Tissue and Cell 44 (2012) 409–417

Contents lists available at SciVerse ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

# Histochemical analysis of glycoproteins in the secretory cells in the epidermis of the head skin of Indian Major Carp, *Labeo rohita*

A.K. Rai, N. Srivastava, U. Kumari, S. Mittal*, A.K. Mittal[1,2]

*Skin Physiology Laboratory, Centre of Advanced Study, Department of Zoology, Banaras Hindu University, Varanasi-221005, India*

ARTICLE INFO

*Article history:*
Received 2 June 2012
Received in revised form 6 August 2012
Accepted 7 August 2012
Available online 30 August 2012

*Keywords:*
Histology
Histochemistry
Glycoproteins
Epidermis
*Labeo rohita*

ABSTRACT

A series of histochemical procedures were employed to localise and characterise glycoprotein (GP) classes produced by the epithelial cells, the type A and the type B mucous goblet cells (MGCs) and the club cells in the epidermis of *Labeo rohita*. The epithelial cells secreted GPs with oxidizable vicinal diols and GPs with sialic acid residues without O-acyl substitution in low concentrations. The type A MGCs and the type B MGCs, in contrast, produced these GPs in high concentrations. Further, these MGCs produced GPs with O-sulphate esters as well. GPs with O-sulphate esters were produced in high concentration by the type A MGCs and in low concentration by the type B MGCs. The club cells produced GPs with oxidizable vicinal diols in trace amounts. Production of more than one type of GPs suggested a basis for functional discrimination in their role in the mucous secretions at the skin surface. This is considered an adaptation to environment inhabited by the fish and is discussed in relation to their role in lubrication, protection and inhibition of the invasion and proliferation of pathogenic micro-organisms.



## 1. Introduction

The skin and mucous layer of fish are of great survival value as they provide the first line of defence against infection by potential environmental pathogens (Ingram, 1980). The epidermis of the skin is equipped with different types of cells which are involved in the secretion of surface mucus – a gel-like, slippery, gluey, viscous substance which is known to contain various biologically active macromolecules, predominantly glycoproteins (GPs). These GPs form a water insoluble layer of adherent mucus on epithelial cells, and have been implicated in many important biological functions such as lubrication, ionic regulation, and protection against microbes, viruses, or proteolytic degradation (Shephard, 1994; Diaz et al., 2001; Raj et al., 2011).

Our current knowledge on the histochemical analysis of GPs in the secretory cells of the skin of fish is limited. Most workers described GPs mainly in one type of secretory cells, the mucous goblet cells (MGCs), using the methods based upon selective localisation of oxidizable vicinal diols by their reactivity with periodic acid Schiff's procedures and the affinity of the anionic groups towards cationic dyes (Mittal and Banerjee, 1975; Sarasquete et al., 2001; Park, 2002; Park et al., 2001, 2003; Arellano et al., 2004; Jeong and Jo, 2007; Garg et al., 2010; Reddy and Benarjee, 2011). These conventional methods, however, fail to identify different classes of GPs.

The present work was therefore, undertaken with the aim to analyse glycoproteins histochemically, in the secretory cells of the epidermis of Indian Major Carp, *L. rohita* (common name – Rohu, Taxonomic Serial Number 163681; Order – Cypriniformes; Family – Cyprinidae). The fish is considered valuable source of food and is cultured extensively in India. A series of histochemical methods in addition to the conventional methods based on periodic acid Schiff's and alcian blue procedures, were employed for the analysis of different classes of GPs, which include GPs with oxidizable vicinal diols, GPs with O-acyl sugars, GPs with O-sulphate esters and GPs with sialic acid residue with or without side chain O-acyl variants. Characterisation of GPs in secretory cells of the epidermis may promote better understanding of the physiological adjustment of skin in the fish in relation to its habits and habitat.

## 2. Material and methods

Live specimens of *L. rohita* (mean ± S.D. standard length, $L_S$, 105 ± 6 mm; weight 26.17 ± 4.15 g; $n = 15$) were collected from local ponds at Varanasi, Uttar Pradesh. The fishes were maintained

* Corresponding author. Tel.: +91 542 6702504; fax: +91 542 2368174.
*E-mail addresses:* drsmittal73@gmail.com (S. Mittal), profakmittal@gmail.com (A.K. Mittal).
[1] Prof. Ajay Kumar Mittal, Retired Professor of Zoology, Banaras Hindu University.
[2] Present address: 9, Mani Nagar, Kandawa, Near Chitaipur Crossing, Varanasi-221106, India. Tel.: +91 9453885563.

0040-8166/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tice.2012.08.004

**Table 1**
Summary of single-dye histochemical methods employed for the visualisation of glycoproteins (GPs) in the type A and type B mucous goblet cells, the superficial layer (SL), the middle layer (ML) and the basal layer (BL) epithelial cells and the club cells in the epidermis of the head skin of the fish, *Labeo rohita*.

| Histochemical methods | Reactions | Interpretation of reactions | Mucous goblet cells | | Epithelial cells | | Club cells |
|---|---|---|---|---|---|---|---|
| | | | Type A | Type B | SL | ML and BL | |
| 1. WO/S | M | Free aldehydes | 0 | 0 | 0 | 0 | 0 |
| 2. PAS | M | GPs with oxidizable vicinal diols and/or glycogen | 4M | 4M | 2M | 1-2M | 1M |
| 3. Acetylation/PAS | 0(M) | Same as '2' | 0 | 0 | 0 | 0 | 0 |
| 4. Acetylation/deacetylation/PAS | M | Same as '2' | 3M | 3M | 1M | 1M | 0 |
| 5. α - Amylase/PAS | M<br>0(M) | GPs with oxidizable vicinal diols<br>Glycogen | 3M | 3M | 1M | 1M | 0 |
| 6. AB2.5 | T | GPs with carboxyl groups and/or GPs with O-sulphate esters | 4T | 4T | 1T | 0 | 0 |
| 7. AM/AB2.5 | 0(T) | Same as '6' | 0 | 0 | 0 | 0 | 0 |
| 8. AM/KOH/AB2.5 | T<br>0(T) | GPs with carboxyl groups<br>GPs with O-sulphate esters | 3T | 3T | 1T | 0 | 0 |
| 9. AB1.0 | T | GPs with O-sulphate esters | 3T | 1T | 0 | 0 | 0 |
| 10. AM/AB1.0 | 0(T) | Same as '9' | 0 | 0 | 0 | 0 | 0 |
| 11. AM/KOH/AB1.0 | 0(T) | Same as '9' | 0 | 0 | 0 | 0 | 0 |
| 12. LID | PBr | GPs with carboxyl groups and/or GPs with O-sulphate esters | 4PBr | 4PBr | 1PBr | 0 | 0 |
| 13. LD | 0(PBr) | Same as '12' | 0 | 0 | 0 | 0 | 0 |
| 14. HID | PBr | GPs with O-sulphate esters | 3PBr | 1PBr | 0 | 0 | 0 |
| 15. HD | 0(PBr) | Same as '14' | 0 | 0 | 0 | 0 | 0 |

Symbols: AB2.5, alcian blue at pH 2.5; AB1.0, alcian blue at pH 1.0; AM, active methylation; GPs, glycoproteins; HD, high diamine; HID, high iron diamine; KOH, saponification; LD, low diamine; LID, low iron diamine; M, magenta; PAS, periodic acid/Schiff; PBr, purplish brown; S, Schiff; T, turquoise; WO, without oxidation; 0, negative reaction; 1–4, feeble to very strong reaction.

**Table 2**
Summary of double or triple-dye histochemical methods employed for the visualisation of GPs in the type A and type B mucous goblet cells, the superficial layer (SL), the middle layer (ML) and the basal layer (BL) epithelial cells, and the club cells in the epidermis of the head skin of the fish, *Labeo rohita*.

| Histochemical methods | Reactions | Interpretation of reactions | Mucous goblet cells | | Epithelial cells | | Club cells |
|---|---|---|---|---|---|---|---|
| | | | Type A | Type B | SL | ML and BL | |
| 16. AB2.5/PAS | M<br>T<br>P | GPs with oxidizable vicinal diols and/or glycogen<br>GPs with carboxyl groups and/or with O-sulphate esters<br>GPs with oxidizable vicinal diols and/or glycogen and GPs with carboxyl groups and/or with O-sulphate esters | 4Bl | 4Bl | 2P | 1M | 0 |
| 17. AM/AB2.5/PAS | M<br>0(T) | GPs with oxidizable vicinal diols and/or glycogen<br>GPs with carboxyl groups and/or with O-sulphate esters | 3M | 3M | 1M | 0–1M | 0 |
| 18. AM/KOH/AB2.5/PAS | M<br>T<br>0(T) | GPs with oxidizable vicinal diols and/or glycogen<br>GPs with carboxyl groups<br>GPs with O-sulphate esters | 3Bl | 3Bl | 1P | 0–1M | 0 |
| 19. AB1.0/PAS | M<br>T<br>P | GPs with oxidizable vicinal diols and/or glycogen<br>GPs with O-sulphate esters<br>GPs with oxidizable vicinal diols and/or with O-sulphate esters | 4Bl/P | 4MP | 1M | 1M | 0 |
| 20. AM/AB1.0/PAS | M<br>0(T) | GPs with oxidizable vicinal diols and/or glycogen<br>GPs with O-sulphate esters | 2M | 2M | 1M | 0–1M | 0 |
| 21. AM/KOH/AB1.0/PAS | M<br>0(T) | GPs with oxidizable vicinal diols and/or glycogen<br>GPs with O-sulphate esters | 2M | 2M | 1M | 0–1M | 0 |
| 22. HID/PAS | M<br>PBr, B | GPs with oxidizable vicinal diols and/or glycogen<br>GPs with O-sulphate esters | 4PBrM | 4MPBr | 1M | 1M | 1M |
| 23. HID/AB2.5 | T<br>PBr, B | GPs with carboxyl groups<br>GPs with O-sulphate esters | 3PBrT | 4T | 1T | 0 | 0 |
| 24. HID/AB2.5/PAS | M<br>T<br>PBr, B<br>P, Bl<br>PBrM | GPs with oxidizable vicinal diols and/or glycogen<br>GPs with carboxyl groups<br>GPs with O-sulphate esters<br>GPs with oxidizable vicinal diols and/or glycogen and GPs with carboxyl groups<br>GPs with oxidizable vicinal diols and/or glycogen and GPs with O-sulphate esters | 4PBrBl | 4Bl | 1Bl | 1M | 1M |

Symbols: AB2.5, alcian blue at pH 2.5; AB1.0, alcian blue at pH 1.0; AM, active methylation; B, black; Bl, blue; GPs, glycoproteins; HID, high iron diamine; KOH, saponification; M, magenta; MP, magenta with purple tinge; MPBr, magenta with purplish brown tinge; P, purple; PAS, periodic acid/Schiff; PBr, purplish brown; PBrBl, purplish brown with blue tinge; PBrM, purplish brown with magenta tinge; PBrT, purplish brown with turquoise tinge; T, turquoise. 0, negative reaction; 1–4, feeble to very strong reaction.