Carbohydrate Histochemistry of Bovine Duodenal Glands

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ABSTRACT. The complex carbohydrates in the bovine duodenal glands were examined histochemically using 5 peroxidase-labeled lectins to achieve a better knowledge of their glycoprotein profiles. The presence of at least 2 different cell types and secretion of neutral mucin were observed in the bovine duodenal glands. The duodenal gland cells located in the central area of the lobules contained neutral glycoproteins with different saccharide residues as β -D-gal-(1-3)-D-galNAc, α -D-galNAc, β -(1-4)-D-glcNAc, NeuNAc and D-galNAc. The other cell types located in the peripheral area of the lobules contained α -L-Fuc, α -D-galNAc, β -(1-4)-D-glcNAc, NeuNAc and D-galNAc. These findings seemed to be characteristic of a unique digestive process of the ruminant.—**KEY WORDS:** carbohydrate, cattle, duodenal gland, histochemistry.

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The duodenal glands or glands of Brunner are said to be present in all mammals and located in the submucosa of the small intestine [10]. In the echidna (*Tachyglossus aculeatus*) [11] and the duckbilled platypus (*Ornithorhynchus anatinus*) [12], however, the glandular cells are present within the submucosa of the most distal portion of the stomach. The duodenal glands secrete an alkaline, highly viscous mucus to protect possibly the duodenal mucosa from the chymous acidity [8]. Although the histochemical properties of the duodenal glands secretion has been studied in various animals [2, 4, 23, 25, 31, 35, 36] to show a high content of neutral carbohydrates, their precise profile has not yet been determined.

In the present study, therefore, the mucus-secreting cells of the bovine duodenal glands have been examined histochemically using peroxidase-labeled lectins with specific affinity for distinct carbohydrate residues.

MATERIALS AND METHODS

Tissue samples of the duodenal glands were taken from 15 normal and adult Holstein cows, fixed in 10% formalin containing 2% calcium acetate for 48 hr, dehydrated through a graded series of ethanol, and embedded in paraffin wax by the conventional methods. The sections were cut at 5 μ m, deparaffinized and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) [31], high iron diamine (HID) [32], low iron diamine (LID) [32], alcian blue (AB) (pH 1.0) [32], AB (pH 2.5) [32], AB (pH 2.5)/PAS [26] and peroxidase-labeled lectins diaminobenzidine (DAB) [26].

Lectins (E. Y. Laboratories, U.S.A.), their specificities and inhibitors used for control studies are summarized in Table 1. The tissue sections were incubated with 2% H₂O₂ for 10 min to block endogenous peroxidase. The sections were covered

Table 1. Lectins, their specificities and control inhibitory sugars

Lectin	Carbohydrate specificity	Inhibitory sugars
Peanut agglutinin (PNA)	β -D-Gal(1-3)-D-GalNAc	D-Gal
<i>Ulex europaeus</i> agglutinin-I (UEA-I)	α -L-Fuc	L-Fuc
<i>Dolichos biflorus</i> agglutinin (DBA)	α -D-GalNAc	D-GalNAc
Wheat germ agglutinin (WGA)	β -(1-4)-D-GlcNAc NeuNAc	D-GlcNAc
Soybean agglutinin (SBA)	D-GalNAc	D-GalNAc

for 10 min with a solution of filtered mouse liver powder at a concentration of 100 $\mu\text{g}/\text{ml}$ in phosphate-buffered saline (PBS), incubated with each peroxidase-labeled lectin for less than 30 min and then washed 3 times in PBS. The horseradish peroxidase was visualized by incubation for 8–10 min in PBS solution containing DAB and H_2O_2 [26].

For control procedures of lectin staining, sections were treated with monosaccharides, as shown in Table 1 as inhibitors [37]. The sections were also treated only with DAB to detect the location of intrinsic peroxidase in the tissue.

RESULTS

The bovine duodenal glands were distributed in the submucosa from the pylorus caudally to about 1.2 m. They were separated into lobules by relatively well-developed interlobular connective tissues. They were branched tubulo-alveolar glands, and their ducts extended through the muscularis mucosa

to open into the base of intestinal crypts. The terminal portion of each lobules was formed by one type of cells indistinguishable by routine tinctorial stainings. These glandular cells were cuboidal, about $7 \times 13 \mu\text{m}$ in size, and had round, basally located nuclei (Figs. 1a, 1b).

Histochemically, the lobules of the duodenal glands consisted of 2 glandular cell types. One was located in the central area of lobules, and the other in the peripheral area of lobules. All areas of the duodenal glands reacted uniformly with PAS (Figs. 2a, 2b). The peripheral area of their lobules was positive to various grades to HID (Figs. 3a, 3b), LID, AB (pH 1.0) and AB (pH 2.5). In the reaction with AB (pH 2.5)-PAS, the peripheral area of the lobules was stained bluish purple and the central area of the lobules was stained purple (Figs. 4a, 4b).

When the duodenal glands were treated with lectins, they showed different staining according to lectins. The duodenal glands were stained uniformly with DBA, WGA and SBA: Golgi area and luminal

Fig. 1. HE staining. a: Duodenal glands in the submucosa. $\times 30$. b: Higher magnification of a. The duodenal glands consist of a large number of mucous cells with basally-located nuclei. $\times 160$.

Fig. 2. PAS staining. a: The duodenal gland cells exhibiting various positive reaction to neutral mucosubstance. $\times 30$. b: Higher magnification of a. Cytoplasm of glandular cells positive to PAS. $\times 160$.

Table 2. Results of the histochemical stainings on the duodenal glands

	Peripheral area of lobules	Central area of lobules
PAS	+/R	+/R
HID	++/Br	—
LID	++/Br	—
AB(pH1.0)	++/B	—
AB(pH2.5)	++/B	—
AB(pH2.5)-PAS	++/BP	+/P
PNA	—	+/Br
UEA-1	+/Br	—
DBA	+/Br	+/Br
WGA	+/Br	+/Br
SBA	+/Br	+/Br

Intensity of staining reactions: ++, moderate; +, weak; —, no reaction. Color of reaction: R, red; B, blue; BP, bluish purple; P, purple; Br, brown.

surface of the glandular cells were positive to DBA, WGA and SBA (Figs. 5a, 5b). When the glands were treated with PNA and UEA-I, the central area of the lobules was positive to PNA (Figs. 6a, 6b), and the peripheral area was positive to UEA-I (Figs. 7a, 7b). PNA-positive cells were unreactive to UEA-I and vice versa. These results are summarized in Table 2.

DISCUSSION

Since the discovery of the duodenal glands by Wepfer in 1697 [10], the secretion of the duodenal glands has been presumed to play an important role in the physiology of digestion. They have been studied in many species to determine the extent of their distribution, density [1, 15–17] and their cell constituents by light [7] and electron microscopy [9, 11–14, 18, 19, 24], and reported to contain a mucous element with classical morphological methods. In spite of the recent progress in the histochemistry,

Fig. 3. HID staining. a: The duodenal gland cells strongly positive to HID. $\times 30$. b: Higher magnification of a. Cytoplasm of glandular cells in the peripheral area of lobules is positive to HID. $\times 160$.

however, only a few papers have been published on the composition of the secretion of the duodenal glands by the use of PAS, AB (pH 1.0), AB (pH 2.5) [2, 4, 23, 25, 28, 31] and lectins [5, 29, 35, 36, 38]. Among them, Crescenzi *et al.* [5] reported 2 different types of neutral mucins in the human duodenal glands. They observed the difference in the location of 2 types of mucous cells in the lobules of the duodenal glands.

In this study, the bovine duodenal glands were examined for carbohydrate residues hitherto unreported. The results obtained by staining with 5 different lectins suggest that the duodenal gland cells in the central area of lobules contained neutral carbohydrates with residues as β -D-gal-(1-3)-D-galNAc, α -D-galNAc, β -(1-4)-D-glcNAc, NeuNAc and D-galNAc in their saccharide moiety, while those of the peripheral area of lobules contained neutral glycoproteins α -L-Fuc, α -D-galNAc, β -(1-4)-D-glcNAc, NeuNAc and D-galNAc in their saccharide moiety. Since all of glandular cells

showed a positive reaction with WGA, this may suggest that WGA-binding D-glcNAc is located within the core of the glycoprotein molecules [5]. Such suggestion has also been made in mucins from different tissues [6, 30] and the duodenal glands of rats [35].

The secretion of duodenal gland cells in the central area of lobules was positive to PNA, DBA and SBA, suggesting to contain a glycoconjugate with terminal D-Gal linked to D-GalNAc [22, 27, 38]. On the other hand, the secretion of duodenal gland cells in the peripheral area, positive to UEA-I, DBA and SBA, might contain terminal α -L-Fuc and β -D-gal both linked to D-GlcNAc residue through 1-3 and 1-4 link, respectively [6]. The occasional presence of a terminal α -L-Fuc 1-2 linked on β -D-Gal [34] may explain the histochemical findings on the presence of a few groups of UEA-I positive cells not stained with PNA [22].

According to the results of the present study, the bovine duodenal glands consist of 2 types of mucous

Fig. 4. AB (pH 2.5)-PAS staining. a: The duodenal gland cells exhibiting various positive reactions. $\times 30$. b: Higher magnification of a. The peripheral area of lobules is stained bluish purple and the central area is stained purple. $\times 160$.

cells containing different carbohydrate residues. These cell types were separately distributed in the central and the peripheral area of the lobules, but showed no clear distinction between them in the transitional zone. These findings have never been reported in the duodenal glands of any other species [4, 5, 13, 25, 28, 29, 35, 36, 38]. As the ruminants show a very unique digestive process [3], it may be considered that the present findings are peculiar to the ruminant duodenal glands. The question remains unsettled whether the existence of the 2 types of carbohydrates is attributable to differences in the composition of secretions caused by functional changes in the same mucous cell.

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Fig. 5. WGA staining. a: The duodenal gland cells all positive to WGA. $\times 30$. b: Higher magnification of a. Golgi area and luminal surface of the glandular cells are positive to WGA. $\times 160$.

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Fig. 6. PNA staining. a: Central area of the duodenal glands positive to PNA. $\times 30$. b: Higher magnification of a. Golgi area, luminal area and luminal surface of the duodenal gland cells in the central area are positive to PNA. $\times 160$.

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Fig. 7. UEA-I staining. a: Peripheral area of the duodenal glands positive to UEA-I. $\times 30$. b: Higher magnification of a. Golgi area, luminal area and luminal surface of the duodenal gland cells in the peripheral area are positive to UEA-I. $\times 160$.