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Aphanius anatoliae sureyanus (Neu, 1937) (Osteichthyes: Cyprinodontidae) Solungaç Glikokonjugatlarının Histokimyasal Özellikleri

Dilek DİLER[*1], Kenan ÇINAR[2]

[1, 2]Department of Biology, Faculty of Science and Art, Süleyman Demirel University, 32260 Isparta, Turkey. (**corresponding author e-mail:* dilekyilmaz@stud.sdu.edu.tr)

Özet: *Aphanius sureyanus* (Neu, 1937) *solungaç mukus hücrelerinde yer alan glikokonjugatların özellikleri klasik yöntemlerle histokimyasal olarak incelendi. Solungaç arkı ve primer filament epitelinde bulunan mukus hücrelerinin asidik ve nötral glikokonjugatları, siyalik asit rezidulleri ve sülfatlı glikokonjugatları içerdikleri ve aynı zamanda PAS/ AB pH 2. 5 ve AF/ AB pH 2. 5 boyama yöntemlerinde AB pH 2. 5 pozitif glikokonjugatın baskın olduğu belirlendi. Sekonder lamele ait goblet hücrelerinin herhangi bir glikokonjugatı (asidik glikokonjugat, nötral glikokonjugat, siyalik asit rezidulleri, sülfatlı, çok sülfatlı ve O- sülfat esterli glikokonjugatlar) içermedikleri belirlendi.*
Anahtar Kelimeler: *Solungaç, glikokonjugatlar, mukus hücreleri, Aphanius sureyanus*

Histochemical Characterization of Glycoconjugates in the Gills of the *Aphanius sureyanus anatoliae sureyanus* (Neu, 1937) (Osteichthyes: Cyprinodontidae)

Abstract: *The characteristics of glycoconjugates in the mucous cells of the Aphanius sureyanus* (Neu, 1937) *gill were investigated histochemically with the help of conventional methods. It was determined that Mucous cells ,which existed in the gill arch and primary filament epithelium contained acidic, neutral glycoconjugates, sialic acid residues and sulphated glycoconjugates and also AB pH 2.5 positive glycoconjugates were dominant in staining procedures of PAS/ AB pH 2.5 and AF/ AB pH 2.5 . It was detected that mucous cells of secondary lamellae did not contain any glycoconjugates (acidic, neutral, sialic acid residues, sulphated, very sulphated and O- sulphate esters glycoconjugates).*
Keywords: *Gill, glycoconjugates, mucous cells, histochemistry, Aphanius sureyanus*

Introduction

The gills which are one of the most important organs of the body of the fish serve a variety of functions to fish such as gas exchange, acid-base balance, osmoregulation and ionic regulation for survival of fish (Fosket et al., 1983; Laurent, 1984; Laurent et al., 1994; Goss et al., 1998; Evans et al., 1999).

Different cell types such as mitochondrion-rich, chloride, pavement and mucous cells in the gill were identified in several studies (Goss et al., 1998; Carmona et al., 2004; Díaz et al., 2005b; Vigliano et al., 2006). Gills are exposed directly to the water and due to their direct contact with environment mucus secreted by mucous cells is a physical barrier which inhibits entry of disease microorganisms from the environment into the fish (Fletcher, 1978; Shephard, 1994).

Mucins the main substance of mucus are high molecular weight glycoconjugates (Strous & Dekker, 1992). The characteristics of the mucous glycoconjugates in the gills of the different fish species (*Coelorhynchus coelorhynchus*, *Cyprinus carpio*, *Micropogonias furnieri*, *Cynoscion guatucupa)* were investigated by conventional and lectin histochemistry methods (Díaz et al., 2001; Calabró et al., 2005; Díaz et al., 2005a; Çınar et al., 2008).

The aim of this work is to analyze the normal glycoconjugate composition in the mucous cells of the gills of the *Aphanius sureyanus* (Neu, 1937) of family Cyprinodontidae which is an endemic species for Burdur Lake/ Turkey. This study has been carried out by using a series of conventional histochemical techniques in order to identify different classes of glycoconjugates

Materials and Methods

In this study ten- specimen- *Aphanius sureyanus* (approximately weight between 0. 473 g, total length between 3. 59 cm) were killed by decapitation. Gill tissue samples were immediately dissected, fixed in 10% buffered formalin solution, dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin. Serial sections (5 μm thick) were stained with conventional techniques for histochemical identification of glycoconjugates (Table 1).

Table 1. Conventional histochemical techniques for identification of glycoconjugates in mucous cells of *Aphanius sureyanus* gill; AB, Alcian blue; PAS, Periodic acid/ Schiff; AF, Aldehyde fuchsin; KOH, saponification; GCs, glycoconjugates.

Procedures	References	GCs revealed
PAS	(McManus, 1948)	Neutral GCs
PAS/ AB pH 2.5	(Mowry, 1956)	Neutral and/ or acid rich GCs
KOH/ PAS	(Culling et al., 1976)	GCs with siailic acid residues
AB pH 0.5	(Lev & Spicer, 1964)	Very sulphated GCs
AB pH 1 0	(Lev & Spicer, 1964)	GCs with O- sulphate esters
AB pH 2. 5	(Lev & Spicer, 1964)	Acidic GCs with carboxylated and sulphated esters
AF	(Gomori, 1952)	GCs with sulphate
AF/ AB pH 2.5	(Spicer & Mayer, 1960)	To separate sulphated GCs from acidic GCs

Results

It was observed that mucous cells were generally located in gill arch and primary filament epithelium and mucous cells of secondary lamellae epithelium did not contain glycoconjugates (Table 2).

Mucous cells in all areas showed a moderate to strong PAS positive reaction (Figure 1). It was observed that a few mucous cells stained purple by showing the presence of a mixture of neutral and acidic glycoconjugates but most mucous cells contained AB pH 2.5 positive material in gill arch and primary filament epithelium.

The histochemical technique (KOH/ PAS) showed that mucous cells of both gill arch and primary filament epithelium (Figure 2) contained sialic acid residues. Moreover, this method resulted in a moderate staining.

Utilizing AB at different pH values, a sequence of procedures displayed that the, mucous cells on the gill sections showed a strong positive reaction to AB pH 2.5 (Figure 3), but they did not contain AB pH 0.5 and pH 1.0 positive glycoconjugates.

Glycoconjugates with sulphate were present in gill arch and primary filament epithelium. A few numbers of mucous cells in two regions gave a weak reactivity to AF

staining method (Figure 4). AF/ AB pH 2.5 application for separating sulphated from carboxylated glycoconjugates method showed that numerous mucous cells were positive to AB pH 2.5 staining, but some contained a mixture of sulphated and acidic glycoconjugates in gill arch and primary filament epithelium (Figure 5).

Table 2. Histochemical staining properties in the mucous cells of *Aphanius sureyanus* gills. Staining intensity is indicated by; +++, strong; ++, moderate; +, weak, -, negative.

Procedure	Staining reaction		
	Primary filament	Secondary lamellae	Gill arch
PAS	++/+++	-	++/+++
PAS/ AB pH 2.5	AB pH 2.5 +++ Dominance	-	AB pH 2.5 +++ dominance
KOH/ PAS	++	-	++
AB pH 0.5	-	-	-
AB pH 1.0	-	-	-
AB pH 2.5	+++	-	+++
AF	+	-	+
AF/ AB pH 2.5	AB pH 2.5 +++ Dominance	-	AB pH 2.5 +++ dominance

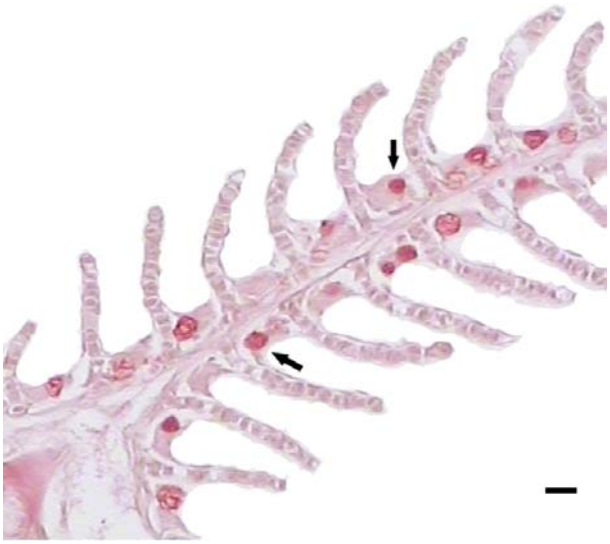

Figure 1. PAS positive cells (arrows) in primary filament. PAS method. 50 µm.

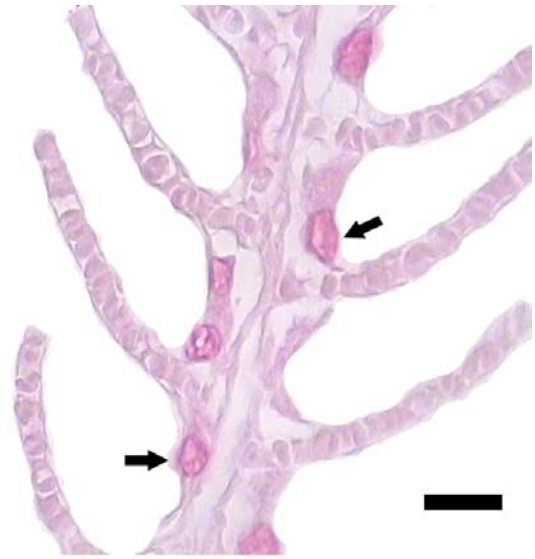

Figure 2. Mucous cells of primary filament (arrows) showing a positive reaction to KOH/ PAS method. 50 µm.

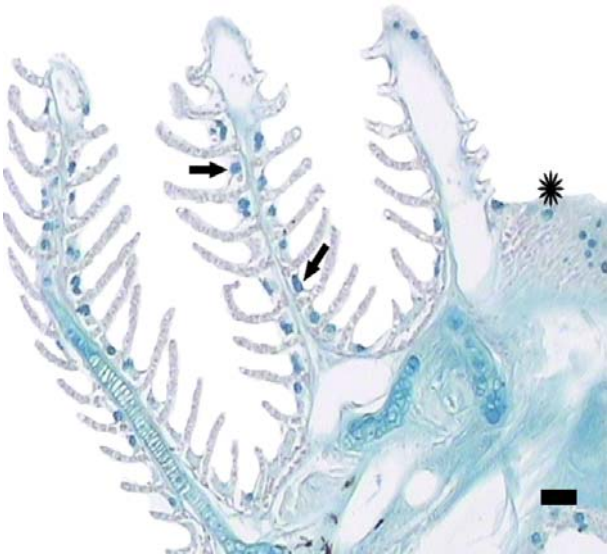

Figure 3. AB pH 2.5 positive mucous cells of gill arch (asterisk) and primary filament (arrows). 50 µm.

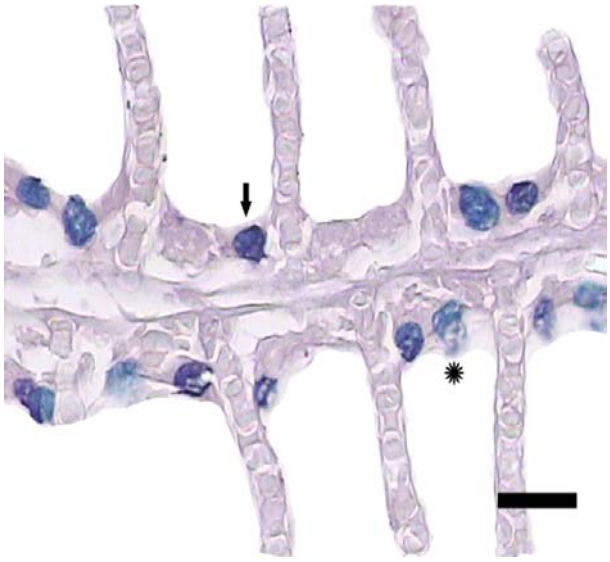

Figure 5. Mucous cell (arrow) in the primary filament shows a mixed reaction to AF/ AB pH 2.5 method. AB pH 2.5 positive cell (asteriks) in the primary filament. AF/ AB pH 2.5 method. 50 µm.

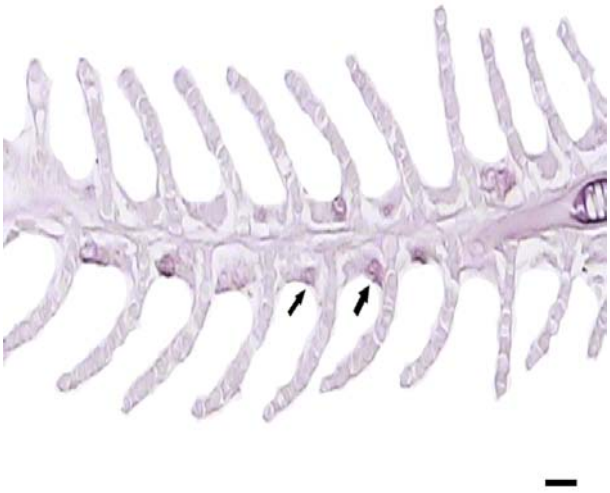

Figure 4. AF positive mucous cells of (arrows) primary filament. 50 µm.

Discussion

According to results above, it could be said that none of Ciwe has toxic and mutagenetic effects time test metho Studies with different fish species showed that mucous cells in secondary lamellae contained different glycoconjugates (Calabró et al., 2005; Díaz et al., 2005a; Çınar et al., 2008). But in the present study, mucous cells were identified to be broadly

distributed in gill arch and primary filament epithelium. Mucous cell density and mucus production generally vary among different fish species and different environmental conditions (Laurent, 1984).

The fact that mucous cells in the *Aphanius sureyanus* gills reacting positively to PAS indicated that cells contained neutral glycoconjugates. This has been demonstrated in other fish species (*Coelorhynchus coelorhynchus, Cyprinus carpio, Micropogonias furnieri* and *Cynoscion guatucupa, Clarias gariepinus, Oreochromis niloticus*) in which cells reacted in the same way with PAS method (Díaz et al., 2001; Zayed & Mohamed, 2004; Calabró et al., 2005; Díaz et al., 2005a; Çınar et al., 2008).

The majority of the mucous cells of *Aphanius sureyanus* were stained blue by the combined PAS/ AB pH 2.5 staining method, as similar to findings observed in *Solea senegalensis* (Arellano et al., 2004) and *Cyprinus carpio* (Çınar et al., 2008) In contrast to these findings, authors (Díaz et al., 2001) have detected that the most mucous cells stained purple, while some mucous cells stained blue.

The histochemical properties of the contents of the mucous cells revealed by the KOH/ PAS staining method confirmed that the presence of sialic acid residues. Authors (Díaz et al., 2005b; Çınar et al., 2008) aggreed that mucous cells of different fish species contained sialic acid residues.

Mucous cells in *Cyprinus carpio* (Çınar et al., 2008) reacted weakly to AB pH 0.5 and mucous cells displayed a moderate positive reaction to AB pH 0.5 in *Cynoscion guatucupa* (Díaz et al., 2005a) but the present result revealed that the mucous cells of *Aphanius sureyanus* did not contain glycoconjugates with very sulphated. The studies of authors (Díaz et al., 2005a; Çınar et al., 2008) have shown that the mucous cells contained glycoconjugates with O- sulphate esters. But mucous cells on gill sections were unreactive with AB pH 1.0 method in this study. According to the present study, the mucous cells of *Aphanius sureyanus* reacted positively to AB pH 2.5 method indicating their contents of acidic glycoconjugates. This result is in accordance with authors (Calabró et al., 2005; Díaz et al., 2005a; Çınar et al., 2008).

The mucous cells detected in the *Aphanius sureyanus* contain glycoconjugates with sulphate. Similar results have been obtained in the mucous cells of *Cyprinus carpio* (Çınar et al., 2008). In this study, most mucous cells stained blue by the combined AF/ AB pH 2.5 sequence indicating that the AB pH 2.5 positive dominance while most mucous cells in *Cyprinus carpio* (Çınar et al., 2008) showed AF positive material dominance.

In conclusion, gills may be one of the most frequently affected organs because they are

exposed directly to the water. Therefore, the data obtained from healthy *Aphanius sureyanus* in this study could serve as a basis for further histopathological and aquatic toxicological studies and to get information for the culture of this endemic species.

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