

Histochemistry of Mucosubstances on the Pedal Sole of Five Abalone Species

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The present study describes histochemical properties of the mucosubstances in the pedal sole of abalone, in relation to two main additional functions known to date. Only neutral mucosubstances were demonstrated in the lateral epithelium of the pedal sole of the abalone, however, two distinct types of cells, one containing neutral and the other containing acidic mucosubstances, were identified in the ventral epithelium. The epithelial mucocytes distributed throughout both epithelial and subepithelial glands located in the ventral subepithelial region showed a mixture of neutral and acidic mucosubstances. However, acidic mucosubstances were found in larger amounts than neutral ones in epithelial mucocytes, and vice versa in subepithelial glands. Among these acidic mucosubstances, sulphated substances were dominant. Lesser amounts of carboxylated substances also occurred together with low levels of neuramic and sialic acid. Collectively, the notable morphological differences between abalone and other gastropoda were observed in the ventral surface of the foot, which showed a tall columnar epithelium, peripherally concentrated mucocytes, and subepithelial glands. Mucosubstances are presumably produced by these cells and may also play a role in clinging to some substances to support abalones in addition to the main functions of lubrication and protection.

Prosobranchs are one of the few groups of animals to inhabit marine, freshwater and terrestrial environment. The edible abalone, *Haliotis gigantea*, *H. sieboldii*, *H. discus*, *H. discus hannai*, and *Sulculus diversicolor aquatilis*, belonging to the subclass Prosobranchia have been widely distributed in Korean coastal waters where various kinds of culture grounds have been intensified. The body of abalone consists of a muscular pedal sole, a head with one pair of tentacles, the viscera, the pedal sole and a mantle surrounding the respiratory organs and the viscera. The gland cells, goblet cells of the typical prosobranch, are clusters of secretory cells embedded in subepithelial spaces, secrete acids for penetration of prey and defense (Carriker and Williams, 1978; Thompson, 1983) and play an important role in shell formation (Crenshaw, 1980). The epithelial mucosubstances which are observed widely in both invertebrates and vertebrates have great diversity both in type and location. Although the function of these materials has not yet to be established, it has been suggested that there are two main functions; lubricating

epithelial surfaces to facilitate movement, and protecting epithelial cells by forming a physical barrier to chemical and physical irritants (Kemper and Specian, 1991). Nearly all gastropods have a flat foot used primarily for locomotion. On some gastropods such as the abalone, the pedal sole is modified for clinging. Epithelial cells and gland cells which function as the main source of mucous in the foot may help in protection and lubrication (Sullivan, 1961; Macha, 1983; Rupavathi et al., 1984; Choi et al., 1995).

The histochemical properties of mucosubstances have been extensively studied in higher vertebrates (Neutra and Forstner, 1987). However, the histochemical nature and physiological role of mucosubstance secretory cells in the foot of molluscs are less studied, particularly in the abalone species as an important marine resource in many countries. In this study we report the histochemical properties of mucosubstances found in five abalone species in Korea.

Materials and Methods

A total of five abalone species (*Haliotis gigantea*, *H. sieboldii*, *H. discus*, *H. discus hannai*, *Sulculus diversicolor aquatilis*) found in Korea were used in this study. Test samples were collected from intertidal rocks

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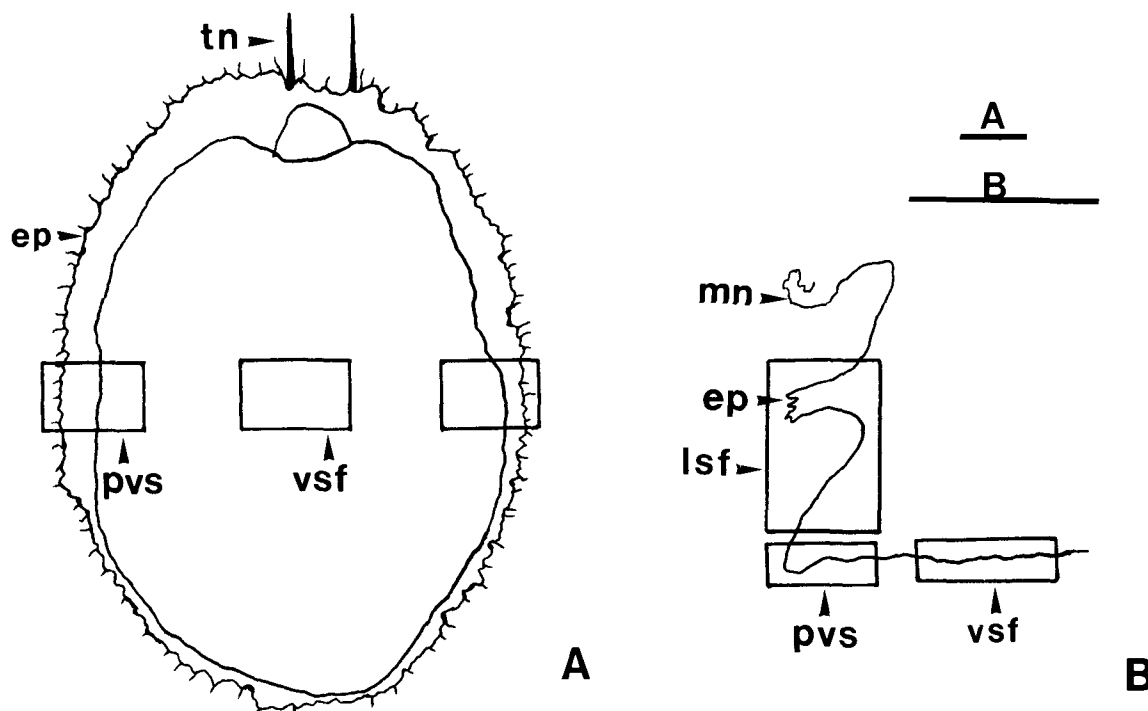


Fig. 1. Schematic diagram of ventral (A) and lateral (B) views of the abalone showing regions collecting the tissue samples. tn; tentacle, ep; epipodium, mn; mantle, lsf; lateral surface of foot, vsf; ventral surface of foot, pvs; peripheral region of ventral surface of foot. scale bar=10 mm.

of Cheju Island for *Sulculus diversicolor aquatilis* and *Haliotis sieboldii*, Pohang for *H. discus* and *H. discus hannai*, and Tongyoung for *H. gigantea*. The foets (Fig. 1) of three abalones for each species were fixed in 10% neutral buffered formalin for 24 h at room temperature. Tissues were dehydrated in a graded ethanol series and embedded in paraffin. Serial 5 μ m thick sections were prepared.

To investigate the histochemical properties of mucosubstances secreted in the foot, the following staining procedures were used: (1) the periodic acid Schiff (PAS) reaction (McManus, 1946) for detecting neutral mucosubstances, (2) PAS reaction after amylase (Sigma, type XII-A) digestion to exclude any possible glycogens, (3) alcian blue (AB) staining at pH 2.5 for the demonstration of acidic mucosubstances, (4) AB at pH 1.0 (Lev and Spicer, 1964) for the demonstration of sulphated mucosubstances, (5) a combined stain using AB at pH 2.5 and PAS reaction (Mowry, 1963), (6) AB at pH 1.0 and PAS reaction (Lev and Spicer, 1964) to separate acidic and neutral mucosubstances, (7) a combined stain of high iron diamine (HID) and AB at pH 2.5 (Lev and Spicer, 1964), and (8) a stain with AB at pH 2.5 following strong methylation plus saponication (MS) (Spicer and Lillie, 1959) to allow the differentiation of carboxylic and sulphated mucosubstances. In addition, enzyme digestion for the elimination of sialic acid (a hyaluronic acid) was carried out with neuraminidase from *Clostridium perfringens* (Sigma type V) (Spicer and Warren, 1960) or bovine testicular hyalu-

ronidase (Sigma, type I-S) (Leppi and Stoward, 1965), each followed by staining with AB at pH 2.5.

Results

The foets of all abalone species studied have been lined by a simple epithelium consisting of tall, ciliated columnar cells on the ventral surface (Fig. 2A and B) and columnar cells on the lateral surface (Fig. 3A and B). At the epipodium, cuboidal cells have replaced the columnar cells. The epithelial mucocytes were scattered throughout the lateral and ventral epidermis. However, these cells have been distributed mainly on the peripheral region of the ventral surface and are rarely observed in other regions (Fig. 2C and D). Connective tissue and smooth muscle are present in the hypodermis. Subepithelial glands have been abundant within the tissue of the ventral surface (Fig. 2E and F). The histochemical properties of mucosubstances in the pedal sole of five abalone species are given in Tables 1-4.

Only neutral mucosubstances are present in the epithelium of the lateral surface of all species. In the epithelium of the ventral surface, cells have been stained either with neutral or acidic mucosubstances (Fig. 2A and B). Cells containing neutral mucosubstances have been predominant in the ventral epithelium of four species. However, in *Sulculus diversicolor aquatilis*, more cells containing acidic mucosubstances have been observed in the ventral epithelium. Epithelial mucocytes

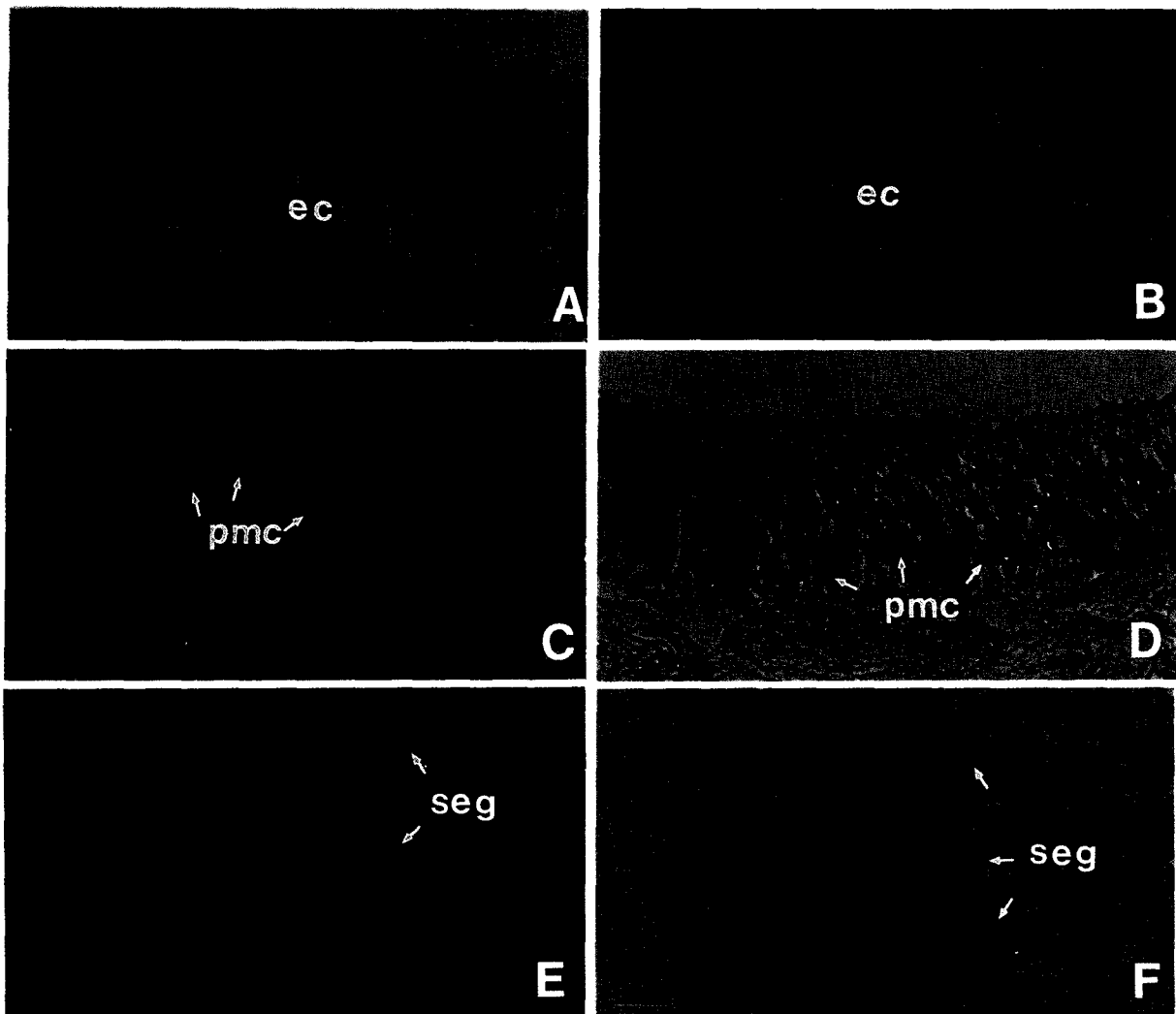


Fig. 2. Photomicrographs showing ventral surface or sole of the foot. Note a tall, ciliated columnar epithelial cell (ec) containing either neutral or acidic mucosubstances and peripherally-concentrated mucocytes (pmc) containing a mixture of neutral and acidic ones. Abundant subepithelial glands (seg) containing more abundant neutral mucosubstances than acidic mucosubstances are also present beneath the hypodermis. A, Sole of *Sulculus diversicolor aquatilis* stained with AB at pH 2.5. B, Sole of *Haliotis gigantea* stained with AB at pH 1.0-PAS. C, Sole of *Haliotis gigantea* stained with AB at pH 2.5-PAS. D, Sole of *Haliotis gigantea* stained with HID-AB at pH 2.5. E, Sole of *Haliotis sieboldii* stained with AB at pH 1.0. F, Sole of *Sulculus diversicolor aquatilis* stained with PAS. Scale bars=30 μ m.

contained a mixture of neutral and acidic mucosubstances, however, the acidic ones were more abundant (Fig. 2C and D). Two types of mucocytes were observed in the lateral surface of *Sulculus diversicolor aquatilis*; one containing neutral mucosubstances and the other containing a mixture of neutral and acidic ones (Fig. 3A and B). Subepithelial glands contained a mixture of neutral and acidic mucosubstances, of which the former is detected more abundantly than the latter (Fig. 2E and F). It is noted that a significant component of acidic mucosubstances is both sulphated and carboxylated substances. Enzymes such as neuraminidase and hyaluronidase for elimination of sialic acid have effects on the staining pattern of mucosubstances.

Discussion

There have been several studies on the structure and histochemical properties of mucosubstances in the pedal sole of the prosobranch (Grenon and Walker, 1978; Bensalem and Chetail, 1982; Lindberg and Dwyer, 1983; Shirbhate and Cook, 1987). Most studies have focused on the mucous glands of the pedal sole which function as the main source of mucous supply (Sullivan, 1961; Macha, 1983; Norenburg and Ferraris, 1990). The pedal sole of gastropoda consists of simple columnar cells at the dorsal surface and ciliated columnar cells at the ventral surface (Kapur and Ginson, 1968; Chang and Lim, 1989). Voltzow (1994) explains that the general surface of the pedal sole in the prosobranch may be scattered with epidermal and subepidermal

Table 1. Histochemical properties of neutral mucosubstances in the foot of five abalone species

Species	Region		PAS	PASa
<i>Haliotis gigantea</i>	Ventral surface	EC	2-3/0	2-3/0
		MC	0-1	0-1
SEG		3	3	
	Lateral surface	EC	0-1	0-1
		MC	2	2
<i>H. sieboldii</i>	Ventral surface	EC	2-3/0	2-3/0
		MC	1	1
SEG		3	3	
	Lateral Surface	EC	0-1	0-1
		MC	1	1
<i>H. discus</i>	Ventral surface	EC	2-3/0	2-3/0
		MC	2	2
SEG		3	3	
	Lateral surface	EC	0-1	0-1
		MC	3	3
<i>H. discus hannai</i>	Ventral surface	EC	2-3/0	2-3/0
		MC	1-2	1-2
SEG		3	3	
	Lateral surface	EC	0-1	0-1
		MC	2	2
<i>Sulculus diversicolor aquatilis</i>	Ventral surface	EC	0/1-2	0/1-2
		MC	1	1
SEG		3	3	
	Lateral surface	EC	1	1
		MC	3/0	3/0

EC, epithelial cell; MC, epithelial mucocyte; SEG, subepithelial gland; PAS, periodic acid Schiff; PASa, PAS after amyliase digestion; /, two distinct types of cells are observed in the same region. Arabic numbers indicate the relative intensity of the staining: 4, very strong; 3, intense; 2, moderate; 1, weak; 0, faint or negligible.

gland cells, as may the sides and dorsal surface of the foot and a specialized area of the dorsal surface only secretes the operculum when present. Available information concerning the properties of mucosubstances of epidermia in abalone is very scarce although the feeding biology, physiology and ecology for abalone are well investigated (Pilson, 1965; McLean, 1970; Bourne and Redmond, 1977; Krajniak and Bourne, 1988; Corazani and Illanes, 1998; Mai et al., 1998).

In this study, the staining properties of mucosubstances of the lateral surface of the foot are similar to the results of previous researchers, which investigated

Table 2. Histochemical properties of acidic mucosubstances in the foot of five abalone species

Species	Region		AB pH 2.5	AB pH 2.5 after MS	AB pH 1.0	HID-AB pH 2.5
<i>Haliotis gigantea</i>	Ventral surface	EC	0/2	0/1	0/1-2	0/1NB
		MC	3-4	1-2	3	3NB
SEG		1	0-1	0-1	1BN	
	Lateral surface	EC	0	0	0	0
		MC	2-3	2	2-3	3NB
<i>H. sieboldii</i>	Ventral surface	EC	0/2	0/1-2	0/2	0/1NB
		MC	3-4	2	3	3NB
SEG		3	1-2	2	1BN	
	Lateral surface	EC	0	0	0	0
		MC	3-4	3	1-2	3NB
<i>H. discus</i>	Ventral surface	EC	0/2	0/1	0/2	0/1NB
		MC	4	1-2	1-2	3NB
SEG		1-2	1	0-1	0-1BN	
	Lateral surface	EC	0	0	0	0
		MC	3-4	1-2	2	2NB
<i>H. discus hannai</i>	Ventral surface	EC	0/3	0/2	0/2	0/1NB
		MC	3	1	2-3	2NB
SEG		1-2	1	1-2	0-1NB	
	Lateral surface	EC	0	0	0	0
		MC	3	2	2	2-3NB
<i>Sulculus diversicolor aquatilis</i>	Ventral surface	EC	3/0	2/0	2/0	1-2NB/0
		MC	3	2	2-3	3NB
SEG		0-1	0-1	0-1	0-1B	
	Lateral surface	EC	0	0	0	0
		MC	3/0	2-3/0	3/0	3NB/0

AB, alcian blue; MS, methylation followed by saponification; HID, high iron diamine; B, blue; N, black. EC, epithelial cell; MC, epithelial mucocyte; SEG, subepithelial gland. Arabic numbers indicate the relative intensity of the staining: 4, very strong; 3, intense; 2, moderate; 1, weak; 0, faint or negligible

the pedal sole of other gastropoda (Banu et al., 1979a; Choi et al., 1995). In contrast, the ventral surface of the foot of five abalone species exhibits two types of cells which secrete either acidic or neutral mucosubstances. The epithelial cells and epithelial mucocytes in the ventral surface of the pedal sole of *S. diversicolor aquatilis* exhibit more abundant cells producing acidic

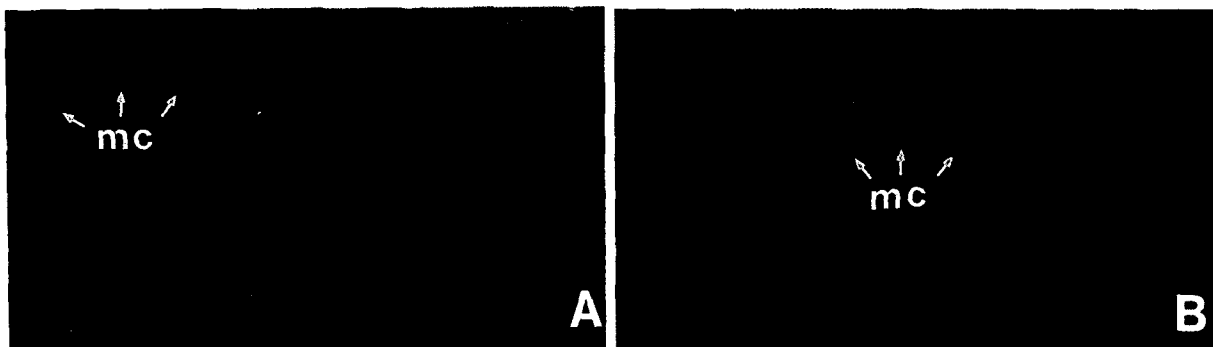


Fig. 3. Photomicrographs showing the lateral surface or side of the foot. Note columnar epithelial cell only containing neutral mucosubstances and scattered mucocytes (mc) containing a mixture of neutral and acidic ones throughout the epidermis. A, side of *Sulculus diversicolor aquatilis* stained with PAS. B, side of *Haliotis sieboldii* stained with HID-AB at pH 2.5. Scale bars=30 μ m.

Table 3. Histochemical properties of neutral and acidic mucosubstances in the foot of five abalone species

Species	Region		AB pH 2.5-PAS	AB pH 1.0-PAS
<i>Haliotis gigantea</i>	Ventral surface	EC MC SEG	2-3R/2B 3B>3BP 3RP	2-3R/2B 3B>3BP 3R
	Lateral surface	EC MC	0-1R 3BP>3B	0-1R 3BP>3B
<i>H. sieboldii</i>	Ventral surface	EC MC SEG	2-3R/2B 3B>3BP 3RP	2-3R/2B 3B>3BP 3RP
	Lateral surface	EC MC	0-1R 3BP, 3B	0-1R 3BP, 3B
<i>H. discus</i>	Ventral surface	EC MC SEG	2-3R/2B 4BP 3RP	2-3R/2B 4BP 3RP
	Lateral surface	EC MC	1R 3BP>3B	1R 3BP>3B
<i>H. discus hannai</i>	Ventral surface	EC MC SEG	2-3R/2B 3BP 3RP	2-3R/2B 3BP 3RP
	Lateral surface	EC MC	0-1R 3BP	0-1R 3BP
<i>Sulculus diversicolor aquatilis</i>	Ventral surface	EC MC SEG	3B/1-2R 3BP 3R	3B/1-2R 2-3BP 3R
	Lateral surface	EC MC	1R 3BP/3R	1R 3BP/3R

R, red; P, purple; >, found in most parts. EC, epithelial cell; MC, epithelial mucocyte; SEG, subepithelial gland; PAS, periodic acid Schiff. Arabic numbers indicate the relative intensity of the staining: 4, very strong; 3, intense; 2, moderate; 1, weak; 0, faint or negligible.

mucosubstances when compared with those of four other abalone species (Table 2). Generally, mucous gland cells appear either singly or in clusters. The occurrence of each cell type differs by species and by habitat (Lim, 1965; Banu et al., 1979b). Unlike bivalves, in which mucocytes called unicellular glands are not found (Chang and Lim, 1989; Choi et al., 1995), abalones contain mucocytes in both the lateral and ventral surfaces. Although the general histochemical properties are similar in both bivalves and abalone, these cells in abalone are predominately observed in the peripheral regions of the ventral surface in the pedal sole. A mixture of neutral and acidic mucosubstances were found in the epithelial mucocytes of the lateral surface on the pedal sole of *Haliotis gigantea*, *H. sieboldii*, *H. discus*, *H. discus hannai*. That of *Sulculus diversicolor aquatilis* possess two types of mucocytes, which secrete neutral mucosubstances and a mixture of neutral and acidic mucosubstances. Therefore, mucosubstances secreted in the lateral surface of the pedal sole of the latter are major histochemical characteristics not found in other abalone species (Tables 2 and 3).

Subepithelial glands are located both subepithelially and within the muscular layer on either side of the pedal sole in pelecypoda (Banu et al., 1979a and b; Choi et al., 1995). However, subepithelial glands of gastropoda are limited to the ventral surface of the foot (Karpur and Gibson, 1968; Chang and Lim, 1989). In this study, abalones show that subepithelial glands are

localized in a thin, ventral sub-epithelial region. It is well known that mucous glands of gastropoda produce acidic and neutral mucosubstances together with glycoprotein (Sullivan, 1961; Lim, 1965; Macha, 1983), and are particularly rich in sulfuric acid groups as Macha (1983), and Norenburg and Ferraris (1990) reported (Table 3). In contrast, the present study shows that the subepithelial glands of the abalone species used in this study secrete some prominent neutral mucosubstances (Table 1). Denny (1983) reports that the mucosubstance of mollusca coats the gills for sorting particles, lines the gut, provides lubrication during reproduction and locomotion, repels potential predators, adheres to substrates, and functions in homing or following conspecifics. According to Banu et al. (1979 a and b), secretion by these cells may function to aid in the protection of the epithelia and lubrication. Lubrication keeps the foot wet and thus enables free sliding movements. They also suggested that secretions of these cells participate in the attachment of the byssus disc. Notable morphological and histochemical differences between the abalone and other molluscs are observed in the ventral surface of the pedal sole. Tall, ciliated epithelial cells, peripherally concentrated mucocytes and subepithelial gland cells are observed from abalones used in this study. It is believed that these structures in ab-

Table 4. Histochemical properties of sialic and hyaluronic acid in the foot of five abalone species using enzyme digestion

Species	Region		AB pH 2.5	AB pH 2.5 after	
				Neur	Hyal
<i>Haliotis gigantea</i>	Ventral surface	EC MC SEG	0/2 3-4 1	0/1-2 3 0-1	0/1-2 3 0-1
	Lateral surface	EC MC	0 2-3	0 1-2	0 3
<i>H. sieboldii</i>	Ventral surface	EC MC SEG	0/2 3-4 3	0/1 2-3 2	0/1 2-3 2
	Lateral surface	EC MC	0 3-4	0 3	0 3
<i>H. discus</i>	Ventral surface	EC MC SEG	0/2 4 1-2	0/1 1-2 1	0/1-2 2 1
	Lateral surface	EC MC	0 3-4	0 2	0 2-3
<i>H. discus hannai</i>	Ventral surface	EC MC SEG	0/3 3 1-2	0/2 2 1	0/2 1 1
	Lateral surface	EC MC	0 3	0 3	0 2
<i>Sulculus diversicolor aquatilis</i>	Ventral surface	EC MC SEG	3/0 3 0-1	2/0 2 0-1	2/0 2 0-1
	Lateral surface	EC MC	0 3/0	0 2/0	0 2/0

PAS, periodic acid Schiff; PASa, PAS after amylase digestion; /, two distinct types of cells are observed in same region; Numbers indicate the relative intensity of the staining: 4, very strong; 3, intense; 2, moderate; 1, weak; 0, faint or negligible; Neur, neuraminidase digestion; Hyal, hyaluronidase digestion. EC, epithelial cell; MC, epithelial mucocyte; SEG, subepithelial gland.

alone produce larger amounts of mucosubstances, compared to other bivalves species. Therefore, it is probable that the mucosubstances produced by the ventral surface of the abalone may have an important role in clinging to some substances in addition to the two main functions of lubrication and protection.

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