Rapid regulatory effect of tri-iodothyronine (T₃) on antioxidant enzyme activities in a fish *Anabas testudineus* (Bloch): Short-term *in vivo* and *in vitro* study

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The short-term action of thyroid hormone tri-iodothyronine (T₃) was studied *in vivo* and *in vitro* on antioxidant enzyme activities in a teleost *Anabas testudineus* (Bloch). T₃ injection *in vivo* (200 ng) in normal fish decreased the lipid peroxidation products and increased superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) activities after 30 min. T₃ *in vitro* (10⁻⁶ M) increased the antioxidant activities of catalase, glutathione reductase (GR), GPx and glutathione level after 15/30 min, except SOD, substantiating *in vivo* effects in normal fish. The results suggest a rapid regulatory effect of thyroid hormone *in vivo* and *in vitro*, in the removal of reactive oxygen species in *A. testudineus*.

**Keywords:** *Anabas testudineus*, antioxidant enzyme, fish, free radicals, lipid peroxidation, thyroid, tri-iodothyronine

Thyroid hormone-mediated short-term interactions on cellular metabolic machinery in ectotherms have not been a subject of intensive research, compared to those of higher vertebrates. Thyroid hormone regulates both long-term and short-term changes in the metabolism. Although the long-term effects of thyroid hormone have been extensively investigated in fish, very little is known of the short-term effect in lower vertebrates. Thyroid hormone tri-iodothyronine (T₃) is reported to directly affect the activity of enzymes of cytosol and mitochondria through extra-nuclear pathways in mammalian systems. These pathways have a faster time of onset and the duration is important in the short-term effect of thyroid hormones. The direct short-term actions constitute an interaction with plasma membrane, cytosolic enzyme and mitochondria. Studies in fish have demonstrated short-term changes in the activities of cytosolic and membrane-bound enzymes and altered mitochondrial respiration in T₃-treated specimens.

This study describes the short-term effect of T₃ on antioxidant enzyme activities in a teleost *Anabas testudineus* (Bloch). T₃ was chosen, since it is the active circulating thyroid hormone in fish. Oxygen radicals and related free radicals are generated during normal metabolism in the mitochondria of aerobic cells. If superoxide radicals are not immediately neutralized, they are converted to reactive oxygen species (ROS). ROS can attack almost all biomolecules including membrane lipids, thereby causing oxidative stress in the cells. Cells have numerous mechanisms to protect themselves from the harmful effect of free radicals. These include antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and glutathione reductase (GR). Other molecules such as glutathione, ascorbate, vitamin E and the enzymes located in the cytosol and membranes are present in the extra-cellular fluid also act as antioxidant agents. SOD effectively dismutates superoxide radicals into hydrogen peroxide, which in turn is neutralized by GPx and catalase. Thyroid hormones are known to influence cellular oxygen consumption, oxidative phosphorylation, and proton leak. The oxygen consumption by mitochondria is greatly influenced by their respiratory state. However, short-term effect of thyroid hormones on antioxidant enzymes remains to be investigated.

The rationale for the selection of the Indian Perch *A. testudineus* as a model animal is that its thyroid structure and function resemble other vertebrates. Besides, a strong antioxidant defense system is reported in the fish. In an earlier study from our laboratory, we established a delayed effect by T₃ on the regulation of antioxidant enzyme activities of *A. testudineus*. In the present study, we have examined the short-term *in vivo* and *in vitro* action of T₃ on *A. testudineus*, to confirm whether thyroid...
hormones also protect the deleterious effect of ROS generated during increased oxidative metabolism.

Materials and Methods
Experimental animal
Adult Anabas testudineus, weighing 50 ± 5 g were collected from a local supplier. They were kept in large cement tanks with flowing dechlorinated water and natural photoperiod (12 L: 12 D) for approx one month. The temperature ranged from 28 to 30°C. The fish were fed ad libitum with 40% protein feed prepared in the laboratory. The components of the feed were rice bran, tapioca, fish meal, groundnut oil cake supplemented with vitamins. One week prior to the experiments, the fish were transferred to experimental aquaria, made of glass (24”×12”×12”). They were maintained in conditions identical to those of stock tanks. Each experimental aquarium accommodated 8 fish. The hormone was administrated for short-term in vivo and in vitro studies.

Short-term in vivo study
The fish were divided into three groups of eight (n=8) each. Group 1: Control — received an intraperitoneal injection of hormone vehicle (alkaline saline). Groups 2 and 3 received 200 ng 3, 5, 3 T3 (Sigma, USA) and fish were sacrificed after 15 and 30 min to study the effect of T3 on normal specimens. All hormone injections were given between 06.00 am and 07.00 am.

Short-term in vitro study
Liver tissue from control fish was cut into very small pieces in 1 ml tissue culture medium (TCM), consisted of RPMI 1640 supplemented with 10% of 200 mM L-glutamine solution and 0.1% of 50 mg/ml gentamycin sulphate solution. Cell suspension (CS) was prepared with slight modification and was placed on ice until the tissue fragments settled down. Cell viability was checked using trypan blue and was found to be 90-95%. Three sets of experiments were prepared, each set containing CS of eight different animals. The CS was transferred to a multi-well culture plates for hormone incubation after being gently stirred using a Pasteur pipette. T3 (10^-6 M) was added to 2nd and 3rd sets of CS in the culture plate. After 15 and 30 min of incubation, the excess TCM was removed with the help of a pipette. Cells were homogenized in the respective medium using the MICCRA D8 homogenizer. The homogenate was then centrifuged, the supernatant was collected and the enzyme activities were determined. The fish of group 1 received hormone vehicle and served as control.

Biochemical analysis
The fish were anaesthetized using MS 222 (1:15,000 w/v) and the dissected liver was used for biochemical analysis. Lipid peroxidation (LPO) in the liver was determined by analyzing peroxidation products malondialdehyde (MDA) and conjugated dienes (CD). The activities of superoxide dismutase (EC. 1.15.1.1), glutathione peroxidase (EC. 1.11.1.9) and glutathione reductase (EC. 1.6.4.2), catalase (EC. 1.11.1.6) and glutathione content were also determined. Protein was estimated with bovine serum albumin (BSA) as the standard. Absorbance was measured using a UV-visible spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan).

Statistical analysis
The data were statistically analyzed by one-way-analysis of variance (ANOVA). The significant difference among means was determined by Duncan’s multiple range test at the level of P<0.05.

Results and Discussion
We report in vivo and in vitro stimulatory effect of thyroid hormone (T3) on the antioxidant defense system in a fish Anabas testudineus. Injection of T3 (200 ng/fish) in normal fish decreased MDA and CD concentrations. T3 treatment did not change SOD activity after 15 min, but at 30 min it increased. Catalase and GPx in the liver increased by T3 treatment at both time intervals, with the maximum activity at 30 min. GR activity and glutathione content decreased in T3-treated group after 15 min, with no change at 30 min (Table 1). T3 treatment in vitro (10^-6 M/fish) increased the activity of GR (30 min), catalase, GPx (15 min) with the maximum activity at 30 min, excepting GPx. SOD activity did not change, whereas glutathione content decreased in T3-treated group (Table 1).

The decrease in MDA and CD after T3 administration in control fish confirms the regulatory effect of the hormone on lipid peroxidation (LPO). This suggests that T3 decreases LPO and increases antioxidant defense mechanism in A. testudineus. A long-term effect of T3 in vivo on these processes in A. testudineus was earlier reported. The reduction in
LPO may be due to the significant change in oxidative metabolism in mitochondria by altered thyroid hormone availability in the body. The respiratory activities of liver mitochondria are decreased, following an anti-thyroid drug propyl thiouracil treatment and increased in response to T3 administration in fish. The hypothyroidism is reported to accelerate free radical production and induces changes in the antioxidant system in rats. The short-term supplementation of antioxidant nutrients improves antioxidant capacity and reduces the LPO products.

SOD is responsible for the primary defence against cellular damage caused by reactive oxygen and its progeny. The increased activity of SOD on T3 administration in normal specimens is indicative of a short-term time-dependent effect of thyroid hormone on the cellular defense mechanism in the teleost. The increased production of hydrogen peroxide as a result of the removal of superoxide by SOD by T3 treatment might have stimulated the catalase activity in this fish. Hydrogen peroxide is formed in the cellular systems, due to dismutation of superoxide radicals by the SOD and its reduction is accelerated by catalase and GPx. The hyperthyroidism induced by T3 caused an elevation of SOD activity in rat. Also, the total dismutase of the post-mitochondrial fraction was elevated after T3 treatment, suggesting that any deviation in the thyroid state of the body might cause subtle change in the ratio of SOD and catalase activity.

Glutathione was reduced, probably due to the change in the availability of NADPH by the activity of glucose-6-phosphate dehydrogenase of the pentose phosphate pathway in vivo condition, since GR activity is dependent on the availability of these reducing equivalents. The accelerated production of H2O2 by T3 in vivo increased the GPx activity, indicating an increased oxidative metabolism. The substantial increase of catalase activity in vivo and in vitro by T3 may...
establish an increased generation of H₂O₂ and its removal. Hyperthyroidism markedly increased the intracellular antioxidant enzymes i.e., catalase and GPx activities as compared to the control. In an earlier study, T₃ level was found increased in selenium-supplemented diet. Selenium is essential for the enzymatic activity of GPx. It clearly reveals T₃ administration increases GPx activity. GR is involved in the redox reaction and is responsible for the conversion of oxidized glutathione to a reduced form.

In this study, the rapid in vivo action of T₃ on antioxidant enzyme activities is substantiated by similar in vitro results in A. testudineus. In hyperthyroid rat liver, besides higher lipid peroxidation, a more active defense mechanism operates as GPx and GR activities are higher than in euthyroid rats. T₃ is capable of combating the oxidative stress by activating the antioxidant enzyme systems by removing the ROS. T₃ appears to have a dual role, as a stimulator of oxidative process and as a regulator of antioxidant enzyme activity. This establishes another example for the multi-functional role of T₃ in lower vertebrates also. However, the precise mechanism of action remains to be understood.

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