et al.</i> , 1967	3 BM	Acidic and neutral glycoconjugate
HID-AB pH 2.5	Spicer <i>et al.</i> , 1967	2 B BI*	Sulfated and nonsulfated glycoconjugates
N ² -AB pH 2.5-PAS	Spicer <i>et al.</i> , 1967	2-3 M	Neu AC lost to stain with AB pH 2.5

1) B = Blue, BI = Black, Br = Brown, M = Magenta
O = negative staining. Number indicates intensity of staining reaction.

2) Neuraminidase

* All mucous cells stain blue. However, there are some pin point staining of black coloration around certain area.

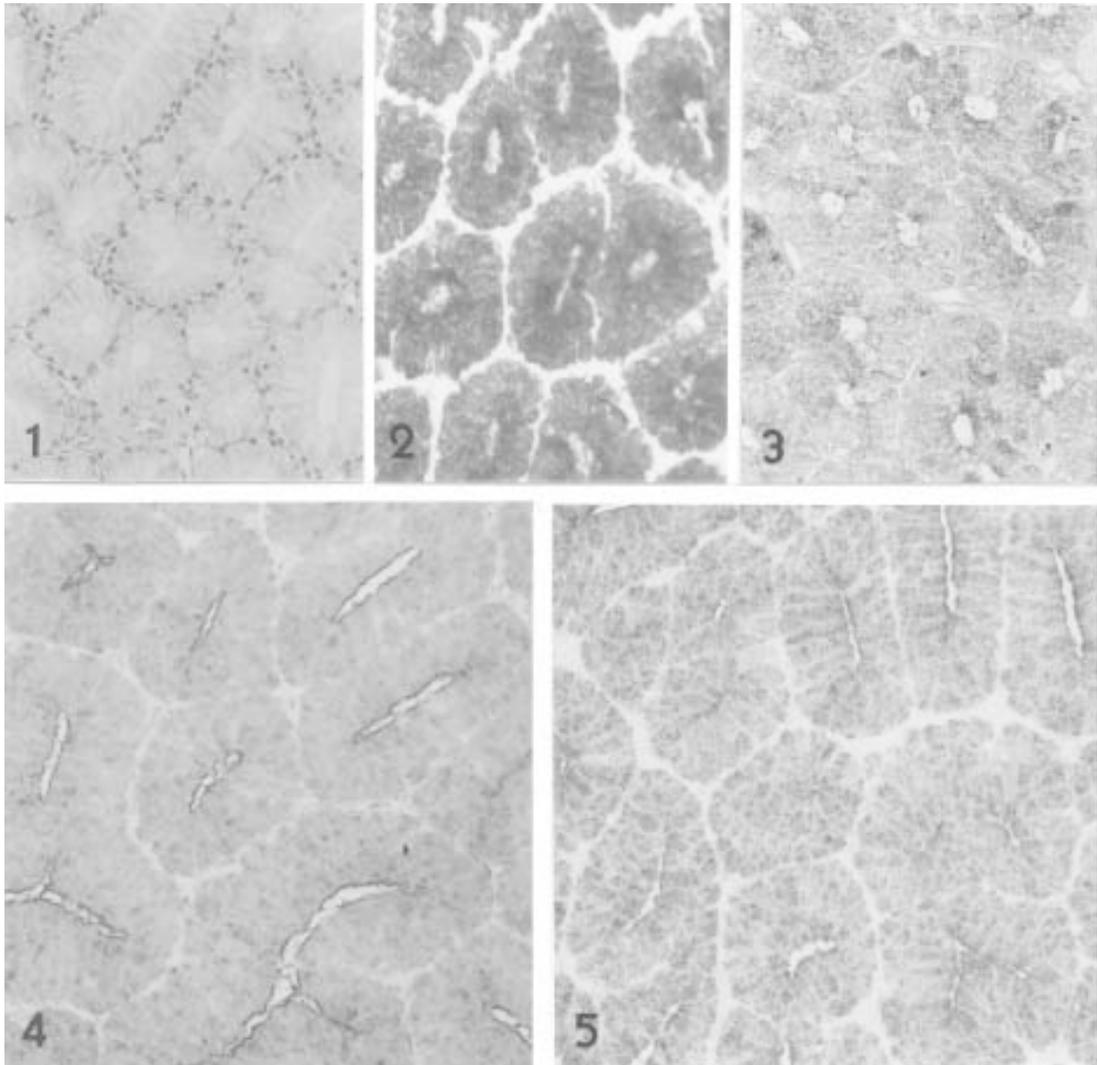


Figure 1 The secretory portions of chicken mandibular gland consist of a single layer of high columnar cells, which contain a flattened nucleus in the basal cytoplasm. The cytoplasm stain lightly with eosin. Hematoxylin and eosin. X 260.

Figure 2 In the mucous cells of chicken mandibular glands, the dual staining with AB pH 2.5-PAS result in deep blue coloration. X 260.

Figure 3 The dual staining with HID-AB pH 2.5 results in blue coloration with some pin point staining of black color around certain areas.

Figure 4 The mucous cells exhibit moderate positive reaction. Con A. X 260

Figure 5 As in Figure 4. PNA. X 260

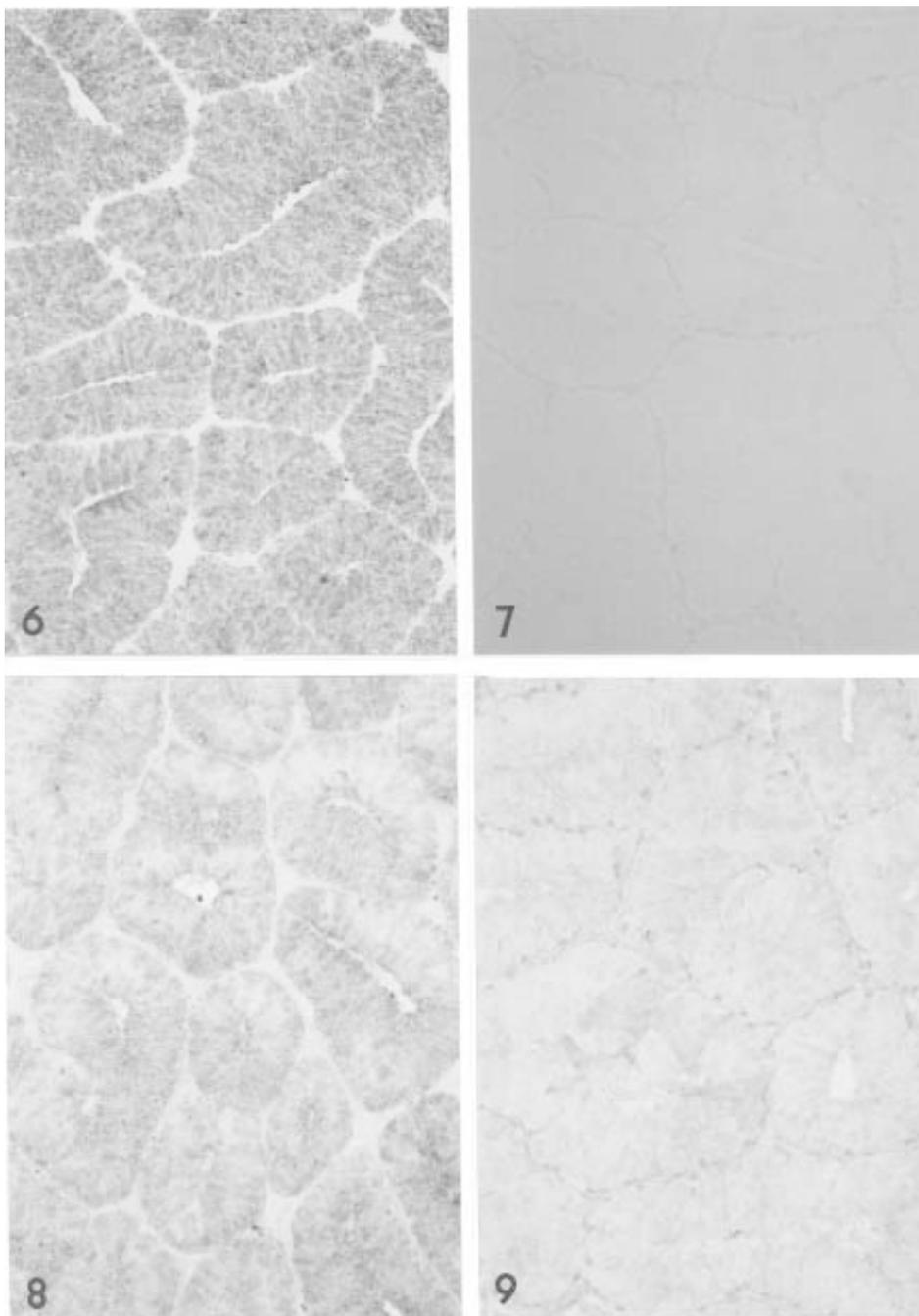


Figure 6 All mucous cells are moderately reactive. LFA. X 260

Figure 7 The mucous cells are stained negatively with LFA after neuraminidase digestion. X 260

Figure 8 The mucous cells exhibit variable intensities of positive reaction. LTA. X 260

Figure 9 The mucous cells are weakly stained with WGA. X 260

(Figure 6) and LTA (Figure 8), was moderately to intensely stained the stored secretion products of mucous cells. However, they were weakly stained with WGA (Figure 9) but negatively stained with DBA and UEA-I.

The staining patterns with PNA, LFA, and LTA corresponded to the presence of terminal galactose-(1-3) N-acetylgalactosamine, terminal sialic acid and α -L-fucose residues respectively. The negative staining with LFA (Figure 7) and the change in color from deep blue to magenta with AB pH 2.5-PAS of mucous granules of mucous cells after neuraminidase digestion further confirmed the presence of acidic glycoconjugates with terminal sialic acid residues (Spicer *et al.*, 1967, Schulte *et al.*, 1984). Furthermore, glycoconjugates with α -D-mannose, α -D-glucose and β -D-galactose were also confirmed the previous study (Suprasert *et al.*, 1986) as judged from their positive staining with Con A and RCA-I. The weakly WGA and negatively DBA reactions are taken to be an evidence that the mucous cells contain small amount of N-acetylglucosamine residues but they are devoid of N-acetylgalactosamine residues.

Comparison the staining specificities of the UEA-I and LTA in the mucous cells of the chicken mandibular gland, each having a nominal binding specificity for α -L-fucose residues, revealed some interesting findings. The UEA-I reactivity could be attributed to the presence of O-glycosidically-linked secretory glycoprotein whereas the LTA binding was restricted to the presence of N-linked glycoprotein (Schulte and Spicer, 1983). The positive LTA but negative UEA-I reactions confirmed the different binding affinity for the same terminal sugar having different glycosidic linkage.

The main function of the mandibular gland, through its salivary secretion is known to be lubrication of food bolus and prevention of microorganism and chemicals in oral cavity. Sialic

acid is believed to play an essential role of the lubrication and protection in the digestive tract (Werner *et al.*, 1982). The physiological role of terminal galactose and terminal fucose residues found in the chicken mandibular gland must await further investigation.

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