DISSERTATION ABSTRACT

Sturve, Joachim, 2005. **Studies of DTD diaphorase in fish; characterization and biomarker application.** Department of Zoology/Zoophysiology, Göteborg University, Box 463, SE-405 30 Göteborg, Sweden.

DT diaphorase (DTD) is an antioxidant enzyme that catalyzes a two-electron reduction of quinones, thus preventing redoxcycling of these compounds. In mammals the enzyme exists in two forms, NQO1 and NQO2. Little is known about DTD properties in fish. The aim of this thesis was to biochemically characterize DTD activity in rainbow trout (*Oncorhynchus mykiss*) and to investigate if DTD responses in fish is suitable as biomarker for oxidative stress in aquatic environments.

Hepatic rainbow trout DTD was purified and partial amino acid sequencing showed homology to the NQO2 form of human DTD. Rainbow trout DTD was strongly inhibited by the anticoagulant dicoumarol, a NQO1 inhibitor, but only slightly inhibited by the NQO2 inhibitor quercetin. In addition, the results show that rainbow trout DTD can utilize both NQO1 and NQO2 specific electron donors, NAD(P)H and NRH respectively, with similar efficiency. It can be concluded that rainbow trout DTD show similarities with both forms of mammalian DTD.

Short term exposure to prooxidants resulted in increases in rainbow trout hepatic DTD activities. The induction of hepatic DTD in rainbow trout injected i.p. with β-naphthoflavone (β-NF) was demonstrated with Western blot. However, β-NF exposure did not affect hepatic DTD activities in other fish species. Also, rainbow trout display higher basal DTD activities compared to feral fish. The reason for this difference is not known. One apparent difference between the rainbow trout and the other fish species is that the rainbow trout is hatchery reared while the other species are not hatchery reared.

Immunolocalization of rainbow trout DTD showed that it is most expressed in epithelial cells in the stomach, intestine and gills and cells lining veins in the liver. This cellular localization of DTD and the fact that DTD activity is increased by prooxidant exposure suggest that DTD has a protective role in fish, as the enzyme have in mammals.

Effluents from Ryaverket sewage treatment plant (STP) caused hepatic biomarker responses in rainbow trout. Elevated EROD activities suggest the presence of pollutants such as polycyclic aromatic hydrocarbons (PAH) and elevated activities of the antioxidant enzymes glutathione reductase (GR), catalase (CAT) and DTD suggest the presence of prooxidants in the effluent. DTD activity was decreased after prolonged exposure to the STP effluent possibly due to specific DTD inhibitors such as coumarins or coumarin derivatives. Coumarin derivatives are naturally occurring in plants and occur also as anticoagulant pharmaceuticals and may end up in sewage.

Extensive dredging of the Göteborg harbour affected hepatic biomarker responses in eelpout (*Zoarces viviparus*). Results from before the commencement of the dredging showed that the harbour area is affected by pollutants such as PAH, metals and genotoxic compounds also during "normal" conditions. Dredging activities did not affect DTD activities in eelpout living in the harbour area.

Keywords: DT diaphorase, oxidative stress, fish, biomarkers
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