Histochemical technique for the detection of chloride cells in fish

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1. Introduction

Fish gills play vital roles, as they are the main site of gaseous exchanges, and are involved in osmoregulation, acid–base balance, excretion of nitrogen compounds, and taste (Machado, 1999). According to Karnaky et al. (1976a) it is well known that the most important osmoregulatory organ in fish is the gill, which is characterized by a relatively slow absorption of Na and Cl in low salinity environments, fresh water, and a relatively rapid secretion of Na and Cl in high salinity environments, seawater.

The gill epithelium is stratified and consists of several cell types, including pavement cells, mucus cells, and chloride cells, in addition to taste buds and undifferentiated supporting cells (Machado, 1999; Garcia-Santos et al., 2007). According to Perry (1997), chloride cells were first identified by Keys and Wilmer (1932), while describing mitochondria-rich cells responsible for the secretion of Cl– in seawater adapted teleosts. Chloride cells are large, scattered on the lamellar surface, rich in mitochondria and participate in the process of active transport of ions (Machado, 1999; Perry, 1997). According to Evans (2008), since early physiological studies, several groups have demonstrated that chloride cells of teleosts express Na–K-activated ATPase.

Because of the large number of mitochondria present, they are also known as mitochondria-rich cells or MRC. According to Perry (1997), studies suggest that chloride cells are the site of active ion transporting processes involved in ionic regulation in marine and freshwater fishes. This regulation, especially in marine fish, can account for a substantial portion of the energy budget, due to the hydrolysis of ATP by the various ion-transport ATPases. These include Na+/K+-ATPase, high-affinity Ca2+-ATPase, and perhaps the poorly defined Cl–/HCO3––ATPase. The chloride cells are probably an important site of NaCl uptake from fresh water adapted fish and therefore chloride cells proliferation in low-[NaCl] water may be an important mechanism contributing to the enhancement of gill NaCl transport capacity. According to Karnaky et al. (1976b) the chloride cells are larger and/or more numerous in sea water than in fresh water fish and the largest cells of all occur in fish adapted to environments more concentrated than sea water.

Under unfavorable ionic conditions or in contact with toxic agents, chloride cells proliferate in the filaments and on the lamellar surface of the gill (Perry, 1997) and according to Perry and Laurent (1989) the most important studies made in this area are related with the physiological and biochemical adaptation adjustments associated with euryhalinity. According to Perry and Walsh (1989), chloride cells have higher metabolic rates than those of other gill cells and the metabolic rate of the remaining of the gill directly affects the population of chloride cells, which may induce their proliferation.
According to Garcia-Santos et al. (2007), histological changes of the gill are recognized as a fast and valid method to determine the damage caused by the exposure to different pollutants in fish, and chloride cells are important tools in these studies. With this purpose, this study aimed at developing a histochemical protocol specific to detect these cells.

2. Materials and methods

Specimens of *Prochilodus lineatus* were collected at CEPTA – Institute Chico Mendes, Pirassununga, São Paulo State, Brazil and maintained in water tanks of 500 l in a constant temperature of 25 °C. For histological analysis of the gill, fish were anesthetized with 0.01% benzocaine and the second gill arch was removed. Gill samples were fixed with neutral formol for 24 h, buffered with sodium phosphate solution for 3 h, dehydrated in a series of increasing concentrations of ethanol, and embedded in historesin. The material was sectioned at 6 μm and stained using the Von Kossa method for the identification of cells with calcium, and counterstained with Hematoxylin; 2.5 μm sections were also obtained for the PAS reaction (Periodic Acid Schiff) (Paulete and Beçak, 1976), for the identification of mucus cells. Slides were observed and photographed using a photonic microscope Leica CM 2000.

The Von Kossa method was modified from the original from Junqueira and Junqueira (1983), and its steps are: the material were stained with silver nitrate for 20 min, washed in water, immersed in Dektol (D-72) for 2 min, immersed in thiosulphate 5% for 30 min, washed in water and stained with Hematoxylin for 2 min.

3. Results

The entire length of the primary lamella was lined by epithelial cells, which in some cases, have the cytoplasm slightly stained with Hematoxylin (Fig. 1A). Only some cells located in the
secondary lamellae (Fig. 1B) and in between them (Fig. 1C) exhibited dark granules throughout the cytoplasm as a result of the Von Kossa staining for calcium. These granules are typical of the Von Kossa method and occur in acidic pH, which was confirmed by the cytoplasm of cells slightly stained purple with Hematoxylin. These cells were identified as chloride cells (Fig. 2A and B), as they coincide with those described by other authors. The mucus cells are in between chloride cells and are strongly PAS stained (Fig. 1D).

4. Discussion

According to Bindon et al. (1994) one of the most often studied and physiologically significant of such morphological adjustments involves variations in the number and/or size of the presumptive ion-regulating cell, the chloride cell. In freshwater teleosts, these cells are thought to perform an essential role in maintaining a balanced internal ionic environment by absorbing Na⁺ and Cl⁻ from the dilute external medium. Consequently, proliferation of branchial chloride cell is utilized by fish as a mechanism for enhancing the ion-transporting capacity of the gills in challenging environments such as ion-poor water and it was recently demonstrated that chloride cells proliferation, induced by chronic treatment of trout with cortisol and ovine growth hormone, caused an increase in the blood-to-water diffusion distance across the lamellar epithelium without a concurrent adjustment (increase) in lamellar surface area. From this observation, it was predicted that gas transfer would be impaired in fish displaying excessive chloride cells proliferation and the accompanying increase in the blood-to-water diffusion distance. According to Perry and Laurent (1989) numerous studies have addressed the physiological and biochemical adaptational adjustments associated with euryhalinity and its relation with chloride cells. These papers show how the chloride cells are important for the fish physiology and how the studies of its proliferation are fundamental to elucidate the impacts of environmental changes, but they do not show a clear method to find and analyze these cells histologically.

According to Perry (1997), chloride cells are responsible for calcium uptake and differ from other epithelial cells, as they have calcium channels in the apical region; with the activity of Ca²⁺-ATPase exceeding the Ca⁺⁺-transporting requirements. Thus, chloride cells have significant quantities of calcium that are not found in other cells of the gill epithelium. This was confirmed by the Von Kossa staining protocol. Several studies aimed at the identification of these cells, such as Mazou et al. (2002), Silva et al. (2003), García-Santos et al. (2007), Motter et al. (2004) and Breseghelo et al. (2004), who used staining protocols with Hematoxylin and Eosin or Toluidine Blue for the identification of these cells, based on the acidic characteristic of chloride cells. However, as demonstrated in our study and according to Perry (1997), epithelial cells in general may have an altered metabolism due to several factors, making them acidic and with similar staining patterns to that of chloride cells, thus stained with basic dyes. The protocol used in our study is more specific and is in agreement with the physiology of these cells.

According to Breseghelo et al. (2004), mucus cells present in the fish gill epithelium are responsible for one of the protection mechanisms of the epithelium, the secretion of a layer of glycoproteins and glycolipids. The PAS reaction was extremely important, as it revealed the exact location of mucus cells, eliminating any possibility of mistaking them as chloride cells, since according to Ahuja (1970) in some species, mucus cells may have similar physiological characteristics during their early stages of development. Thus, this technique is of extreme importance for the localization of chloride cells due to its specific reaction to calcium deposits that have only been found and stored in fish gill chloride cells.

Acknowledgments

The authors are thankful to FAPESP for financial support and to CEPTA – Institute Chico Mendes for providing the specimens used in this experiment.

References


