

**LIPOXYGENASE AS AN INDICATOR OF FISH STRESS**  
**FROM THE AQUATIC ENVIRONMENT**

Bonnie Sun Pan  
Department of Food Science  
National Taiwan Ocean University  
Keelung, Taiwan, R.O.C., 202  
phone: 886-2-2462-9781 / fax: 886-2-2462-9781 /  
email: bonnie@mail.ntou.edu.tw

**EXTENDED ABSTRACT ONLY- DO NOT CITE**

Three types of lipoxygenases (LOX) were identified in the gill of grey mullet (*Mugil cephalus*). The bacteria-infected mullet sampled from the culturing ponds that showed exophthalmia, hemorrhaged eyes, and pink-discolored gills have LOX activity 5 fold of that in the normal fish. Activity of 5-LOX was higher in the infected fish than in the normal ones. However, in both cases, 5-hydroxyeicosatetraenoic acid (5-HETE), the product of 5-LOX was not found in gill tissue.

Content of 12-HETE was lower in the infected fish, while 15-HETE was found not being affected by the infection. In addition, our in vitro studies showed that three inhibitors of 5-LOX, diphenyl sulfide, nordihydroguaiaretic acid (NDGA), and green tea extract inhibited leukotriene A<sub>4</sub> synthase at the same time, indicating that both enzyme activities are from a single protein. Therefore, 5-LOX is expected to function in fish gill in a two-step process similar to that found in the leukotriene biosynthetic pathway in mammals. It first catalyzes the dioxygenation of arachidonic acid to hydroperoxyeicosatetraenoic acid (5-HpETE), then catalyzes the dehydration of 5-HpETE to form leukotriene A<sub>4</sub>. In mammals, this unstable intermediate is further converted to leukotriene B<sub>4</sub> which has potent pro-inflammatory property.

Physiological responses of fish to environmental stress from heavy metal were

also studied. When tilapia and grey mullet were exposed to acute toxicity concentration of cadmium (Cd), accumulation of Cd increased continuously in liver with duration of exposure, while Cd in gill also increased initially then reached a plateau. No accumulation was found in the muscle or blood. Discoloration of liver, saprolegniosis, fin rot and lesions of scales appeared in these Cd-intoxicated fish. Hematological indices including leukocytes, erythrocytes, hematocrit, and hemoglobin decreased with increased Cd concentration or prolonged exposure. Mean corpuscular hemoglobin concentration (MCHC) decreased upon exposure to Cd.

In tilapia, after 19 h of exposure, activity of 5-LOX in gill increased to 23 times and 12-LOX increased to 17 times. Both of them decreased afterwards. 15-LOX decreased in activity with exposure time to 25% of the initial activity after 49 h. Similar trend of changes in LOX activities was found in grey mullet gill. Both tilapia and grey mullet gill showed that activities of 5-LOX and leukotriene A<sub>4</sub> synthase changed in a similar pattern with duration of exposure to Cd. Leukotriene metabolites were identified in gill tissues of both fishes. This evidence again indicated that 5-LOX was induced by the stress from the heavy metal pollution and produced 5 HpETE which was immediately transformed to leukotriene A<sub>4</sub>. Other metabolites were formed possibly via the same mechanism as found in human and rats.

In medicinal studies, evidence has shown that leukotrienes play a putative role in human diseases while leukotriene antagonists and lipoxygenase inhibitors especially that against 5-LOX have therapeutic potentials. Our observations showed that fish bear similar lipoxygenase and leukotriene mechanisms to those in humans. Natural antioxidants, e.g., flavonoids, inhibited lipoxygenase and improved significantly the blood fluidity and low temperature adaptation for grey mullet to maintain growth through winters.

For fishes, the environmental stress factors may come from chemical or microbial pollutants, each of which may fall under action levels. But abnormality or mortality may come as a result of the collective effects. Therefore, we propose that the overall stress developed in the fish habitat may trigger the defense mechanisms reflected by the synthesis of LOX and the accumulation of the 5-LOX catalyzed leukotriene and the further metabolized products. Our objective is to find a feasible stress indicator to serve as an “environmental watch” before outbreak of mass mortality, and human health hazard by consumption of the marine organisms under stress.