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ORIGINAL ARTICLE

Identification of Glycoproteins in Mucous Cells of Epidermis and Gill of *Tinca tinca* Linnaeus, 1758 (Cypriniformes: Cyprinidae)

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SUMMARY

The present article reports of the glycoproteins in the mucous cells of gill of the *Tinca tinca.* As material, twenty five uninfected *Tinca tinca* were used. 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. Histochemical analysis showed that the gills mucous content included acidic (AB pH 2.5 +), neutral (PAS +), neutral or acid-rich (PAS/AB pH 2.5 +), strong acid sulphated (AF +) and strong sulphated (AF/AB pH 2.5 +) glycoproteins (GPs).

Key Words *Histochemistry, Gill, Tinca tinca*

Kadife Balığının *(Tinca tinca)* Linnaeus, 1758 (Cypriniformes: Cyprinidae) Solungaç Epitel Mukus Hücrelerinde Glikoproteinlerin Belirlenmesi

ÖZET

Bu çalışmada kadife balığı *(Tinca tinca)* solungaç epitelindeki mukus hücrelerinde glikoproteinlerin belirlenmesi rapor edilmiştir. Materyal olarak 25 adet kadife balığı kullanıldı. Işık mikroskobik incelemeler için solungaçlar %10'luk formaldehit içerinde tespit edildi. Örnekler rutin doku takibinden geçirilip parafinde bloklandı. Parafin bloklardan karbonhidrat bileşenlerinin yoğunluğunu ve farklılığını belirlemek için histokimyasal teknikler uygulandı. Solungaç mukus içeriğinin asidik (AB pH 2.5 +), nötral (PAS +), nötral-asidik (PAS/AB pH 2.5 +), güçlü asidik sülfatlı (AF +) ve güçlü sülfatlı (AF/AB pH 2.5 +) glikoprotein olduğu gözlendi.

Anahtar Kelimeler *Histokimya, Solungaç, Tinca tinca*

INTRODUCTION

The fish gill consists of several kinds of epithelia and many diverse cell types. The primary epithelium covers the primary lamella including the interlamellar region, and the secondary gill epithelium covers the free part of the secondary lamellae (Diaz et al.2005). Gills are the main sites of gas exchange in almost all fishes. In addition to their respiratory function, the gills play an important role in the excretion of certain waste products and in the maintenance of the fish's salt balance (Zayed and Mohamed 2004).

Gills of fish are organs exposed directly to the water and because of their direct contact with the environment, their structure and function has been investigated in the several studies. Gills secrete mucus, which protects the animal but is also involved in respiration, ion and osmoregulation and is an important factor in disease resistance (Burkhardt-Holm 997).

Our current knowledge on the histochemical analysis of GPs in the secretory cells in the epithelium at different regions of fish gills – the gill arches, the gill rakers, the gill filaments and the secondary lamellae is limited (Kumari et al.2009). Many studies on the mucous cells of vertebrates and their secretion products, both from the histochemical and the ultrastructural point of view, have been cited (Diaz et al.2001). The purpose of this work was to describe the morphology of mucous cells paying special attention to the glycoproteins secreted by the gills of *Tinca tinca.*

MATERIALS and METHODS

In this study we choosed the omnivorous fish species *Tinca tinca*. We obtained these fish at Eğirdir lake. As material, twenty five uninfected *Tinca tinca*, length between 25–30 cm and weight between 320–350 g, were used. The gills were rapidly excised and fixed by immersion in 10% buffered formalin for light microscopic studies. Tissues samples were routinely processed to parafin wax blocks and five micrometer sections were prepared.

Table 1. Performed the histochemical techniques in the gill epithelium of *Tinca tinca*

Procedures	Carbohydrate moieties
1. PAS	GPs with oxidizable vicinal diols and/or glycogen
2. PAS/AB pH 2.5	Neutral and/or acid rich GPs
3. AB pH 2.5	GPs with carboxyl groups (sialic acid or uranic acid) and/or with sulphate esters
4. AF	GPs with sulphate
5. AF/AB pH 2.5	Strong sulphated GPs

AB, Alcian blue; PAS, periodic acid/Schiff; AF, Aldehyde fuchsin; GPs, glycoproteins.

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Sections were stained for general morphological purposes with haematoxylin and eosin (H & E) stains and histochemical techniques were performed for the density and differentiation of carbohydrate moieties (Table 1). It was applied for acidic mucosubstance in alcian blue pH 2.5 methods, for neutral mucosubstance in periodic acid/schiff and for sulphate mucosubstance in aldehyde fuchsin.

RESULTS

The main structural component of the epithelium at different regions of the gills - the gill arches, the gill rakers, the gill filaments and the secondary lamellae consists of the epithelial cells. Mucous cells were detected mostly among other epithelial cells of the primary and secondary gill lamellae. The mucous cells appeared characteristically depressed in the epithelium surface of the epithelial cells which covered them almost completely.

Histochemical analysis showed that the gills mucous content included carboxyl groups and/or with sulphate esters (AB pH 2.5 +), glycogene and/or oxidable dioles (PAS +), neutral or acid-rich (PAS/AB pH 2.5 +), strong acid sulphated (AF +) and strong sulphated (AF/AB pH 2.5 +) glycoproteins (GPs).

The histochemical staining properties of glycoproteins present in mucous cells are summarized in Table 2.

Table 2. Histochemical reactions of glycoproteins in the gills of *Tinca tinca*

Histochemical method	Histochemical reactions
AB pH 2.5	++
AF	++
AF/AB pH 2.5	++
PAS	++
PAS/AB pH 2.5	++

Mucous cells with periodic acid Schiff (PAS) reaction showed a moderate positive response, in which the coloration disappeared after acetylation and was recovered after saponification. Alcian blue at pH 2.5 was moderately positive, indicating the moderate presence of carboxyl-rich glycoconjugates (Fig. 1).

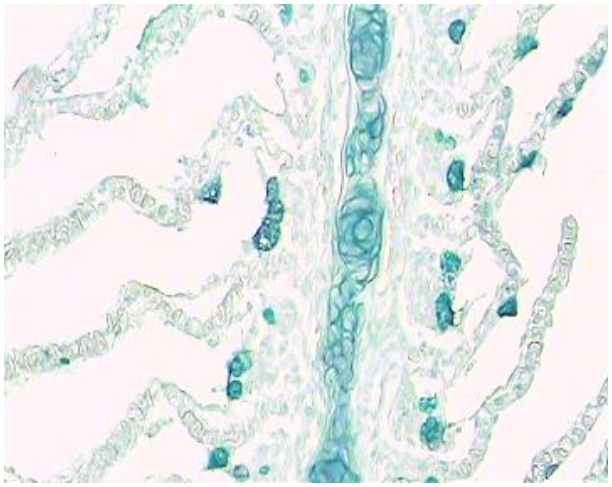

Figure 1. AB pH 2.5 positive mucous cells, X 400

When AB pH 2.5-PAS reaction was performed for identification of neutral and acidic glycoconjugates, most mucous cells were stained purble, indicating a combination of neutral and acid glycoconjugates, whereas some mucous cells were only stained with blue. The reaction with AF indicates the presence of GPs with sulphate as well in these cells (Fig. 2). For separating sulphated glycoconjugates from carboxylated those when the AF/AB pH 2.5 stain was performed, most mucous cells were strongly AF-positive, although some mucous cells stained blue.

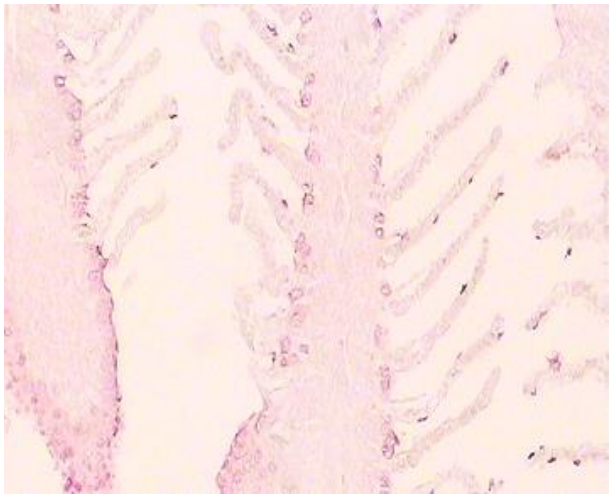

Figure 2. AF positive mucous cells, X 200

As a result of histochemical staining methods was observed positive reaction in primary and secondary lamellae and gill arc in *Tinca tinca*.

DISCUSSION and CONCLUSION

The analysis of the results obtained by present histochemical methods employed in this study has elucidated significance differences in the composition and the concentrations of GP classes eloborated

The general morphology of gill cells and the histochemistry of the mucosubstances from fish gills and different types of mucous cells in the gill epithelium have been described by Diaz et al. (2010), Kumari et al. (2009), Mittal et al. (2002), Rosety-Rodriguez et al.(2002).

It has been documented that freshwater rainbow trout have gill mucous cells consisting of mostly neutral mucins, followed by carboxylated mucins, a comparable finding to that of Tinca tinca. Our results are also in general agreement with that of patterns observed in epidermal mucous cells of other freshwater teleosts, which generally contain a greater proportion of carboxylated mucins than do marine species (Roberts and Powell 2008).

As in the present study, reaction of AB pH 2.5 have been determined in the gills of many fish species, such as *Odontesthes bonariensis* (Diaz et al. 2010), *Rita rita* (Kumari et al. 2009), *Scophthalmus maximus* (Rosety-Rodriguez et al. 2002), *Cyprinus carpio* (Çınar et al. 2008), *Pseudophoxinus antalyae* (Çınar et al. 2009), *Micropogonias furnieri* (Diaz et al. 2001).

Similar to study, in *Micropogonias furnieri* (Diaz et al. 2001) acclimated to sea, great portion of mucous cells are observed to react with PAS (+). A mixture of neutral and acidic glycoproteins, both sulphated and sialylated, has been found in the gill of the fish species *Odontesthes bonariensis* (Diaz et al. 2010), *Rita rita* (Kumari et al.2009), *Salmo salar* (Roberts and Powell 2003). Similar results on

Tinca tinca showed that the production of acidic GPs, mainly sulphated GPs, predominates in their mucous cells.

In this study the reaction with AF indicates the presence of GPs with sulphate as well in these cells of *Tinca tinca*. For separating sulphated glycoconjugates from carboxylated those when the AF/AB pH 2.5 stain was performed, most mucous cells were strongly AF-positive, although some mucous cells stained blue. Similar to *Solea senegalensis* (Sarasquete et al. 1998) adults, in *Tinca tinca* moderate sulphated GPs were encountered with AF/AB applications. Likewise glycoprotein with sulphate groups seen in *M. furnieri* (Diaz et al. 2001) and *Salmo salar* (Shane and Powell 2003) acclimated to sea water were also observed in *Tinca tinca* in this study.

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