Histochemistry of the Mucous Cells in the Skin of *Pseudobagrus fulvidraco* and *Leiocassis nitidus* (Bagridae, Siluriformes)

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Skin mucous cells in the three regions of the body in *Pseudobagrus fulvidraco* and *Leiocassis nitidus* were investigated using three histochemical methods (PAS, AB-PAS and HID). In the two species, components of mucous cell were not distinguished in each region, but presented a differences in position and type, size, and count in all regions. Mucous cells of *P*. fulvidraco were located in two layers, a superficial epithelial cell and the space within alarm substance cells (ASCs). Mucous cells of *L*. nitidus were situated only in the superficial epithelial cell layer. The size of mucous cells in the ASC layer were larger than those of the superficial epithelial cells for all measured values: diameter, length, width, and area. In the superficial epithelial cell layer, the average number of mucous cells within the three regions did not differ significantly between species by unpaired T-test, but the average number of those within two cell layers of *P*. fulvidraco had a similarity at each region by paired T-test. The dissimilarity in dorsal and ventral regions in average number of ASCs between *P*. fulvidraco and *L*. niditus was confirmed by unpaired T-test.

Key words: Histochemistry, mucous cell, skin, *Pseudobagrus fulvidraco*, *Leiocassis nitidus*, Bagridae

**Introduction**

The fishes inhabit at unique environment as it is called water, a accommodation of the epidermis that is direct in contact with their environment have been maintain stability to the interior milieu in water (Matoltsy and Bereiter-Hahn, 1986). The skin of the fishes protects themselves and its inside condition maintain uniformly from outside surrounding environment (Park et al., 1995a) and the main function of the integument of vertebrates is to control and to protect the organism from the environment (Matoltsy and Bereiter-Hahn, 1986). Most skin of the fishes is covered with scales and it does protect itself from mechanical wounds and predators (Lagler et al., 1977; Moyle and Cech, 1996). However, some species have no scale, but they secrete mucus from specialized mucous cells in epidermis to the outside surface of skin continually (McKim et al., 1996). The secreted mucus constitutes the primary biological interface between fish and the aqueous environment and plays an important role of protection against a mechanical injury, friction reducing properties, possibly in ionoregulation, and first barrier against infection, because of having defence factors in the mucus, such as immunoglobulin, lysozyme and lectin (Iger et al., 1994; Strüssmann et al., 1994; Moon, 1995a; Sabóia-Moraes et al., 1996; Burkhardt-Holm et al., 1997; Ottesen and Olafsen, 1997; Quiniou et al., 1998; Burkhardt-Holm et al., 2000; Zhang et al., 2000). *Pseudobagrus fulvidraco* and *Leiocassis nitidus*
appertaining to the family Bagridae are species having no scale and keep a lot of mucus to an exterior of epidermis from a mucous cell. Mucous cells can be classified by various stain methods (Whitear, 1986; Mittal et al., 1994) based on qualitative difference of a chemical component in both interspecies and intraspecies (Ottesen and Olafsen, 1997).

Already many study of mucous cell at epidermis of fishes have been performed between male and female (Irving, 1996) or genera (Gona, 1979; Satō, 1979; Singh and Mittal, 1990). On the other hand, nowadays P. fulvidraco and L. nitidus were reappraised the taxonomic position by Lee (1988), which is having very interesting aspects, although it was decided into different genus clearly based on their osteological characters.

The aim of this study is to make a check on components, location, count and size of mucous cells in epidermis between two species by light microscope.

**Materials and Methods**

In this study, six specimens of P. fulvidraco (102.0~175.2 mm) and ten of L. nitidus (161.7~171.4 mm) were collected at Guiam, Buyeo-up Buyeo-gun, Chungcheongnam-do, Korea in 2000. All specimens were fixed in 10% neutral buffer formalin at the living condition immediately and each specimen were dissected to obtaining about 5×5 mm of skin fragments from three region, that is dorsal, lateral, and ventral respectively. Dissected fragments carried out the successive course of dehydration in ethanol for paraffin embedding, clearing in xylene, and paraffin infiltration in paraffin wax. Embedded samples were sectioned at thickness of 5 μm by rotary microtome. To identify a ingredients of mucous cell was stained with three methods, periodic acid Schiff (PAS), alcian blue at pH 2.5–periodic acid Schiff (AB–PAS), and high iron diamine (HID) at pH 2.5 of alcian blue (McManus, 1946; Mowry, 1963; Spicer, 1965). The section was chosen randomly from each fish and the number of mucous cells and alarm substance cells (ASCs) counted in a captured image (the dimension is about 75,000 μm², n = 40) by Image plus pro 4.0 (400×). Average number of mucous cells and ASCs obtaining from random area were compared by T–test.

**Results**

1. **Component of mucous cell**

We confirmed that P. fulvidraco and L. nitidus contained neutral mucous cells in dorsal region by PAS stain method (Fig. 1). Consequently, in the three regions of P. fulvidraco and L. nitidus, it was not discovered a different components in their mucous cells. Mucous cells in two species, in general, contained a mixture of neutral and acidic by AB–PAS (purple in colour) (Fig. 2), and sulfonic and sialic components by HID (brown in colour) (Fig. 3), but a few mucous cells showed the only neutral or acidic and sulfonic or sialic component. However, most mucous cells had sulfonic component than sialic in two species relatively (Fig. 3).

2. **Size of mucous cell**

Mucous cells of ASC layer of epidermis in P. fulvidraco were larger than those within superficial epithelial cell layer for several measured values : diameter and length, over two times; width, over three; area, over six (Table 1). And it appeared that the mucous cells of L. nitidus was some larger than those of P. fulvidraco of the superficial epithelial cell layer relatively (Table 1).

3. **Location and type of mucous cell**

In P. fulvidraco, There are two types of mucous cell in three regions; the one presented in the superficial epithelial cell layer and the other did in the space of ASC layer of epidermis (Figs. 1A–3A), in L. nitidus, however, mucous cells situated only in the superficial epithelial cell layer in three regions (Figs. 1B–3B). In P. fulvidraco and L. nitidus, some mucous cells in the superficial epithelial cell layer showed the extended shape horizontally, and sometimes others appeared the

| Table 1. Measurements of the mucous cell (n=40) in the skin of Pseudobagrus fulvidraco and Leiocassis nitidus (mean±S.D.) |
|-----------------|-----------------|-----------------|
| Cell layer      | P. fulvidraco   | L. nitidus      |
| Division        | superficial     | alarm           | superficial |
| Diameter (μm)   | 7.1±2.0         | 17.9±3.2        | 8.5±2.1     |
| Length (μm)     | 9.5±3.2         | 22.6±5.2        | 10.6±2.9    |
| Width (μm)      | 6.0±1.9         | 18.9±7.3        | 8.2±2.2     |
| Area (μm²)      | 44.1±23.0       | 266.5±96.9      | 64.1±33.0   |
extended shape vertically. However, mucous cells in the ASC layer of epidermis showed almost round shape. Mucous cells of *L. nitidus* of superficial epithelial cell layer were denser than those of *P. fulvidraco*. So, in the ASC layer of epidermis of *P. fulvidraco*, it was very sparsely distributed.

4. **Count of alarm substance cells (ASCs)**

The average number of ASCs between *P. fulvidraco* and *L. nitidus* in three regions showed similarity each other (in *P. fulvidraco*: dorsal and lateral, *P* < 0.05; lateral and ventral, *P* < 0.05; dorsal and ventral, *P* < 0.05; in *L. nitidus*: dorsal and lateral, *P* < 0.05; lateral and ventral, *P* < 0.05; dorsal and ventral, *P* < 0.05) (Fig. 4A). In the intraspecies the average number of ASCs did not have a similarity among all regions. But in the interspecies, there was not similarity at dorsal and ventral regions except lateral: dorsal, *P* > 0.05; lateral, *P* < 0.05; ventral, *P* > 0.05 (Fig. 4B). At dorsal and ventral region, the average number of ASCs of *L. nitidus* was more than those of *P. fulvidraco* (Fig. 4B).

5. **Count of mucous cell**

Three regions of between two species and two kinds of mucous cells within *P. fulvidraco* were compared by T-test. First, in the superficial epithelial cell layer the average number of mucous

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**Fig. 1.** Periodic acid Schiff stained sections of dorsal skin of *Pseudobagrus fulvidraco* (A) and *Leiocassis nitidus* (B). Scales indicate 20 µm. ASC, alarm substance cell; E, epidermis; NM, neutral mucous cell; S, superficial epithelial cell layer.

**Fig. 2.** Alcian blue (pH 2.5)–periodic acid Schiff stained sections of lateral skin of *Pseudobagrus fulvidraco* (A) and *Leiocassis nitidus* (B). Scales indicate 20 µm. ASC, alarm substance cell; E, epidermis; NM, neutral mucous cell; S, superficial epithelial cell layer.
cells had not a similarity between *P. fulvidraco* and *L. nitidus* at the three regions: dorsal, $P < 0.05$; lateral, $P < 0.05$; ventral, $P < 0.05$ (Fig. 5A), but it of *L. nitidus* was much more than it of *P. fulvidraco* relatively.

Second, mucous cell in the ASC layer in the epidermis in *P. fulvidraco* had a similarity at the three regions for it in the superficial epithelial
cell layer: dorsal, $P > 0.05$; lateral, $P > 0.05$; ventral, $P > 0.05$ (Fig. 5B). As a results, $P$. fulvidraco largely differed from $L$. nitidus in the average number of mucus cells at all regions.

**Discussion**

The present study was performed for investigation of ingredient, size, location, type and count of mucus cells in the skin between $P$. fulvidraco and $L$. nitidus of belonging to the family Bagridae by histochemical methods.

Whitear (1986) mentioned that each species of fishes usually secrete a different type of mucus according to the nature of mucus cells. Betta splendens has a only neutral mucus cells and Macropodus opercularis showed acid but non-sulfated ed ones, while Colisa lalia and a species of the genus Trichogaster had mixed populations of mucus cells differing in the proportion with neutral glycoprotein in skin (Gona, 1979). Moon (1995b) reported that in the gill epithelium the guppy (Poecilia reticulata) also has the single mucus cell contained either neutral or acid glycoproteins alone or in combination. However, the components of the mucus cell was changed from sulfomucin to sialomucin by a influence of seawater (Fletcher et al., 1976; Gona, 1979; Moon, 1995b). $L$. nitidus inhabits at the freshwater having a effect upon seawater (Kim, 1997), if so, this species may be have a lot of sialomucin than $P$. fulvidraco. Whitear (1986) mentioned that taxonomy is no guide to the type of mucus secreted. However, A differences of mucus cell may be due to an adaptation to the vastly diverse habitats of a teleost fishes and a bottom-dwellers produce, with a few exceptions, exclusively sulfated glycoproteins and a sulphation confers increased charge on the mucin, which may affect the rheological properties of the mucus (Ottesen and Olafsen, 1997), while a species with a pelagic habitat produce merely a carboxylated mucus (Whitear, 1986). But $P$. fulvidraco and $L$. nitidus have a lot of sulfated mucous cells than sialoid (Fig. 3) relatively. It is proved that they may be a bottom-dweller indirectly. The sulfated mucus may, in particular, have a role of protection from an abrasive environment for benetic species (Mittal et al., 1994). When designing a cultivating unit for two species, such fact should be taken in to consideration.

Mucous cells of $P$. fulvidraco are distributed at two locations, which are the superficial epithelial cell and ASC layer of epidermis, but $L$. nitidus was only one, that is the superficial epithelial cell layer. According to a species, some species have only one type or more two type of mucus cells (Satoh, 1978). Such differences of the position and type may be due to have a different habitat between two species. Generally $P$. fulvidraco dwells in a mid-stream and downstream of river with the slow speed of current, but $L$. nitidus live lower area of river where somewhat influenced by seawater (Kim, 1997). Park et al. (1995b) mentioned that when the freshwater eel (Anguilla japonica) of a adapted condition in freshwater came in contact with a seawater, a vacuoles with agranule and space of among the cell within epidermis increase at the inside of epidermis, while such happenings didn’t appear between epithelial cells and mucus cells. But $P$. fulvidraco and $L$. nitidus have a similar habit as a bottom-dweller (Lee, 1990; Kim and Kang, 1993; Kim, 1997). And in a alteration of osmotic balance mucus cell must be take charge of a role of protection against a wound or abrasion (Moon, 1995a). If so, because of $L$. nitidus is adapted to often changing water environment, according to the habitat, it may be have this position. We are considered that $L$. nitidus had a unique arrangement of mucous cell separately from $P$. fulvidraco (Figs. 1–3).

In Cyprinus carpio var. communis the presence of a large number of mucus cells in the outer regions of the epidermis suggests that the overall production of mucus in this fish is very high and this may be as an adaptation in relation to its peculiar bottom-scooping habits for increasing efficiency in the fish keeping its surface clean (Singh and Mittal, 1990). In mucus cells within the superficial epithelial cell layer of $P$. fulvidraco and $L$. nitidus, ones of the latter is more than ones of the former. From that point view, $L$. nitidus may be considered as an adaptation to their bottom feeding habits than $P$. fulvidraco.

In $P$. fulvidraco, mucous cells in ASC layer were larger than that within the layer of other epidermis cells and it was presented the two position, which are the superficial epithelial cell and ASC layer of epidermis. Singh and Mittal (1990) reported that mucous cells of $C$. carpio var. communis were located mainly in the outer region of the epidermis, Labeo calbasu and Cirrhina mrigala, in surface layer, and Catla catla was found in the superficial and under deeper
layers of the epidermis. Also a certain freshwater catfish (Ictalurus punctatus) had a same position with mucous cell of P. fulvidraco (Quiniou et al., 1998).

The number of ASCs in P. fulvidraco was fewer than those of L. nitidus in the dorsal and ventral region. In a comparative study of the epidermis of the craps, Singh and Mittal (1990) reported that numerical relation of ASC and mucous cell is a interdependence, and high density of ASCs may compensate for the smaller and sparser mucous cells in providing and effective defense mechanism. Therefore, P. fulvidraco and L. nitidus of dissimilarity of ASCs in number may be relative the whole number of mucous cells. And, in two regions of P. fulvidraco, relationship of mucous cells may be suggest that its ASC layer at epidermis supplement a few mucous cells in the superficial epithelial cell layer for providing a lacking mucus production.

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