ニワトリ食道腺に含まれる複合糖質の組織化学

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Lectin Histochemistry of Glycoconjugates in Esophageal Mucous Gland of the Chicken.

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In the esophageal submucous gland of different mammals, numerous histochemical studies have been made on glycoconjugates elaborated by their secretory cells [1, 3, 5, 6]. However, little information is available as to the detailed histochemical properties of glycoconjugates in the comparable esophageal mucous gland of the chicken. Recently, various kinds of lectins have been employed for the detection of sugar residues in the glycoconjugates [7, 8, 12]. In view of circumstance mentioned above, attempts have been made to analyze glycoconjugates involved in esophageal mucous gland of the chicken, employing a wealth of currently available light microscopic methods of peroxidase-conjugated lectin and correlated procedures.

Esophagus from male White Leghorn chicken was fixed by immersion with [1] 10% formalin containing 2% calcium acetate for 12 hours at 4°C, [2] Carnoy's fluid for 6 hours at room temperature or [3] Rossman's fluid for 12 hours at 4°C. A variety of staining procedures was applied to paraplast sections. Vicinal diol groups of glycoconjugates were shown by periodic acid-Schiff (PAS) method. Sulfated glycoconjugates were demonstrated by alcian blue (AB) pH 1.0

Table 1. Histochemical reaction of glycoconjugates in esophageal mucous glands of the chicken

Staining	Intensity[a)]
AB pH 1.0	3B
AB pH 2.5	2–3B
HID	3–4Bl
LID	3Bl
PAS	4M
AB pH 2.5-PAS	3–4MB
HID-AB pH 2.5	3–4BBl
Con A	2–3Br
RCA-I	1–3Br
WGA	1Br
DBA	0
UEA-I	0
LFA	2Br
PNA	1–3Br
N[b)]-AB pH 2.5	1–2B
N[b)]-LFA	0–1Br
N[b)]-PNA	2–3Br

a) B=Blue, Bl=Black, Br=Brown, M=Magenta, 0=Negative reaction. Number indicates intensity of staining reaction.
b) Neuraminidase.

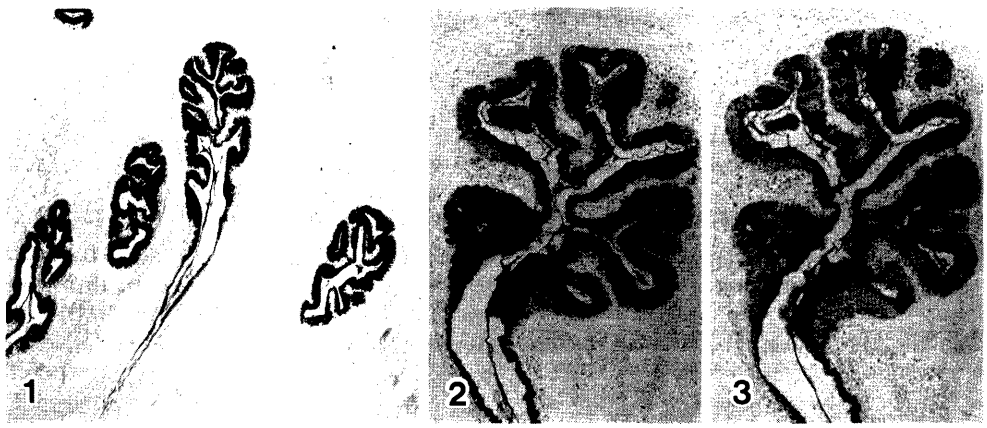

Fig. 1. The secretopry epithelium consists exclusively of strongly stained mucous cells. PAS ×25.
Fig. 2. The mucous cells exhibit strong positive reaction. AB pH 2.5. ×60.
Fig. 3. The alcianophilia of mucous cells is weaker in intensity, as compared with that illustrated in Fig. 2. AB pH 2.5 following neuraminidase digestion. ×60.

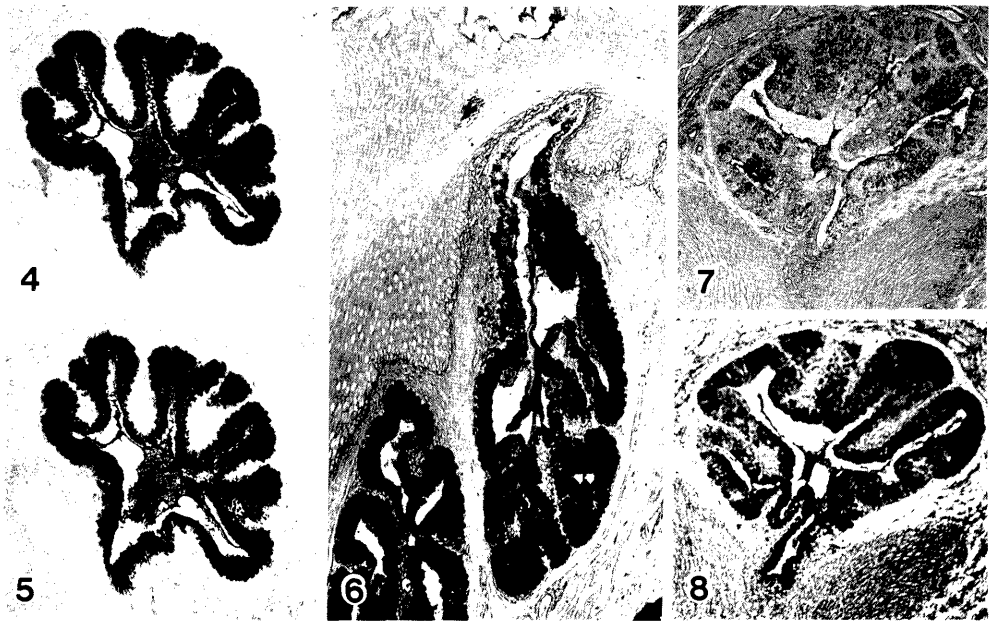

Fig. 4. HID. As in Fig. 1. ×60.
Fig. 5. LID. As in Fig. 1. ×60.
Fig. 6. All the mucous cells are strongly reactive. PNA. ×60.
Fig. 7. All the mucous cells are weakly reactive. WGA. ×60.
Fig. 8. All the mucous cells exhibit strong positive reaction. LFA. ×60.

and high iron diamine (HID), and acidic glycoconjugates by AB pH 2.5 and low iron diamine (LID). In addition, combined AB pH 2.5-PAS staining method was performed for demonstrating acidic and neutral glycoconjugates, and HID-AB pH 2.5 for differentiating sulfated from carboxylated glycoconjugates.

To assess further the saccharide residues, the peroxidase-conjugated lectin-diaminobenzidine procedure was performed. 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) and Peanut agglutinin (PNA). All these lectin preparations conjugated with peroxidase were purchased from E.Y. Laboratory (San Mateo, California, USA.). 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 AB pH 2.5 or PNA.

The tunica propria of the chicken esophagus contains numerous mucous glands which are similar in structure to the mandibular gland of the chicken [13]. The mucous glands differ from the mandibular gland only in that individual esophageal gland is composed of merely one or two glandular lobules. The secretory epithelium of the chicken mucous glands was found to consist exclusively of mucous cells, in contrast to that of various mammalian submucous glands, which contain both mucous and serous cells [1, 3, 5, 6]. The staining results in the mucous cells are listed in Table 1. All mucous cells stained intensely with the PAS (Fig. 1), AB pH 1.0 and HID (Fig. 4) methods, indicating the presence of vicinal diol and sulfate groups of glycoconjugates [4, 9, 10]. The mucous glands are also believed to involve a relatively large amount of acidic glycoconjugates as AB pH 2.5 (Fig. 2) and LID (Fig. 5) procedures resulted in a strong positive reaction in all the mucous cells [9, 10]. In addition, the acidic glycoconjugates are thought to contain terminal sialic acid residues since AB pH 2.5 reaction decreased in intensity after digestion with neuraminidase (Fig. 3) [10]. The deep purple color obtained with AB pH 2.5-PAS

method in the mucous cells are considered to be due to a mixture of acid and neutral glycoconjugates [10]. The acid glycoconjugates are predominantly sulfate-containing carbohydrate as confirmed by the HID-AB pH 2.5 [9]. The presence of vicinal diol- and sulfate-containing glycoconjugates with terminal sialic acid residues in the mucous glands are keeping in line with mandibular gland of the chicken [13].

Except for DBA and UEA-I, the lectins used in the present study showed binding affinities with the mucous cells. The negative DBA and UEA-I reactions are taken to be an evidence that the mucous cells are devoid of terminal α-N-acetylgalactosamine and α-L-fucose residues [2]. However, the presence of terminal or internal α-D-mannose and α-D-glucose, β-D-galactose and N-acetyl-D-glucosamine residues in mucous epithelium was indicated by the staining results with Con A, RCA-I and WGA (Fig. 7) respectively [2].

Staining profiles of serial sections with different lectins might provide information as to whether a single mucous cell contains glycoconjugates with more than one type of terminal sugar. The glycoconjugates stored by mucous granules in the mucous cell contained large amount of galactose-(1–3)N-acetylgalactosamine disaccharide together with terminal sialic acid residues as evidenced by its affinity for PNA (Fig. 6), RCA-I and LFA (Fig. 8). In addition, the glycoconjugates contain a penultimate dimer of galactose-(1–3)N-actylgalactosamine underlying terminal sialic acid, as evidenced by strong PNA reaction after removal of sialic acid [11, 12]. The existence of glycoconjugates with various saccharide residues in mucous gland of the chicken esophagus is reported for the first time in this study.

The main functions of the esophageal mucous glands may be lubrication of boli and prevention of microorganism and chemicals. Sialic acid and sulfate groups are believed to play an essential role of the lubrication and protection in the digestive and respiratory tracts, and eleswhere [14]. The physiological roles of terminal galactose residues found in the chicken esophageal mucous gland must await further investigation.

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要　約

ニワトリ食道腺に含まれる複合糖質の組織化学（短報）：アピナン・スパサート，藤岡俊健（名古屋大学農学部生体機構学講座）——ニワトリ食道腺の粘液細胞には，中性および硫酸とシアル酸残基を含む酸性糖質がみられた。レクチンを用いて検討したところ，複合糖質には α-D-glucose, α-D-mannose, β-D-galactose と N-acetyl-D-glucosamine 残基および末端位の galactose-(1-3)N-acetylgalactosamine 二糖が認められた。