DISSERTATION ABSTRACT

Stephensen, Eiríkur, 2002. **The use of glutathione and glutathione-dependent enzymes as indicators of chemically induced oxidative stress in fish.** Department of Zoology/Zoophysiology, Göteborg University, Box 463, SE-405 30 Göteborg, Sweden.

Oxidative stress is a condition that aerobic organisms are faced with, due to their utilization of molecular oxygen in the synthesis of ATP. Partly reduced oxygen species are reactive in biological systems and can cause damage to cellular macromolecules. To keep these reactive oxygen species (ROS) in check, aerobic organisms have developed a complex antioxidant defence system, comprising both low-molecular antioxidants as well as enzymes.

Glutathione (GSH) is a central molecule in antioxidant defences. It can react directly with ROS but also as a cofactor for enzymes, such as GSH-peroxidase (GPx) and GSH-S-transferases (GST), which reduce peroxides or conjugate GSH to breakdown products of organic peroxides to facilitate their excretion. When GPx/GST reduce peroxides, GSH is oxidised to GSH-disulfide (GSSG). In order to maintain the reducing power of GSH, glutathione reductase (GR) reduces GSSG to GSH, using NADPH as a cofactor. Synthesis of GSH by glutamate cysteine ligase and GSH-synthetase can also maintain GSH levels.

Many xenobiotics can lead to increased production of ROS. Organisms exposed to such chemicals may respond by increasing their antioxidant defences. Many fish populations are exposed to urban pollution containing chemicals with the potential to increase ROS. It is of interest to develop biomarkers for oxidative stress in order to facilitate the identification of prooxidative compounds in chemical mixtures.

Fish treated in the laboratory, with known prooxidants increased their GR and GST activities as well as GSH content, which may therefore be useful as biomarkers for oxidative stress. Further examination of antioxidant responses to known prooxidants showed that nutrition status affects the responsiveness of especially GR activity and GSH content, these biomarkers being more sensitive in food-deprived fish.

Laboratory exposures of trout to chemical mixtures in the form of rubber leachates resulted in marked increases in these biomarkers, indicating the presence of prooxidative compounds. Also, CYP1A activity and CYP1A content were increased, due to the presence of planar aromatic compounds. Rubber additives include accelerators, antioxidants and antiozonants, many of which are reactive, and may lead to oxidative stress. Among those, 2-mercaptobenzothiazole and diphenylamine were identified in fish bile exposed to rubber leachates. Treatment of rainbow trout with these two compounds led to increased GR activity and GSH content.

Shorthorn sculpin from Icelandic harbours had elevated DNA adducts in liver and CYP1A content, indicating exposure to PAH compounds. Upregulation of catalase, GR, and GPx activities indicated the presence of prooxidative chemicals in the harbour pollution. However, these responses were not as marked as in the laboratory studies, possibly indicating that the use of these markers is more convenient in controlled laboratory studies were fish are treated with high doses of chemicals for a short time.

Treatment of hepatocytes with prooxidants did not result in increased GR activity. However, hepatocytes can import GSH from medium when treated with menadione.

Keywords: oxidative stress, glutathione, glutathione reductase, glutathione S-transferase, rainbow trout, pollution

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