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Histochemical Analysis of Glycoconjugates in the Mucous Cells in the Gill of Rainbow trout (Oncorhynchus mykiss,W., 1792)

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Abstract: In the present observation, histochemical methods were utilized to understand the structural organization and to categorize the diverse classes of glycoproteins eleborated by the epithelium in various regions of gills of a Rainbow trout, Oncorhynchus mykiss. Samples were taken from the gills. The samples were routinely processed and embedded in parafin. Histochemical techniques were performed for the density and differentiation of carbohydrate moieties. The presence of neutral (PAS), acidic (AB pH 2.5) and sulphate (AF), neutral and/or acid rich (PAS/AB pH 2.5), strong sulphated (AF/AB pH 2.5) of glycoconjugates were identified by means of conventional histochemistry in all regions.

Key words: Glycoconjugates, gill, *Oncorhynchus mykiss,* rainbow trout.

Gökkuşağı Alabalığı (*Oncorhynchus mykiss,*W., 1792) Solungaç Mukus Hücrelerindeki Glikokonjugatların Histokimyasal Analizi

Özet: Bu çalışmada histokimyasal tekniklerle gökkuşağı alabalığı *(Oncorhynchus mykiss)* solungaç dokusunun farklı bölgelerindeki glikokonjugatların belirlenmesi amaçlanmıştır. Solungaçlardan örnek alımı gerçekleştirildi. Örnekler rutin histolojik doku takibinden geçirilip parafinde bloklandı. Farklı karbonhidrat gruplarının yoğunluğunu belirlemek için histokimyasal boyama yöntemleri uygulandı. Yapılan histokimyasal gözlemlerde solungaç bölgelerinin tümünde nötral (PAS), asidik (AB pH 2.5), sulfatlı (AF), nötral yada asidik (PAS/AB pH 2.5), güçlü sülfatlı (AF/AB pH 2.5) gilikoproteinler belirlendi.

***Anahtar Kelimeler*:** Glikokonjugat, gökkuşağı alabalığı, *Oncorhynchus mykiss,* solungaç.

1. Introduction

Rainbow trout (*Oncorhynchus mykiss*) are included in the group of carnivorous fish. Living in natural waters of trout always feed worms, filies, zooplankton and other fish [1]. Gills epithelium takes form the first barier between the external and the internal ambient and is a composite tissue [2]. In teleosts, the supporting structure of gills is the branchial arch; the next level of organization is the primary lamella that finally supports the terminal secondary lamella [3]. Gills include diverse kinds of epithelia. There are different cell types such as mucous cell and chloride cell in the fish gills. They are responsible for many functions [4]. Gas exchange is provided by the gills which are the main sites, almost all fishes. The feeding habit effects the gill dimensions and organization of its filaments [5]. The morphology of gill cells and the mucosubstances and kind of mucuos cells in the gill epithelium have been described [6].

Mucus is secreted by the fish gills. The mucus protects the animal but it is also involved in repiration, ion and osmoregulation. The gills mucous is an important factor in disease

resistance. Mucins, the main constituents of mucus are high molecular weight glycoproteins [7]. Mucins compound a peptide backbone with oligosaccharide side chains (attached in **O**-glycosidic linkages through **N**-acetylgalactosamine to threonine or serine residues) [8]. Members of the mucin family can differ substantially, in size. Some are small, containing a few hundred amino acid residues, whereas others contain a few thousands of residues and are amid the largest known proteins [9].

In the present observation, histochemical methods were utilized to understand the structural organization and to categorize the diverse classes of glycoproteins eleborated by the epithelium in various regions of gills of a Rainbow trout, *Oncorhynchus mykiss*.

2. Material and Methods

In this study, we chose the carnivores fish species, rainbow trout (*Oncorhynchus mykiss*). Five adult rainbow trout fishes, length between 25 and 30 cm and weight between 150 and 200 g, were collected. Samples were taken from the gills. Gill tissues were fixed for 24 h in 10 % buffered formalin. After dehydration by passing tissues through a series of alcohol solutions, the samples were vacuum embedded in paraffin. Sections (6–7 μ) were stained for general morphological purposes with haematoxylin and eosin (H and E) [10]. Histochemical techniques were performed for the localization and differentiation of carbohydrate moieties (Table 1).

Table 1: Performed the histochemical techniques in the gill tissues of Rainbow trout (*Oncorhynchus mykiss*).

Procedures	**References**
1. PAS	Mc Manus (1948)
GCs with oxidizable vicinal diols and/or glycogen	
2. PAS/AB pH 2.5	Mowry (1956)
Neutral and/or acid rich GCs	
3. AB pH 2.5	Lev and Spicer (1964)
GCs with carboxyl groups (sialic acid or uranic acid) and/or with sulphate esters	
4. AF	Gomari (1952)
GCs with sulphate	
5. AF/AB pH 2.5	Spicer and Meyer (1960)
Strong sulphated GCs	

AB, Alcian blue; PAS, periodic acid/Schiff; AF, Aldehyde fuchsin; GCs, glycoconjugates.

3. Results

Gill arch structure is resemble that of other teleosts. Two epithelial types are distinctly classified in the gills of Rainbow trout (*Oncorhynchus mykiss*): primary and secondary lamellar. The mucous cells of primary and secondary lamellae also gill arch were observed with histochemistry procedures.

The presence of neutral (PAS), acidic (AB pH 2.5) and sulphate (AF), neutral and/or acid rich (PAS/AB pH 2.5), strong sulphated (AF/AB pH 2.5) of glycoconjugates were identified by means of conventional histochemistry in all regions (Table 2).

Table 2. Histochemical reactions of glycoconjugates in the gills of Rainbow trout (*Oncorhynchus mykiss*), Staining intensity is indicated by; +++, strong; ++, moderate; +, weak, +/-, rather weak.

Procedures	Gill arch	Primer lamellae	Sekonder lamellae
PAS	+++	**+++**	+++
PAS/AB pH 2.5	**+++ (PAS dominance)**	**+++ (PAS dominance)**	**+++ (PAS dominance)**
AB pH 2.5	++	+	++
AF	+	+/-	+
AF/ AB pH 2.5	**++ (AB dominance)**	**+ (AB dominance)**	**++ (AB dominance)**

PAS staining (Figure 1 a, b) showed that the reaction stronger than AB pH 2.5 (Figure 1 c) and AF (Figure 1 d) stainings. The same density of neutral (PAS) glycoconjugates were investigated in primary, secondary lamellae and gill arch. Acidic (AB pH 2.5) glycoconjugates were moderate density but the density decreased in pimer lamellae. A single goblet cell contained either neutral or acid glycoconjugates alone or in combination. Mucous cells containing PAS were dominance in PAS/AB pH 2.5 application (Figure 1 e).

A procedure sequence using aldehit fuchsin showed the presence of sulphate glycoconjugates. In all regions the mucous cells included glycoconjugates with sulphate weakly; in the primary lamallae mucous cells this glycoconjugates occured rather weak. For separating sulphated glycoconjugates from carboxylated those when the AF/AB pH 2.5 stain (Figure 1 f) was performed, most mucous cells were moderately AF/AB pH 2.5 combination-positive, although some mucous cells stained blue (AB).

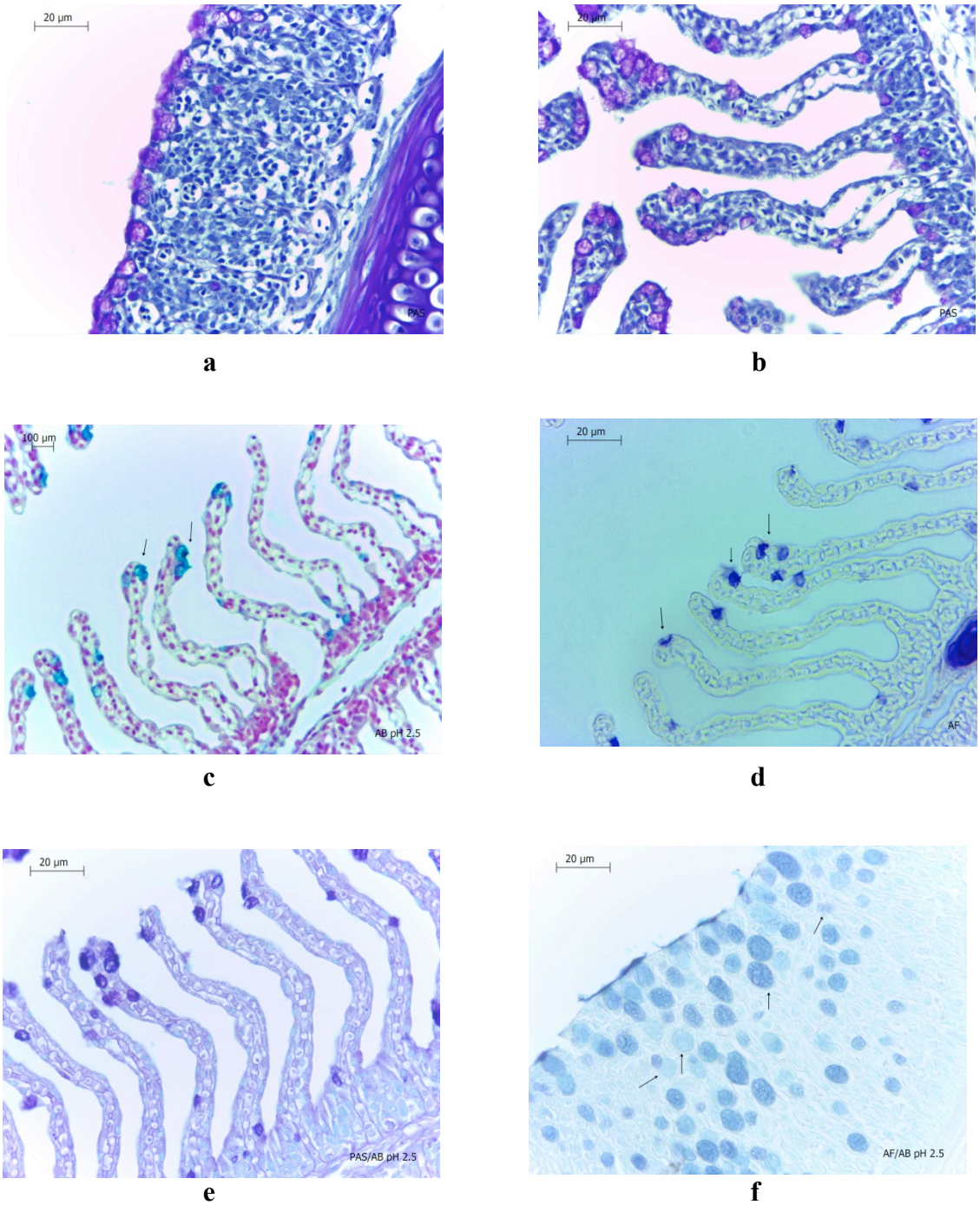

Figure 1. a) PAS positive cells in gill arch. **b)** PAS positive cells in pimary and secondary lamellae. **c)** AB pH 2.5 positive cells in pimary and secondary lamellae. **d)** AF positive cells in pimary and secondary lamellae. **e)** PAS/AB pH 2.5 positive cells in pimary and secondary lamellae. **f)** AF/AB pH 2.5 positive cells in gill arch.

4. Discussion

In rainbow trout (*Oncorhynchus mykiss*), as in other teleosts, the gill are located on either side at the boundry of the pharynx and the opercular chamber. We have also shown that although the mucous cell characteristics can be very similar in the same fish family [2].

The gill structure in *P. antalyae* passessed four arches of gills and each gill arch consisted of a double row of well- developed gill filaments as do almost bony fishes [4]. The first four pairs of gill arches are pulmonary function. Each gill arch relocates two lines of gill rakers on pharyngeal border and two lines of gill filaments on opercular border [2].

In some fish species, mucous cells exhibited different histochemical characters by positioning the gill [6]. In rainbow trout (*Oncorhynchus mykiss*) the histochemical method exposed that mucous cells from gill filaments or secondary lamellae have the same histochemical characters.

The mucous cells of primary as in secondary lamellae were researched by means of familiar histochemistry. Mucuous cells strong positive reaction occurred with all used reactions [3]. Similar findings were described in rainbow trout after conventional histochemistry.

The histochemical tecniques show that mucous cells of both primary and secondary lamellae contain sialic acids and some their side-chain variants and neutral glycoconjugates [3]. Glycoconjugates in mucus are majör determinants of mucus viscosity and the acidic glycoconjugates of the sialylated type are indicative of a rather fluid mucosal secretion [11].

Similar to study, in *Micropogonias furnieri* [12]. *Cirrhinus mrigala* [2] 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* [6]. *Rita rita* [13], *Salmo salar* [14], *Cirrhinus mrigala* [2]. Similar results on rainbow trout (*Oncorhynchus mykiss*), showed that the production of acidic GPs, mainly sulphated GPs, predominates in their mucous cells.

In *Cyprinus carpio* [15], *Pseudophoxinus antalyae* [4], AF positive cells were observed in gill arch, primary and secondary lamellae. This sutdy, in all regions the mucous cells included glycoconjugates with sulphate weakly; in the primary lamallae mucous cells this glycoconjugates occured rather weak. Diler and Çınar [16], were reported that a few numbers of mucous cells in gill arch and primary filament gave a weak reactivity to AF satining method in *Aphanius anatoliae sureyanus*. In rainbow trout (*Oncorhynchus mykiss*) most mucous cells were moderately AF/AB pH 2.5 combination-positive, although some mucous cells stained blue (AB). Similar results in *Dicentrarchus labrax* [16], acidic glycoconjugates were dominant in the mixture of sulphated and acidic glycoconjugates when treated with AF/AB pH 2.5.

Although the implication of this is not clear, the present study could be a good model to evaluated, by functional approaches, the biology of fish gill mucous cells.

5. References

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