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Histochemical characterization of glycoproteins in the gills of the carp *(Cyprinus carpio)*

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Summary: The characteristics of the mucous cells located in gills of the fish *Cyprinus carpio* were investigated. Mucous cells were determined from gill arc, gill body, primary and secondary lamellae. Mostly mucous cells had oval-globular shape throughout all regions. Histochemical analysis of the gill of *Cyprinus carpio* showed that mucous content included glycogene and/or oxidizable dioles (PAS +), neutral or acid-rich (PAS/AB pH 2.5 +), sialic acid residues (KOH/PAS +) and strong acid sulphated (AF +) glycoproteins (GPs). Except these mucosubstances, carboxyl groups and/or with sulphate esters (AB pH 2.5 +) strong sulphate (AB pH 0.5 +) (AF/AB pH 2.5 +), O-sulphate esters (AB pH 1+) glycoproteins were also determined.

Key words: *Cyprinus carpio,* density, gill, glycoproteins, mucous cells

Sazan *(Cyprinus carpio)* solungaç glikoproteinlerinin histokimyasal özellikleri

Özet: *Cyprinus carpio* solungaçlarında yer alan karakteristik mukus hücreleri incelendi. Mukus hücreleri solungaç arklarında, solungaç lamellerinde, primer ve sekonder lamellerde tespit edildi. Tüm bölgeler boyunca mukus hücrelerinin çoğu oval-yuvarlak bir şekle sahipti. Histokimyasal analizler solungaçlardaki mukus hücrelerinin glikojen ya da oksidizable diolleri (PAS +), nötral ya da asitce zengin (PAS/AB pH 2.5+), siyalik asid rezidulleri (KOH/PAS +) ve asitce zengin sulfatlı (AF +) glikoproteinleri içerdiği gösterildi. Bu mukosubstanslar dışında karboksil gruplu ya da sülfat esterli (AB pH 2.5 +) güçlü sülfatlı (AB pH 0.5 +) (AF/AB pH 2.5 +), O-sülfat esterli (AB pH 1+) glikoproteinler tespit edildi.

Anahtar sözcükler: *Cyprinus carpio,* glikoproteinler, mukus hücreleri, solungaç, yoğunluk.

Introduction

The fish gill epithelium consists of several cell types. Many studies of fish gills have described the morphological and functional characteristics of gill epithelial cells: respiratory cells, chloride or mitochondria-rich cells, pavement cells and mucous cells (4, 9, 19, 22). As well as some studies is reported the branchial lamellae epithelium consists of granular cells, ciliated cells, leydig cells, bazal cells (17), undifferentiated cells (4), and accessory cells (1). Mucus producing cells integrate a variety of critical functions. Physiologically, mucus is important for protection and inhibition of micro-organisms (5, 27, 36). This function is occurred by mucous glycoproteins (GPs) (5, 15, 24, 35), as well as the GPs are identified with O-sulphate esters are responsible for the lubrication (8). In addition, mucus membran is engaged important functions, such as osmoregulation, diffusion and protection of dehydration (8,19, 24, 36).

Mucus producing cells are numerically and morphologically affected by different conditions as other cells localized in gill epithelium. Increase of mucous cells number is reported with different conditions such as bacterial gill disease (12), amoebic gill disease (26, 30, 33), high concentrations of ammonia (12, 16), salinity (2, 13), acidity (6, 20), high pressure and low temparature (10). On the other hand, in conditions of high concentrations of ammonia (16), low pH (37), high concentrations of aluminum (29), heavy metals (18), substrat of diazinon (11) and acid plus aluminum (6), mucous cells size is increased. It is claimed that, numerically and morphologically the difference in mucous cells probably relates to period of secretion. In this study, our aim was to determine the mucous cells of the gills of the Carp *(Cyprinus carpio)* with histochemical technique

Materials and Methods

In this study we choosed the omnivorous fish species, *Cyprinus carpio*. We obtained these fish at Eğirdir lake. As material, twenty five uninfected *Cyprinus carpio*, length between 25 – 30 cm and weight between 320 – 350 g, were used. Water conditions were showed in table 1. The gills were rapidly excised and fixed by immersion in 10% buffered formalin for light microscopic studies. The samples were routinely processed and embedded in parafin. Histochemical techniques were performed for the density and differentiation of carbohydrate moieties (Table 2).

Table 1. Water conditions (Photometer method, soil pool)
Tablo 1. Su değerleri (Fotometre yöntemi, toprak havuzu)

Water conditions	Abbreviations	Water assets
Potassium	(K)	2.7 mg/l.
Sulphate	(SO_4)	5 mg/l.
Phosphate	(PO_4)	5 mg/l.
Chlorine	(Cl)	4 mg/l.
Ammonium	(NH_4)	0.06 mg/l.
Nitrate (nitrogen of nitrat)	(NO_3)	0.6 mg/l.
Nitrate (total nitrat)	(NO_3)	3.6 mg/l.
Alkalinity	($CaCO_3$)	300 mg/l.
Salinity		% 3
pH		7.5
Conductor		522 µs

Table 2. Performed the histochemical techniques in the gill epithelium of *Cyprinus carpio*;AB, Alcian blue; KOH, saponification; PAS, periodic acid/Schiff; AF, Aldehyde fuchsin; GPs, glycoproteins.
Tablo 2. *Cyprinus carpio* solungaç epitelinde uygulanan histokimyasal teknikler; AB, Alsiyan mavisi; KOH, saponifikasyon; PAS, periyodik asit/Shif; AF, Aldehit fuksin; GPs, glikoproteinler.

Procedures	References
1. PAS GPs with oxidizable vicinal diols and/or glycogen	Mc Manus (1948)
2. PAS/AB pH 2.5 Neutral and/or acid rich GPs	Mowry (1956)
3. AB pH 2.5 GPs with carboxyl groups (sialic acid or uranic acid) and/or with sulphate esters	Lev and Spicer (1964)
4. AB pH 1.0 GPs with O-sulphate esters	Lev and Spicer (1964)
5. AB pH 0.5 Very sulphated GPs	Lev and Spicer (1964)
8. KOH/PAS GPs with sialic acid residues	Culling et al. (1976)
9. AF GPs with sulphate	Gomari (1952)
10. AF/AB pH 2.5 Strong sulphated GPs	Spicer and Meyer (1960)

Results

As a result of histochemical studies, mucous cells were determined from gill arc, gill body, primary and secondary lamellae. Density in gill arc and body was higher. Respectively primary and secondary lamellae followed these (Table 3). At the bases of secondary lamellae, such cells were detected as single cells or sometimes in groups. Mucous cells were observed to occur towards the outer surface, but sometimes in deeper layers. Mostly mucous cells had oval-globular shape throughout in all regions. Exceptionally some were in goblet shape, rather commonly in the gill arc. Despite foamy or cloudy appearance at mucous cell cytoplasms, seldomly cells had granular cytoplasms.

Table 3. Histochemical reactions of glycoproteins in the gills of *Cyprinus carpio;* AB, Alcian blue; KOH, saponification; PAS, periodic acid/Schiff; AF, Aldehyde fuchsin; GPs, glycoproteins.
Tablo 3. *Cyprinus carpio* solungaç epitelinde histokimyasal reaksiyonlar; AB, Alsiyan mavisi; KOH, saponifikasyon; PAS, periyodik asit/Shif; AF, Aldehit fuksin; GPs, glikoproteinler.

Procedures	Staining reactions			
	Gill arc	Gill body	Primer lamellae	Sekonder lamellae
PAS	+++	++	++	+
PAS/AB pH 2.5	+++	+++	++	+
AB pH 2.5	++	+++	++	+
AB pH 1.0	++	+++	++	+
AB pH 0.5	++	+++	++	+
KOH/PAS	+++	++	++	+
AF	++	+++	++	+
AF/AB pH 2.5	++	+++	++	+

Alcian blue (AB) pH 1 (Figure 1), AB pH 2.5 (Figure 2), periodic acid/schiff (PAS) and aldehyde fuchsin (AF) applications were intense in all regions, while AB pH 0.5 (Figure 4, 5) was somewhat weaker. AB (+), PAS (+) and AF (+) mucosubstances were dominant in PAS/AB pH 2.5 (Figure 3), saponification / periodic acid/schiff (KOH/PAS), AF/AB applications respectively. In gill arc of some cells only AB (+) mucosubstances were observed in PAS/AB pH 2.5. similar results were obtained in AF/AB application. But in AF/AB application, cells away from lumen surface also showed AF (+) reaction at secondary lamella.

Discussion and Conclusion

Studies with different fish species shows that gill mucous cells distributed in different areas and different densities. In *Poecilia vivipara* (31) these were observed in apical of gill filaments only in interlamellar region and gill arc. In *Micropogonias furnieri* (9), mucous cells were observed in primary and secondary lamellae, while in *Acipenser naccarii* (4) along the filaments, rarely between pavement cells. In this study, mucous cells were identified to be broadly distributed in gills, rather in gill arc. Mucous cells were mostly in oval-globular in shape less commonly in goblet or pear-shaped, similar to *Morone saxatilis* or *M. chrysops* (28).

In accordance with Diaz *et al* (8), except for primary and secondary lamellae, mucous cell glycoprotein characterization was almost the same. Also in PAS/AB pH 2.5 applications similar results were obtained (1, 3). The AB dominance also has been obtained in *Solea senegalensis* (1), as in *Cyprinus carpio* in this study.

In AB pH 2.5 applications glycoprotein with carboxyl groups are more common, while freshwater adapter *Salmo* species (33) they are found less

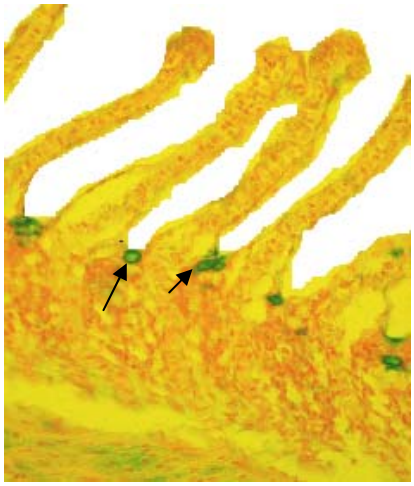

Figure 1. Secondary lamella, GPs with O-sulphate esters in mucous cells. AB pH 1.0. X 200
Şekil 1. Sekonder lamel, O-sülfat esterli glikoprotein içeren mukus hücreleri AB pH 1.0. X 200

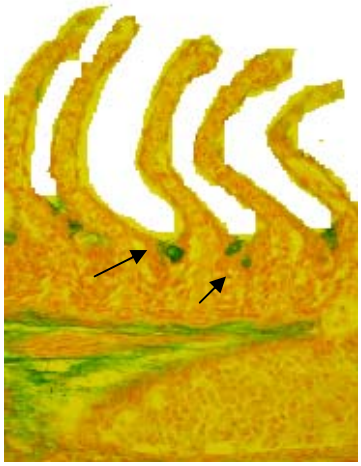

Figure 2. Secondary lamella, GPs with carboxyl groups (sialic acid and/or with sulphate esters in mucous cells. AB pH 2.5. X 200
Şekil 2. Sekonder lamel, karboksil gruplu sülfat esterli glikoprotein içeren mukus hücreleri AB pH 2.5. X 200

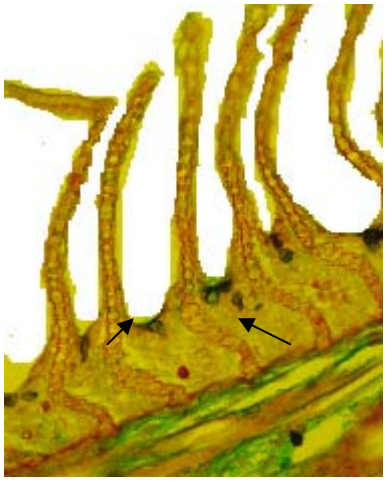

Figure 3. Secondary lamella, GPs with carboxyl groups and/or with sulphate esters in mucous cells PAS/AB PH 2.5 (AB dominant) X 200
Şekil 3. Sekonder lamel, karboksil gruplu sülfat esterli glikoprotein içeren mukus hücreleri PAS/AB pH 2.5 X 200

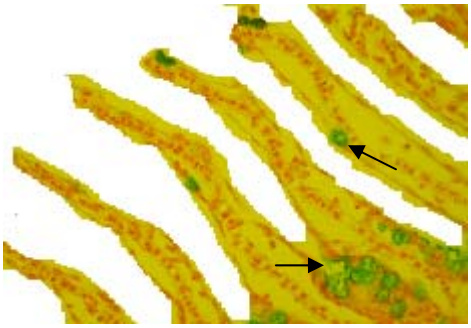

Figure 4. Secondary lamella, very sulphated GPs in mucous cells AB pH 0.5 X 200
Şekil 4. Sekonder lamel, güçlü sülfatlı glikoprotein içeren mukus hücreleri AB pH 0.5 X 200

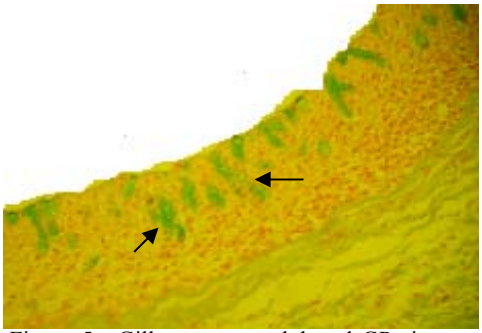

Figure 5. Gill arc, very sulphated GPs in mucous cells AB pH 0.5 X 200
Şekil 5. Solungaç yayı, güçlü sülfatlı glikoprotein içeren mukus hücreleri AB pH 0.5 X 200

frequently. Similar is case for *Solea senegalensis* (1), differing in that there were also some cells equally comprising AB pH 2.5 and PAS (+) mucosubstances.

Similar to study, in *Acipenser naccarii* (8) acclimated to sea, great portion of mucous cells are observed to react with PAS (+). Also similar to *M. furnieri* (8), sialic acid residues, glycoproteins with oxidizable vicinal diols are observed. Similar to *Solea senegalensis* (32) adults, in *Cyprinus carpio* strong sulphated GPs were encountered with AB pH 0.5 and AF/AB applications. Likewise glycoprotein with sulphate groups seen in *M. furnieri* (8) and *Salmo salar* (33) acclimated to sea water were also observed in *Cyprinus carpio* in this study.

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