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Mucosubstance Histochemistry of the Epidermis in Yellowtail, Striped Beakperch, Brown Spotted Grouper, Sea Chub, and Multicolorfin Rainbowfish

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Abstract: To investigate the properties of mucosubstances of the epidermis in various teleostean species, conventional histochemical stainings were used on the skin in five species of order Perciformes, i. e., yellowtail, *Seriola quinqueradiat*, striped beakperch, *Oplegnathus fasciatus*, brown spotted grouper, *Epinephelus chlorostigma*, sea chub, *Ditrema temmincki* and multicolorfin rainbowfish, *Halichoeres poecilopterus*. The following methods were used: periodic acid Schiff (PAS), alcian blue (AB) pH at 2.5, AB pH at 1.0, AB pH at 2.5-PAS, AB pH at 1.0-PAS, aldehyde fuchsin (AF) pH at 1.7-AB pH at 2.5 and high iron diamine (HID)-AB pH at 2.5. The epidermis of all five species consisted of three layers: superficial, middle, and basal layer. The superficial layer was comprised of rather flattened cells. In particular, the outermost layer of striped beakperch and middle layer of sea chub consisted of mucus-secreting cells. Mucous cells, the unicellular glands, were found in epidermis but varied in number in different body regions and species. Although there was a slight difference in the amount in various species and body regions, the secretory contents of the mucous cells in the five teleostean species contained acidic mucopolysaccharides. In yellowtail, striped beakperch, and multicolorfin rainbowfish, the property of mucosubstances was identified as sialomucin, while it was sulphomucin in brown spotted grouper and sea chub.

Key words: epidermis, mucosubstance, fishes, mucous cells, histochemistry

The skin of fish consists of dermis and epidermis. The epidermis is a stratified squamous or cuboidal epithelium covering the external surface like other vertebrates, but it is particular in that there are unicellular or multicellular glands, e.g., mucous cells, in fish skin. Terrestrial vertebrates have keratinized epithlia, which act as an effective barrier against infection. On the other hand, the epithelia of fish skin is consistantly exposed to a great variety of pathogens in the aquatic environment. In addition, fish can take up antigens than the environment into the body via the skin (Moore *et al.*, 1998).

The skin of fish is covered with mucus, which is generally believed to serve as a mechanical as well as biochemical barrier against pathogen invasion. The skin mucus of fish contains immunoglobulins as well as various innate-defense factors such as complement proteins, C-reactive protein, lysozyme, hemolysin, and anti-bacterial peptides (Ingram, 1980; Alexander and Ingram, 1992; Shephard, 1994).

Athough there have been numerous studies on the skin structure of various teleosts, literature on properties of mucosubstances is limited. Previous histochemical studies on the mucosubstances of the fish epidermis have shown that the properties are diverse in species and inhabitants (Kazuyori and Motoyoshi, 1975; Blackstock and Pickering, 1980; Singh and Mittal, 1990; Jeong and Moon, 1994; Park *et al.*, 1995; Park and Kim, 1999; Lee and Kim, 1999; Lee *et al.*, 2000a, 2000b; Kim *et al.*, 2002; Park and Kim, 2000, 2003; Park *et al.*, 2001, 2002, 2005).

The present report is a comparative histochemical study on mucopolysaccharides of the epidermis in five teleostean species, i. e., yellowtail, striped beakperch, brown spotted grouper, sea chub, and multicolorfin rainbowfish.

MATERIALS AND METHODS

Live adult specimens of yellowtail, *Seriola quinqueradiata*, striped beakperch, *Oplegnathus fasciatus*, brown spotted grouper, *Epinephelus chlorostigma*, sea chub, *Ditrema temmincki*, and multicolorfin rainbowfish, *Halichoeres poecilopterus*, all belonging to the order Perciformes, were

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collected. Skin pieces (c. 5 × 8 mm) excised from the abdominal, thoracic, and dorsal regions were fixed in 10% neutral phosphate buffered formalin. Paraffin sections were cut at 6 μm, and were stained with hematoxylin-eosin (H-E) and by periodic acid Schiff (PAS) reaction (McManus, 1968).

For general histochemistry of mucosubstances, the following staining procedures were used: PAS reaction (McManus, 1968) to detect neutral mucosubstances, alcian blue (AB) staining at pH 2.5 (Spicer *et al.*, 1967) to demonstrate acidic mucosubstances, AB at pH 1.0 (Lev and Spicer, 1964) to selectively detect sulphated mucosubstances, and combined stainings with AB at pH 2.5 and PAS (Mowry, 1963) to demonstrate a composition of neutral and acidic mucosubstances, aldehyde fuchsin (AF) at pH 1.7 and AB at pH 2.5 (Spicer and Meyer, 1960), and high iron diamine (HID) and AB at pH 2.5 (Spicer, 1965) to distinguish between sulphated and nonsulphated (sialyated) mucosubstances.

RESULTS

The histological structure of the skin from five teleostean species

In three different body regions, the skin structure of five teleostean species consisted of epidermis and dermis. The epidermis exhibited a stratified epithelium which consisted fundamentally of the superficial layer, middle layer, and the stratum germinativum, basal layer. This principal structure is similar in all three regions of the five species although there are differences in their thickness.

The superficial layers of the epidermis of the five species were composed of rather flattened cells arranged in 1 to 3 rows of cells. In general, the middle layer of the epithelial cells, arranged in several layers, are in general polygonal with centrally placed round nuclei. In yellowtail, striped beakperch, brown spotted grouper, and multicolorfin rainbowfish, the layers were difficult to distinguish. In sea chub, however, the basal layer of epithelial cells, columnar in shape with apically placed, round or elongated nuclei, arranged in a single layer on a basement membrane: it was easy to distinguish the basal layer from the middle layer.

In the five teleostean species, between epithelial cells, small spherical or flask-shaped unicellular mucous cells were present. The mucous cells opened to the exterior by a short narrow neck opened to the surface by a wide pore. The mucous cells in striped beakperch and sea chub were relatively larger in dimension than the ones in yellowtail, brown spotted grouper, and multicolorfin rainbowfish. In yellowtail, they were relatively few, near to surface layer (Figs. 1 and 2). In striped beakperch (Figs. 3 and 4), brown spotted grouper (Figs. 5~7), and sea chub (Figs. 8 and 9), the density of mucous cells was much lower, located mainly in the outer regions of the epidermis and also in the underlying deeper layers of the epidermis. In contrast, in multicolorfin rainbowfish, abundant mucous cells were present, restricted to surface layer of epidermis, close together, in a single row (Figs. 10~12).

Below the mucous cells other glandular cells, the club cells, were found in striped beakperch, and were oval or spherical in shape (Figs. 3 and 4). The middle layer of epidermis in brown spotted grouper (Figs. 5~7) and sea chub (Figs. 8 and 9) consisted of a few voluminous cells, adjacent to the epithelial cells, called the swollen cells. The voluminous cells have an oval nucleus and a homogenous cytoplasm, and their boundary is clear. Occasionally, vesicles or vacant acelluar structures were apparent due to a loss of nucleus. The voluminous cells did not show any histochemical reactions for general histochemical stain and mucopolysaccharide staining. The voluminous cells in brown spotted grouper were numerous and multi-layered. These stratified voluminous cells in the middle layers in brown spotted grouper produced a web-shaped structure in appearances.

The histochemical properties of mucosubstances of the skin in five teleostean species

Results of the histochemical properties of mucosubstances in the five teleostean species are outlined in Tables 1~5.

The cytoplasm of the epithelial cells in the epidermis in the five teleostean species was homogenous, stained light pink in H-E, but did not stain significantly for mucosubstances in general. On the other hand, the superficial epithelial cells in striped beakperch and middle epithelial cells of dorsal region in sea chub gave positive reaction to acidic mucopolysaccharides, although weak (Figs. 3 and 4).

The mucous cells in the five teleostean species stained blue with AB at pH 2.5 and AB at pH 2.5-PAS. Therefore, their secretory products, in general, contained acidic (sulfated and sialylated) mucopolysaccharides. The mucous cells of yellowtail (Fig. 1), striped beakperch (Fig. 3), and multicolorfin rainbowfish (Fig. 10) stained blue with AF at pH 1.7-AB at pH 2.5 and HID-AB at pH 2.5. Their secretory products were likely to be sialomucin (Fig. 2, 4, 11, and 12). In the brown spotted grouper and sea chub, their secretory products were likely to be sulphomucin, staining blue with AB at pH 1.0, purple with AF at pH 1.7-AB at pH 2.5, and black with HID-AB at pH 2.5 (Figs. 5~9).

The amount of mucins in mucous cells of epidermis varied in species and body regions; in yellowtail, the amount of sialomucin was small to moderate in abdominal region, and moderate to considerable in thoracic and dorsal region. In striped beakperch and multicolorfin rainbowfish, it was small to moderate and moderate, respectively. In brown spotted grouper, the mucous cells of epidermis contained a small to moderate amount of sulphomucin in thoracic epidermis, and a moderate amount in abdominal and dorsal

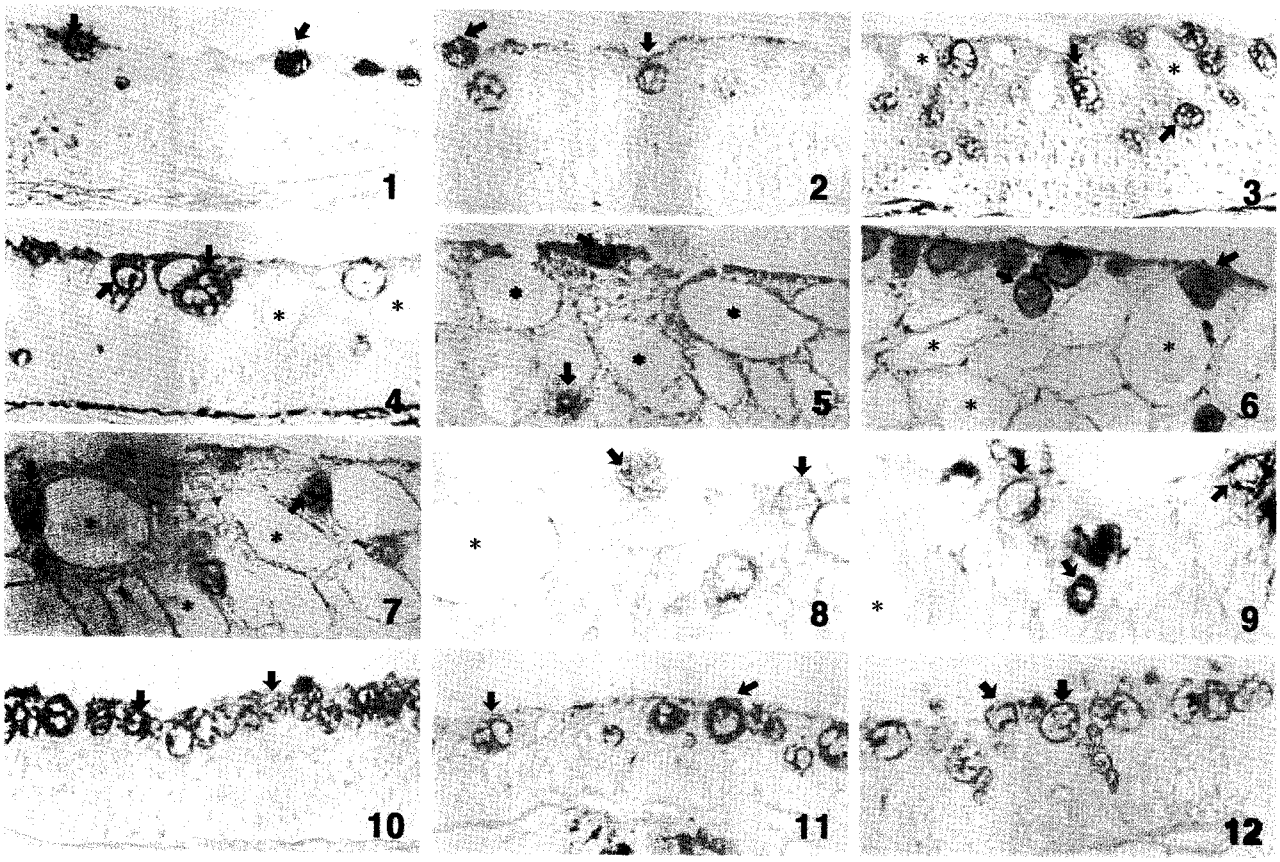

Fig. 1. AB at pH 2.5-PAS staining of the abdominal skin in yellowtail, *Seriola quinqueradiata*. ×400. The mucous cells (arrows) showed a weak to moderate reaction.
Fig. 2. HID-AB pH at 2.5 staining of the abdominal skin in yellowtail, *Seriola quinqueradiata*. ×400. The mucous cells (arrows) showed a weak to moderate blue coloring.
Fig. 3. AB at pH 2.5 staining of the dorsal skin in striped beakperch, *Oplegnathus fasciatus*. ×400. The superficial epithelial cells stained trace blue, and the mucous cells (arrows) showed a weak to moderate reaction. The club cells (asterisks) were unreactive.
Fig. 4. AF at pH 1.7-AB at pH 2.5 staining of the dorsal skin in striped beakperch, *Oplegnathus fasciatus*. ×400. The superficial epithelial cells stained bluish purple or blue, and the mucous cells (arrows) showed a trace to moderate blue coloring. The club cells (asterisks) were unreactive.
Fig. 5. AB at pH 2.5-PAS staining of the abdominal skin in brown spotted grouper, *Epinephelus chlorostigma*. ×400. The mucous cells (arrows) showed a weak to moderate black coloring. The swollen cells (asterisks) were unreactive.
Fig. 6. AF at pH 1.7-AB at pH 2.5 staining of the abdominal skin in brown spotted grouper, *Epinephelus chlorostigma*. ×400. The mucous cells (arrows) showed a moderate purple coloring. The swollen cells (asterisks) were unreactive.
Fig. 7. HID-AB at pH 2.5 staining of the abdominal skin in brown spotted grouper, *Epinephelus chlorostigma*. ×400. The mucous cells (arrows) showed a moderate purple coloring. The swollen cells (asterisks) were unreactive.
Fig. 8. AB at pH 2.5 staining of the dorsal skin in sea chub, *Ditrema temmincki*. ×400. The mucous cells (arrows) showed a weak to moderate reaction and a few middle epithelial cells stained trace blue. The swollen cell (asterisk) was unreactive.
Fig. 9. AF at pH 1.7-AB at pH 2.5 staining of the dorsal skin in sea chub, *Ditrema temmincki*. ×400. The mucous cells (arrows) showed a weak to moderate purple coloring and a few middle epithelial cells stained trace purple. The swollen cell (asterisk) was unreactive.
Fig. 10. AB at pH 2.5-PAS staining of the thoracic skin in multicolorfin rainbowfish, *Halichoeres poecilopterus*. ×400. The mucous cells (arrows) showed a moderate reaction.
Fig. 11. AF at pH 1.7-AB at pH 2.5 staining of the thoracic skin in multicolorfin rainbowfish, *Halichoeres poecilopterus*. ×400. The mucous cells (arrows) showed a moderate blue coloring.
Fig. 12. HID-AB at pH 2.5 staining of the thoracic skin in multicolorfin rainbowfish, *Halichoeres poecilopterus*. ×400. The mucous cells (arrows) showed a moderate blue coloring.

area. A small to moderate amount of sulphomucin was observed in the mucous cells of all epidermis in sea chub.

DISCUSSION

The epidermis of fishes was composed of the three layers of stratified epithelium interspersed with round to ovoid or elongated mucous cells (Sato, 1979; Whitear and Mittal, 1986; Lee and Kim, 1999). The primary function of the epidermis is protection against environmental hazards. In fish, this function is generally attributed to the gland cells secreting their contents on surface. Fish epidermis is in

Table 1. Staining properties with histochemistry of epithelial elements of the epidermis in yellowtail

Regions	Epithelial elements of epidermis	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5 -PAS	AF pH 1.7 -AB pH 2.5	HID -AB pH 2.5
Abdominal skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	1R	1B~2B	0	1~2B	1~2B	1~2B
Thoracic skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	1~2R	2~3B	0	1B	2~3B	1~2B
Dorsal skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	1~2R	2~3B	0	1~2B	1~2B, ±B	1~2B

Abbreviations: B, blue: R, red: P, purple: BP, bluish purple: RP, reddish purple: N, black: BN, blue to black: NB, black to blue: ESL, epithelial cells of superficial layer: EML, epithelial cells of middle layer: EBL, epithelial cells of basal layer: MC, mucous cells: AB, alcian blue: PAS, periodic acid Schiff: AF, aldehyde fuchsin: HID, high iron diamine: >, most marked
Numbers indicate the degree of staining: 4, very intense; 3, intense; 2, moderate 1, weak; ±, trace; 0, absent

Table 2. Staining properties with histochemistry of epithelial elements of the epidermis in striped beakperch

Regions	Epithelial elements of epidermis	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5 -PAS	AF pH 1.7 -AB pH 2.5	HID -AB pH 2.5
Abdominal skin	ESL	±R	0	0	±B	±B	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	1~2R	1~2B	0	1~2B	1~2B	1B
	CC	0	0	0	0	0	0
Thoracic skin	ESL	0~±R	0	0	0~±B	0~±BP	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	1~2R	1~2B	0	2B	1~2B	1B
	CC	0	0	0	0	0	0
Dorsal skin	ESL	±R	0	0	±B	±BP, ±B	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	1~2R	1~2B	0	1~2B	±~2B	1~2B
	CC	0	0	0	0	0	0

Abbreviation: CC, club cells
Numbers and other abbreviations are the same as Table 1.

direct contact with the environment and mucus is considered to be a first line of defense protecting the epidermis (Shephard, 1994; Zaccone *et al.*, 2001). External and internal epithelial surfaces of fish are covered with a mucous layer providing protection against environmental factors like microorganisms, toxins, pollutants, acidic pH and hydrolytic enzymes. Secretory mucins are the major constituents of the mucus layer in which several biochemical compounds have been identified; like lysozyme (Fletcher and White, 1973a), antimicrobial peptides (Cole *et al.*, 1997), and antibodies (Fletcher and White, 1973b).

The epidermis of five teleostean species investigated in the present study as in most fishes, was mucogenic in nature. In particular, the number of mucous cells in multicolorfin rainbowfish was much higher than the remaining species.

Singh and Mittal (1990) noted that in the epidermis of Indian major carps, *Labeo rohita* and *Cirrhina mrigala*, the arrangement of mucous cells closed together in a single row in the superficial layer, secreting profusely at the surface, may also be considered as an adaptation to their bottom feeding habits. However, the low number of mucous cells in the epidermis of yellowtail suggests that these cells may confer less protection in terms of mucus production than in other teleostean species.

Conventional histochemical studies have shown that the mucous cells in epidermis of fish are merocrine glands secreting mucus, and contained acidic and neutral glycoprotein. Kazuyori and Motoyoshi (1975) demonstrated that epithelial goblet cells of eel elaborate a neuraminic acid-containing mucosaccharide with vicinal hydroxyl, sulfate and carboxyl groupings. Singh and Mittal (1990) reported that secretory contents of the mucous cells in the four species of carp (common carp and three Indian major carp), in general, contained a mixture of neutral and acidic (non-sulphated

Table 3. Staining properties with histochemistry of epithelial elements of the epidermis in Brown spotted grouper

Regions	Epithelial elements of epidermis	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5 -PAS	AF pH 1.7 -AB pH 2.5	HID -AB pH 2.5
Abdominal skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	1R	2B	2B	2B	2P	1~2N
	CC	0	0	0	0	0	0
	SC	±R	0	0	0	0	0
Thoracic skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	1R	1~2B	1~2B	1~2B	1~2P	1~2N
	CC	0	0	0	0	0	0
	SC	±R	0	0	0	0	0
Dorsal skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	±~1R	2B	2B	2B	2P	1N
	CC	0	0	0	0	0	0
	SC	±R	0	0	0	0	0

Abbreviation: CC, club cells: SC, swollen cells
Numbers and other abbreviations are the same as Table 1.

Table 4. Staining properties with histochemistry of epithelial elements of the epidermis in sea chub

Regions	Epithelial elements of epidermis	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5 -PAS	AF pH 1.7 -AB pH 2.5	HID -AB pH 2.5
Abdominal skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	0	1~2B	1~2B	1~2B	1~2P	1~2N
	SC	0	0	0	0	0	0
Thoracic skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	0	1~2B	1~2B	1~2B	1~2P	1~2N
	SC	0	0	0	0	0	0
Dorsal skin	ESL	0	0	0	0	0	0
	EML	±R>0	0>±B	0>±B	0>±P	0>±P	0>±N
	EBL	0	0	0	0	0	0
	MC	0>±R	1~2B	1~2B	1~2B	1~2P	1~2N
	SC	0	0	0	0	0	0

Abbreviation: SC, swollen cells
Numbers and other abbreviations are the same as Table 1.

and sulphated) mucopolysaccharides, although a few mucous cells contained only neutral mucopolysaccharides. They noted that in *Catla catla* and *Cirrhina mrigala,* the mucous cells in initial stages of development, located in deeper layers, appear to synthesize only neutral mucopolysaccharides, the acid mucopolysaccharides moieties, both sulphated and non-sulphated, being gradually incorporated as they are further differentiated and pushed toward the surface. Ottesen and Olafsen (1997) also reported that Atlantic halibut had a mixture of neutral and sulphated glycoproteins, but qualitative changes of the mucus composition from predominantly neutral to a mixture of neutral and sulphated glycoproteins occurred during the development from a pelagic larva to a bottom-dwelling flat fish. Lee *et al.* (2000a) reported that mucous cells in the epidermis of blenny, *Pholis nebulosa* contained only neutral mucopolysaccharides, and also Kim *et al.* (2002) demonstrated neutral mucin in bastard halibut, *Paralichthys olivaceus*. Acidic mucosubstances in the mucous cells of epidermis were reported in eel (Kazuyori and Motoyoshi, 1975; Park *et al.*, 1995), goldfish, *Carassius auratus* (Jeong and Moon, 1994), mud loach, *Misgurnus anguillicaudatus* (Park and Kim, 1999), and marbled sole, *Limanda yokohamae* (Lee *et al.*, 200b). Fish inhabiting muddy waters and of burrowing habit have also been reported to secrete copious mucus (Mittal and Munshi, 1971; Mittal and Banerjee, 1974). Acidic sulphated mucopolysaccharides in mucous cells also have been reported in the following

Table 5. Staining properties with histochemistry of epithelial elements of the epidermis in multicolorfin rainbowfish

Regions	Epithelial elements of epidermis	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5 -PAS	AF pH 1.7 -AB pH 2.5	HID -AB pH 2.5
Abdominal skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	2R>1R	2B	0	2B	2B	2B
Thoracic skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	2~3R	2B	0	2B	2B	2B
Dorsal skin	ESL	0	0	0	0	0	0
	EML	0	0	0	0	0	0
	EBL	0	0	0	0	0	0
	MC	2R	2B	0	2B	2B	2B

Numbers and other abbreviations are the same as Table 1.

cutaneous respiratory fishes as *Monopterus, Mastacembelus, Amphipnous, Misgurnus, Liobagrus, Iksookimia, Pseudobagrus, Luciogobius, Orthrias* (Mittal and Munshi, 1971; Mittal and Banerjee, 1974; Mittal *et al.*, 1980; Park and Kim, 1999, 2000, 2003; Park *et al.*, 2001, 2002, 2005). These benthic fishes inhabit stagnant ponds or reservoirs and substrates such as bottoms of pebbles, sands, and rocks that are frequently subjected to be dry.

In general, sulphation confers increased charge on the mucins, which may affect the rheological properties of the mucus. The sulphated mucus may, in particular, have a role in protection from an abrasive environment for benthic species (Blackstock and Pickering, 1982; Mittal *et al.*, 1994). Sulphated mucus may also have a role in defense aganist pathogens (Solanki and Benjamin, 1982). Bottom-dwellers produce, with a few exceptions, exclusively sulphated glycoproteins (Whitear, 1986).

The epidermis mucous cells in the epidermis of five teleostean species considered in the present study contained acidic mucopoly-saccharides; yellowtail, striped beakperch, and multicolorfin rainbowfish had sialomucin, while brown spotted grouper and sea chub had sulfomucin. For brown spotted grouper and sea chub, the existence of these sulphated mucus-producing cells in the epidermis may suggest that habitats of these species may confer abrasive environment.

Qualitative differences, both inter- and intraspecies, have been observed in the chemical composition of the epidermal mucus of fish. In the present study, goblet cells show species variations in number and localization of epidermal mucosubstances, which are recognized as sulfomucins and sialomucins. Such differences may reflect an adaptation to the vastly different habitats of teleosts and further studies should be conducted.

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