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Alkaline and Acid Phosphatase Activities in the Mosquitofish Gill

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Alkaline (ALP) and acid (ACP) phosphatases have been widely studied among many different species, such as amphibians, birds, and mammals [1-2]. However, few studies have focused on ALP and ACP activities in fish or fish gills. ALP is an important marker for the circulatory system [1] and serves as a marker enzyme for lysosomes [3]. Our objectives in the present study were to map distribution patterns of ALP and ACP activities in mosquitofish (*Gambusia holbrooki*) gills.

Mosquitofish were sacrificed, their gills removed and separated, and fixed in ice-cold buffered paraformaldehyde for 6 hrs. A naphthol AS-MX phosphate-stabilized diazonium salt method was used for demonstrating ALP activity (fast blue BB) and ACP activity (fast red violet LB) for staining. The reaction products displayed a blue color for ALP and a red color for ACP [4]. We incubated the gills at room temperature (22-25º C) for 30 min for ALP and 25 min for ACP. For controls, L-tetramisole (50 mM) and cupric sulfate (50 mM) served as inhibitors for ALP and ACP, respectively [4]. Control specimens were first soaked in buffered inhibitor for an hour and then placed in substrate that also contained inhibitors. After incubation, specimens (experimental and controls) were placed in fixative overnight containing inhibitors to prevent enzymatic reactivation. We placed fully hydrated gills in Nanoplast^{R}, a water-soluble embedding medium to eliminate dehydration steps that extracted reaction products from cells. Sections were photographed with bright field and differential interference microscopy, adjusting white balance and gamma to improve contrast and color separations.

ALP has long been used as a circulatory marker enzyme because it specifically stains arteries, arterioles, and arterial ends of capillaries [1-5] although the enzyme's vascular role remains uncertain. Our study confirmed that ALP is a circulatory marker enzyme in mosquitofish gills because it specifically stains pillar cells and vascular elements in the primary lamellae and gill rakers (FIG. 1); it did not stain other cell types. Pillar cells fill the interiors of the secondary lamellae [6] and form tunnel walls (sinusoids) within the secondary lamellae through which blood flows (FIG. 1). Furthermore, pillar cells are contractile and can regulate the gill microcirculation by constriction (reducing blood flow) and dilation (increasing blood flow) [6]. Chloride cells did not stain for ALP. Thus, the conclusion that ALP and transport ATPases were co-localized must be viewed cautiously unless the investigators used multiple controls and inhibitors to distinguish cross-reactions between ALP and transport ATPases [7].

ACP, a marker enzyme for lysosomal activity, was distributed among all gill cell types because of the presence of lysosomes within the cells (FIG. 2). ACP staining intensities in chloride cells and mucous cells were higher than other cell types, which reflected the elevated lysosomal activities of *these* metabolically active cells [2], i.e., staining consistent with lysosome involvement in ion transport and secretion, respectively.

Cellulose gel electrophoretic data revealed three ALP isoforms and two ACP isoforms in gills as identified by band patterns, migration distances, and sensitivity to enzyme inhibitors [8]. Our

electrophoretic results are in accord with evidence obtained using other species for multiple gill ALP isoforms. In short, the histochemical study showed that, unlike the distribution of ACP, ALP is present in relatively few cell types (mainly vascular).

References

[1] M. Grim and B.M. Carlson, J. Histochem. Cytochem. 38 (1990) 1907-1912.
[2] G.M. Cohen and T. Kido, Microsc. Microanal. 8 (Suppl. 2) (2002) 1008CD-1009CD.
[3] Y. Ohsumi et al., J. Biochem. (Tokyo) 93 (1983) 547-556.
[4] D.C. Sheehan and B.B. Hrapchak, *Theory and Practice of Histotechnology*, 2nd ed. C.V. Mosby Co., St. Louis, 1980. Pgs. 106, 296, 298.
[5] H. Shimoda et al., Arch. Histol. Cytol. 60 (1997) 215-224.
[6] K.R. Olson, J. Exp. Zool. 293 (2002), 214-231
[7] L.L. Vacca, *Laboratory Manual of Histochemistry*, Raven Press, NY, 1985. Pg. 487.
[8] Jiang, C. et al., Southeastern Biologist 53 (2006) in press
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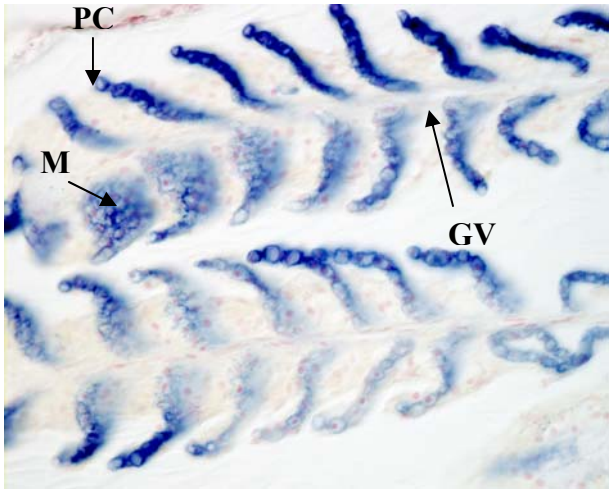

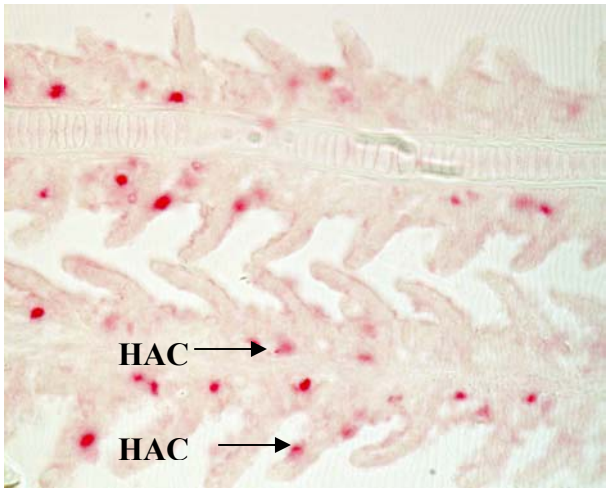

FIG. 1. ALP staining in mosquitofish gills was limited to pillar cells (PC) and gill vascular elements (GV). The mesh structure (M) of pillar cells represents the horizontal view of pillar cells. Counterstained with Safranin O.
FIG. 2. ACP staining activity in seawater-adapted gills. HAC, high ACP activity cells.

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