LECTIN BINDING IN ORAL MUCOSA OF MAMMALS

MATSUJI HOSAKA, NORIYASU MURASE, YOSHIKI TAKAI, SHIN FUKUI AND MASAHIKO MORI

Department of Oral Surgery, Gifu College of Dentistry, Hozumi, Motosu-gun, Gifu 501–02

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The binding of horseradish peroxidase (HRP)-conjugated lectin was studied in the oral mucosa of several mammals. The following lectins were used: Con A, PNA, SBA, DBA, RCA-1, UEA-1, and WGA. Con A staining was exhibited strongly in the connective tissue cells, moderately in germinal cells, and negatively in the keratinized layer. However, following periodate oxidation (PA/Con A) it revealed a different distribution patterns as compared to single Con A staining; i.e., PA/Con A was limited to the spinous cell epithelium. Lectin binding gave weak results in basal cells, strong in spinous cells, and showed no effect in keratinized layers. Using lectins—PNA, SBA, RCA-1, UEA-1, and WGA—, the effects in the oral squamous epithelium revealed zonal or regional distribution in their respective individual layers except for a few exceptions.

Recently, it has been shown that blood group substances A, B, and H antigen are present on cellular surface and cell coats of oral epithelial cells (5, 6). The immunogenecity of blood type antigens has long been known to be based on specific sugar residues on the red blood cell surface. Carbohydrate components of A-antigen consist of Gal and GalNAc; those of the B-antigen, Gal and GlcNAc; and those of the H-antigen, Fuc, Gal, and GlcNAc. These blood group antigens have a high affinity for the lectins which bind specifically to these sugars.

Lectins isolated from various plant sources specifically bind to certain sugar residues of complex carbohydrates. By immunohistochemical techniques, HRP-conjugated lectins have been utilized to detect sugar residues in tissue sections. Histochemically detectable lectin-binding sites in oral tissue have been described in oral mucosa and salivary glands (3, 4).

The Present histochemical study describes lectin-binding patterns in various parts of oral mucosa of several rodents and domestic animals and compares these findings with those of epithelia having varying degrees of keratinization.

MATERIALS AND METHODS

Materials

Animals Oral mucosa from the mouse, rat, hamster, guinea pig, rabbit, dog, and

Abbreviation Gal: galactose; Fuc: fucose; GalNAc: N-acetyl-D-galactosamine; GlcNAc: N-acetyl-D-glucosamine; ManNAc: N-acetyl-D-mannosamine; NeuNAc: N-acetyl-neuraminic acid.
monkey (male and female) were used.

Tissue Sections The mucosa from the tongue, buccal, and palatal regions were obtained immediately after sacrifice. The tongue specimens consisted of dorsal surface mucosa including base, middle, anterior, and marginal regions, and they were cut sagittally or at cross sections. The palatal mucosa includes that of the hard and soft palate. Gingiva mucosa was extracted only from the dog. All tissues were fixed in Carnoy’s solution for 6 hr at 20°C, embedded in paraffin and cut into 4 µm serial sections.

Lectin Histochemistry From each specimen, 10 serial sections were cut to examine the hematoxylin/eosin (HE) staining (1), Con A staining (3) and lectin-conjugate staining (6). For the Con A-HRP staining, the method of Katsuyama and Spicer (17) was employed with minor modifications. The sections were reacted with a 0.1% Con A/PBS solution for 30 min at 20°C following immersion in 0.3% H2O2/methanol solution for 20 min in order to inactivate endogenous peroxidase. They were rinsed 3 times in PBS and then immersed in 0.0005% HRP (type VI, Sigma Chem. USA) solution for 30 min and rinsed well in PBS. Finally the slides were immersed for 10 min in 100 ml of 0.05 M Tris-HCl buffer, pH 7.6, containing 30 mg of DAB and 0.3% H2O2 solution. For the periodic acid oxidation/Con A-HRP method (PA/Con A-HRP method), the sections were oxidized prior to the Con A-HRP staining by immersion in 1% periodic acid for 30 min at 20°C. For the periodic oxidation, borohydride reduction, and Con A-HRP method (PA/Red/Con A-HRP method, the sections were immersed in a 0.2% borohydride (NaBH4) for 2 min after periodic oxidation.

HRP-Conjugated Lectins

The following lectins were used as HRP-conjugates: PNA (Arachis hypogaea, peanut lectin) and SBA (Glycine max, Soy bean), for detecting galactose (Gal) and N-acetyl-D-galactosamine (GalNAc); DBA (Dolichos biflours, horse gram), for N-acetyl-D-galactosamine (GalNAc); RCA-1 (Ricinus communis, caster bean), for galactose (Gal); UEA-1 (Ulex europeus, gorse), for L-fucose (Fuc); and WGA (Triticum vulgaris, wheat germ), for N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-neuraminic acid (NeuNAc). The sections were reacted with HRP-conjugated lectin solutions for 40 min at 20°C, rinsed with PBS, and then reacted with a DAB/H2O2 solution for 10 min. Concentration of lectin solutions were as follows; Con A—250 µg/ml, UEA-1 and WGA—100 µg/ml, PNA, DBA, SBA, and RCA-1—50 µg/ml. The lectins used were purchased from E.Y. laboratory, Inc. San Mateo, Ca. USA.

Control Test

Competitive sugars—D-galactose, GalNAc, GlcNAc, and α-methyl mannoside (Sigma)—were used at 0.1 M and 1.0 M for inhibition of lectin-binding activities.

RESULTS

The epithelial structure of oral mucosa showed varying degrees of keratinization. The epithelium of the hard palate from all animals revealed the greatest degree of keratinization. Dorsal tongue epithelia also showed abundant hornification,
whereas the buccal mucosa were not so keratinized.

**Buccal Epithelia**

There were varying epithelial strata which consisted of 2 to 4 cell layers with a single layer of orthokeratinized or parakeratinized cells in mouse, rat, hamster, and rabbit. In the cat, dog, and monkey, 4 to 6 or more cell layers were observed. Guinea pig specimens had numerous spinous cell layers with well developed rete pegs and parakeratosis. Of the animal species examined the thinnest mucosa was found in the hamster cheek pouch; and the thickest in the guinea pig.

Con A binding was moderate in the superficial cell layer, slight in the spinous layer, and absent in the basal layer of the animals examined (Fig. 2d). Staining for PNA, SBA, DBA, and RCA-1 as Gal- and GalNAc-binding lectins was negative in the superficial keratinized layer and moderate to slight in the spinous and basal layers, although it was negative in the basal layer in some cases (Figs. 2g, 6a, 6b). Staining by the above mentioned lectins was only moderate in the spinous layer of the guinea pig. WGA and UEA-1 binding reactions were generally weak or negative in buccal epithelia (Figs. 6c, 6d).

**FIGS. 1a-d. Palatal mucosa of mice. ×100**

1a. Con A/HRP staining. The basal layer cells in the upper spinous cells show weak Con A binding; keratinized layer, no Con A binding. The subepithelial connective tissue shows an intense Con A staining.

1b. PNA binding. Spinous cell layer is strongly positive, upper spinous layer slightly positive and superficial keratinized layer negative. Note the cytoplasmic distribution of PNA of intercellular substances. Connective tissue shows negative stain to PNA.

1c. RCA-1 binding. RCA-1 staining appears in the basal layer cells to upper spinous cells, no RCA-1 reaction in keratinized cells, connective tissue elements react moderately to RCA-1 lectin.

1d. DBA binding. DBA staining revealed a moderate appearance in the upper spinous cells of palatal epithelial area of the mouse.

**FIGS. 2a-i. Oral mucosa of hamsters. ×100**

2a. Con A/HRP staining. Palatal mucosa, Con A binding is moderate in spinous cells, particularly in upper spinous cells, low in basal cells and negative in keratinized cells. Staining is also moderate in subepithelial connective tissue.

2b. PA/Con A/HRP staining. Palatal mucosa stainability is reduced in spinous cells, but increased in the connective tissue.

2c. PA/Red/Con A staining. Palatal mucosa, stainability reduced in the basal layer cells to keratinized layer cells, but moderate to connective tissues.

2d. Con A/HRP staining. Buccal pouch mucosa, Con A staining is strongly positive in upper spinous cells, weak in basal and adjacent spinous cells. Connective tissue and muscle fiber showed moderate positive Con A binding.

2e. PA/Con A/HRP staining. Buccal pouch mucosa staining reaction was present in basal to upper spinous cells, none in connective tissue but increased markedly in muscle fiber.

2f. PNA binding. Palatal mucosa, PNA binding, strongly positive in cellular surface in the lower spinous cells to upper spinous cells, and negative in keratinized layer cells. There was no PNA binding in the connective tissues.

2g. PNA binding. Buccal pouch mucosa, PNA binding is limited germinal cell surfaces.

2h. RCA-1 binding. Palatal mucosa, weak RCA-1 binding seen in cellular surface of basal cells to upper spinous cells. Connective tissue cells and intercellular substance of spinous layer display a weak to moderate RCA-1 staining.

2i. SBA binding. Tongue mucosa (dorsal surface), SBA staining is positively strong in upper spinous cells, low in basal layer cells and negative in fully keratinized cells.
Palatal Epithelia

Palatal mucosa was divided into two parts—the hard palate with a highly keratinized epithelium and the soft palate with little or no keratinization. The epithelial layer of the hard palate had a wave-like appearance except for the monkey specimen which displayed an orthokeratinized epithelia. On the contrary, the soft palate was smooth in shape with ortho- or parakeratosis or almost lacking keratin. Epithelial strata generally consisted of 5–6 cell layers in the mouse, rat, and rabbit, and 8–12 cell layers in the guinea pig, hamster, cat, dog, and monkey. In the soft palate of the rabbit and cat, there were few or no layers of keratinized epithelia without rete peg formation. Con A binding in the palatal epithelia was slight to moderate in the basal to spinous cell layers and negative in the keratinized layer (Figs. 1a, 2a, 5a). PNA staining was negative to weak in the basal layer (Figs. 1b, 2f) except for the cat specimens which showed moderate staining in the spinous layer, though the keratinized layer was negative (Fig. 5d). SBA binding in the basal layer was negative or showed only traces in the mouse, rat, cat (Fig. 5a), and dog, with slight evidence in the hamster, guinea pig, and monkey. The spinous layer displayed moderate SBA binding, which did not give a clear picture in the keratinized layer. DBA and RCA-1 staining patterns were generally similar to that of SBA (Figs. 1c, 1d, 2h). WGA binding in the palatal epithelium was moderate in the spinous layer of the monkey, however it was noted that it gave a weak or very weak stain in other specimens. UEA-1 binding was moderate in the basal and spinous layers of the monkey and cat although it was very weak in the layer of other animals.

Tongue Epithelia

Histochemical observation of lectin-binding sites was confined to the sublingual and dorsal mucosa. The former showed slight or no keratinization in squamous epithelium; whereas the latter, which consisted of tongue papillae had a well keratinized epithelium. Tongue papillae exhibited a high degree of keratinization in mouse, rat, hamster, and guinea pig and a lesser degree in rabbit. Papillae of the cat’s tongue showed a horny form with well developed orthokeratinization; the dog and monkey revealed a large cleft with flat papillary processes, the top portion of which were fully keratinized.

Lectin-binding patterns in the sublingual epithelia of almost all the specimens were similar to those in the buccal mucosa. Papillary regions of the dorsal tongue mucosa gave the most prominent lectin staining among the oral epithelia, and it was further observed that lectin conjugate staining gave more intense results as compared to that of none or slightly keratinized epithelium. Con A-HRP staining was comparatively weak in basal cells of all specimens, weak to moderate in the spinous cells of mouse, rat, guinea pig, and rabbit, but moderate to high in spinous cells of hamster, cat, and dog (Figs. 3a, 4a). Lectin binding using PNA, SBA, DBA, and RCA-1 in the dorsal epithelium showed similar patterns in the mouse, rat, guinea pig, hamster, and rabbit; i.e., binding sites were mainly distributed in the spinous cell layers with weak to moderate staining (Figs. 2i, 3c, 3d, 4b). PNA, SBA, and DBA binding was low to moderate in the spinous and granular cells of dorsal tongue epithelium of cat, dog, and monkey. The RCA-1, WGA, and UEA-1 conjugate staining in these mammals were comparatively stronger in the spinous cell
layer than in basal cell layers. It was observed that the monkey's dorsal tongue epithelium was characterized by basal and spinous cells as revealed by the different stains used, however the UEA-1 binding level of cat's tongue gave a markedly high response particularly in the spinous cells. It was noteworthy that the lectin binding pattern was generally restricted to the spinous and granular cells layers, and SBA binding existed only in the basal layer cells. Fully hornified layers did not show any lectin stain (Table 1).

**Lectin Binding in Subepithelial Connective Tissue**

Con A-HRP staining of connective tissue fibers was markedly positive in all the specimens studied (Figs. 2a, 2b, 2e, 5b), and PNA and RCA-1 conjugates gave slight to moderate staining. Lectin staining using SBA and DBA conjugates was

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**Figs. 3a–d.** Tongue mucosa of rabbit. ×100

3a. Con A/HRP staining. Con A binding is localized in basal layer to upper spinous or parakeratinized cells, as well as in connective tissues.

3b. WGA binding. Staining to WGA conjugate is moderately present in upper spinous or parakeratinized cells and weak in basal to lower spinous cells. Connective tissue cells show low to moderate positive to WGA binding.

3c. SBA binding. SBA staining is positive in basal layer cells to spinous cells, and weak in connective tissue.

3d. PNA binding. PNA staining is positive in basal to spinous cells and weak in connective tissues. Distribution patterns of lectins, SBA and PNA are similar in tongue epithelia and connective tissue fibers.

**Figs. 4a–d.** Tongue mucosa of cats. ×40

4a. Con A/HRP staining. A moderate level of stainability is present in basal to spinous cells of the epithelium, and subepithelial connective tissue and tongue muscle fibers.

4b. WGA binding. WGA conjugate is moderately positive in upper spinous cells and low in basal cells of the epithelium. Connective tissues and muscle fibers of tongue are also positive.

4c. RCA-1 binding. RCA-1 conjugate is slightly positive in upper spinous cells and very weak in basal cells. Connective tissue fibers show slight reaction to RCA-1 binding and muscle fibers are almost negative.

4d. PNA binding. PNA conjugate is intense in upper spinous cells and weak in basal cells. Connective tissue fibers appear moderately and none in muscle fibers.

**Figs. 5a–d.** Palatal mucosa of cats. ×100

5a. Con A/HRP staining. Con A staining is moderately positive in basal to upper spinous cells of the epithelium, as well as in connective tissue fibers.

5b. SBA binding. Strong SBA staining is present in basal to upper spinous cells. Connective tissue shows no stain reaction.

5c. PA/Con A/HRP staining. Con A staining prior to PA oxidation disappears in the epithelial staining, and is seen moderately in subepithelial connective tissue.

5d. PNA binding. Strong PNA staining is present in basal to lower spinous cells and moderate in upper spinous cells.

**Figs. 6a–d.** Buccal mucosa of the monkeys. ×100

6a. RCA-1 binding. RCA-1 staining is usually positive, as displayed moderately in basal cells and its adjacent spinous cells, and intermittent reactions in spinous and upper strata.

6b. PNA binding. PNA staining is confined in the basal cells. Connective tissue fibers shows moderately positive reaction.

6c. WGA binding. WGA staining is positive in basal to spinous cells and negative in upper spinous cells.

6d. UEA-1 binding. UEA-1 staining is irregular in basal and spinous cells.
slight or nil except for sublingual mucosa in the monkey. WGA staining was negative to slight except in the dorsal and sublingual mucosa of cat and rabbit. UEA-1 staining was negative in almost all specimens, except for monkey's sublingual mucosa which was positive.

**Control Test**

When the Con A solution contained 0.1 M α-methyl-mannoside or 0.1 M D-mannose, it gave weak reaction. When 0.1 M GalNAc was added to RCA-1, PNA, SBA, or DBA solutions, it resulted in decreased staining as compared to the original. The incubation of WGA with 0.1 M GlcNAc blocked the staining.

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### Table 1. Regional distribution of lectin binding in oral mucosa

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Con A</th>
<th>PA/Con A</th>
<th>PNA</th>
<th>SBA</th>
<th>DBA</th>
<th>RCA-1</th>
<th>WGA</th>
<th>UEA-1</th>
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<tbody>
<tr>
<td>Man Glc</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>1</td>
<td>2-3</td>
<td>2</td>
<td>1-2</td>
<td>±-2</td>
<td>±</td>
<td>1-2</td>
</tr>
<tr>
<td>Gal GalNAc</td>
<td>±-2</td>
<td>1</td>
<td>2-3</td>
<td>2</td>
<td>1-2</td>
<td>1-2</td>
<td>1</td>
<td>1-2</td>
</tr>
<tr>
<td>Gal GalNAc</td>
<td>±</td>
<td>0</td>
<td>0-±</td>
<td>1</td>
<td>±</td>
<td>0-±</td>
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<td>±</td>
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<tr>
<td>Gal</td>
<td>2-3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1-2</td>
<td>0</td>
<td>±</td>
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<tr>
<td>NeuNAc</td>
<td>±</td>
<td>±</td>
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<td>±</td>
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<tr>
<td>α-L-Fuc</td>
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<td>±</td>
</tr>
</tbody>
</table>

0; negative  ±; trace  1; slight  2; moderate  3; strong

Tabulated effects of lectin binding stain in their respective location indicated by the greatest common measurement of intensities to the different specimens used.
reaction of lectin. A complete disappearance of staining occurred when 1.0 M sugar was used (Fig. 7).

DISCUSSION

The stratified squamous epithelium of oral mucosa consists of the basal, spinous, granular, and keratinized cells, and that stratification is one of the characteristic expressions of epithelial maturation. Normal stratification in the oral epithelium differed in various parts of oral cavity; i.e., the epithelia of tongue and palate were most highly keratinized in rodents and in many domestic animals. Irrespective of different degrees of hornification in oral epithelia, cellular division was limited to the basal cells, cellular proliferations confined to the spinous cells, keratohyalin formation occurred in granular cells, and finally hornification was completed in the superficial layer cells. These epithelial differentiations disclosed a specific distribution of polysaccharides and oxidative enzymes.

It has been reported that PAS-positive polysaccharides, mainly glycogen, in oral epithelia are distributed in spinous and granular layer cells, whereas basal cells are devoid of these materials. Intercellular polysaccharides in human gingival epithelium were positive for PAS and toluidin blue stainings (2, 34). The structure and biochemical properties of the surface coat of biological membranes have been well documented in many reviews (27–29, 33). Light microscopic histochemical methods such as PAS, PA-methenamine (PA-Ag), and ruthenium red stainings have been used to visualize surface coats in the epithelial cells. Mercer et al. (22) pointed out that the PA-Ag technique showed little staining of the surface coat of basal cells but that reaction was increased in desmosomes of spinous cells, and no stain occurred in superficial keratinized cells. Those findings are similar to the present lectin-binding patterns.

Since lectins were isolated from various plant sources, it has first been shown to have ability to agglutinate red blood cells, and lectin histochemistry has been used to detect sugar residues in tissue sections (32). For example, the lectin, Con A, interacts specifically with hexose group sugars (D-glucose, D-mannose, and D-fructose) and oligosaccharides (maltose and so on) (14). PNA and RCA-1 are linked to D-galactose (Gal); DBA and SBA, to N-acetyl-D-galactosamine (GalNAc); WGA to N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-D-neuraminic acid (NeuNAc); UEA-1, to L-fucose (Fuc) (8). It has been pointed out that lectin-conjugates bind to intercellular materials, cells coats, and basement membranes of oral mucosa (3–6) and skin (1, 9, 15, 16, 25, 26, 30). These experiments were conducted to determine if the cell coat or intercellular materials in oral epithelia contain Gal, GalNAc, and GlcNAc residues in the spinous cells and hence determine if they are positive to PAS staining. Lectin-binding activity in oral epithelia was comparatively lower in the basal layer cells than in the spinous cells. Intercellular staining using lectin-conjugates in oral epithelial cells probably represented desmosomes among spinous cells and hemi-desmosomes that connect basal cells. A higher degree of lectin binding also occurred in upper spinous layer cells and granular cells, but not in fully keratinized cells. Regional and zonal distributions of lectin-binding sited in keratinized squamous epithelia are thought to be an expression of epithelial stratification resulting from keratinocytic differentiation from the basal to horny cell level.
Biosynthesis of glycoproteins in the plasma membranes of epithelial cells has been observed by the use of radioisotope labelled sugars; e.g. Mann et al. (21) and King et al. (18) reported that $^3$H-L-fucose was incorporated into the plasma membrane and that $^3$H-glucosamine was found in cell-surface-associated glycoconjugates of epidermis in cultured pig skin. Davies and Trotter (7) have also demonstrated that $^3$H-GlcNAc and $^3$H-ManNAc becomes localized in the internal membrane system in human cultured epidermal cells. Fuc, Gal, GalNAc, and GlcNAc are sugar residues of carbohydrate chains found in the cell surface or cell coat glycoproteins. The lectin-conjugate localization from the present and preceding studies resembles that biochemically determined with the use of radioactive isotopes.

Histochemically demonstratable enzymes, dehydrogenases and transglucosidases, in stratified squamous epithelium also exhibit a zonal distribution in their individual stratum (10–13, 19, 20, 23, 31). Dehydrogenase histochemistry has shown that basal cells are positive for SDH activity; and spinous cells, for lactate dehydrogenase (LDH) and G-6-P-dehydrogenase (G-6-PDH). The latter was particularly high in upper spinous and granular cells, but horny cells were devoid of enzymes (10–13, 20, 23, 31). Klingberg and Butcher (19) have reported that phosphorylase in rodent oral mucosa displayed a basal to spinous layer distribution. Enzymatic localization in epithelial strata implies that the substrate such as hexoses and other mono- and oligosaccharides are present in the cytoplasm. Nieland et al. (24) have described that glucose, galactose, galactosamine, and glucosamine produce PAS-positive cytoplasm in organ cultures cells as revealed by electron microscopy. PAS-positive substances in the epithelial cells were also reactive in the PA/Con A method in the present study. The spinous and granular cells in squamous epithelia exhibited a cytoplasmic lectin stain, suggestive of the presence of hexoses and or oligosaccharides. These lectin-binding sugar residues in keratinocytes may interact with macro-molecules in glycoproteins or complex carbohydrates as magnified histochemically by detectable PAS-positive materials.

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REFERENCES


