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Effect of dissolved oxygen (DO) on superoxide dismutases (SOD) activities

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ABSTRACT: Superoxide dismutases (SOD) are the antioxidant scavenging enzymes that exist in virtually all oxygen respiring organisms. SOD metabolize reactive oxygen species (ROS) into a safe product, and hence prevents cellular damage. Fishes are exposed to daily and seasonal fluctuations in both temperature and oxygen availability. A comparative study shows diversity in the response of SOD activities between two different fishes in response to total dissolved oxygen. The observed result also shows significant differences in the SOD activity among different tissues types and in response to change in the content of dissolved oxygen from different experimental sites. Further, results suggest that the total SOD activities positively correlated with the content of dissolved oxygen in the water samples.

KEYWORDS: Superoxide dismutases, dissolved oxygen, fish, reactive oxygen, Catla catla, Clarias batrachus

I. INTRODUCTION

Reactive oxygen species (ROS) are continuously produced as bye products of metabolism, such as formation of superoxide anion and hydrogen peroxide (H_2O_2) during metabbolism of xenobiotics. These ROS attack on the nitrogen bases in nucleic acids, the amino acid side chains in protein and double bounds of unsaturated fatty acids and damage it, and hence cellular integrity and functions. To combat such menaces, organisms have developed antioxidant defense systems. Two important strategies are (i) non-enzyme defense strategies and (ii) enzyme defense strategies. In non-enzyme defense strategies, low molecular weight substances, such as ascorbic acid, α-tocopherol and glutathionine are produced and directly interact with ROS and neutralize their effects. The enzyme defense strategies use variety of enzymes that metabolize ROS to make it non-toxic and prevent the macromolecular damages. One important enzyme is superoxide dismutatses (SOD) that exist in virtually all oxygen respiring organisms. Superoxide dismutases (SOD) are the antioxidant scavenging enzymes that catalyze the dismutation between two moles of superoxide anion to yield one mole of oxidized product (oxygen) and one mole of reduced product (hydrogen peroxide) (Klug et al., 1972; Halliwell and Gutteridge, 1989; Babich, 1993). SOD form first line of defense against oxygen-derived free radicals and its activity can be rapidly induced when cells or organisms are exposed to oxidative stress (Crapo and Tierney, 1975). Hence this catalysis process often called primary defense because this enzyme prevents the further generation of free radicals. SOD target to different cellular and sub-cellular locations that reflect the slow diffusion process. This spatial specificity point to the need of SOD for fine local control of ROS signaling.

SOD control and maintain the levels of a variety of reactive oxygen and nitrogen species that produced through several metabolic processes and hence protect the system (Crapo and Tierney, 1974; Fridovich 1978, 1986). The degree of toxicity also depends on the concentration of pure oxygen. A prolonged elevated concentration of pure oxygen may result in to damage of central nervous system. The lack of SOD1 may results in hepatocellular carcinoma (Elchuri et al., 2005), age-related muscle loss (Muller et al., 2006), earlier incidence of cataracts, and reduced lifespan. The absence of SOD2 may results in death due to massive oxidative stress (Li et al., 1995). While the absence of SOD3 may increase the chances of hyperoxic injury (Sentman et al., 2006). The SOD activities also vary from tissues to tissues in the different body part. High activity of SOD is observed in liver, while intermediate level of activities in adrenal tissues and RBCs (Fried and Mandel, 1975). In most of the other tissues, a low activity of SODs was observed. Thomas et al., (1976) observed relatively homogenous distribution of SOD in brain; however it was about two fold ranges from the highest area to the lowest area (cortex). The present work describes the study of

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SOD activities and its comparative analyses between two different species of fishes in response to total dissolved oxygen in water of river Ganga. An attempt has also been made to do a detail comparative study of SOD activities among different active tissues.

II. MATERIAL AND METHODS

The SOD activities were studied in two different types of fish: Clarias batrachus (air breathing) and Catla catla (non-air breathing) which were collected from four different point of river Ganga: Buxar, Patna, Mokama and Barh. Fishes were sacrificed by decapitation for the extraction of different tissues, viz, liver, adrenal gland and gill. Tissues were thoroughly washed in chilled saline water to clean the blood and adhering tissues. Homogenate of tissues in the concentration of 10% (w/v) were prepared using potassium phosphate buffer (0.05M; pH 7.0) with the help of Yorks homogenizer fitted with Teflon plunger. Lowry et. al., (1951) method was used for protein estimation in post nuclear fractions and cytosolic supernatant. 10 mg of crystalline bovine serum was dissolved in 100 ml of deionized water for the preparation of standard protein solution. After 30 minutes, 0.5 ml of Folin's reagent was added. Optical density of blue colour developed was read at 625nm exactly after 30 minutes. Standard solution (BSA, 20-100 µg) and blank were run simultaneously.

Total SOD from different tissues were estimated and calculated by using McCord and Fridovich (1969) method. The generated superoxide anion reduce the nitroblue tetrazolium (NBT) forming a blue formazan which is measured at 560 nm. SOD inhibited the reduction of NBT and thus enzyme activity is measured by monitoring the rate of decrease in optical density at 560 nm. 2.0 ml of tissue was homogenate with distilled water and dispensed in centrifuge tubes. The tissue homogenate were diluted 1:9 for gills and adrenal gland, 1:4 for other tissues. The tubes were centrifuged at 1000 rpm for 15 minutes at 4°C. Solid 313 mg/ml ammonium sulphate was added into supernatant of each tube to make the final concentration of 50%. The tubes were shaken thoroughly and kept for 4 hrs at 4°C. The supernatant was dialyzed three times against deionized water using 0.3 ml nitroblue tetrazolium, 0.2 ml phenazine methosulphate, 1.0 mi pyrosulphate buffer, and 2.0 ml enzyme source. After 90 second, 1.0 ml of glacial acetic acid was added for checking the reaction. The absorbance were read at 560 nm on a spectrocolorimeter (Elico CL 171) against blank (NBT + PMS + Buffer + deionized water). Protein content in enzyme sources was also estimated by the method of Lowry et al., (1951). The unit of enzyme activity was defined as the amount of enzyme required to inhibit the optical density at 560 nm in one minute under the assay condition. Results were expressed as units/mg protein.

The total dissolved oxygen (DO) in the water at four different points was estimated by Winkler's modified azide method. The measurement was done by precipitating as manganese basic oxide and dissolved by concentrated sulphuric acid forming manganese sulphate. Then it reacts with potassium iodide to liberate iodine which is estimated by titration with sodium thiosulphate (0.025 N).

III. RESULTS AND DISCUSSION

Total 20 water samples were collected from each of the four different point of river Ganga. The measurement shows variation in the content of total dissolved oxygen (DO) among four sampled site (Table 1). Highest DO content was 12.0± 1.97 mg/lt in the water samples collected from river Ganga near Barh, while lowest content was 7.6.0± 0.98 mg/lt in the sample collected near Buxar.

Table 1: Measured dissolved oxygen (DO) in water samples collected from four sites of river Ganga.

Experimental site	DO (mg/lit)*
Near Buxar	7.5 ± 0.78
Near Patna	10.9 ± 1.03
Near Mokama	11.2 ± 0.33
Near Barh	12.1 ± 1.17

* Value indicate mean ± standard deviation of 20 measurement

Clarias batrachus (air breathing) is an important cat fish of Indian subcontinent widely distributed in the river, lake, and ponds. While Catla catla (non-air breathing) is common edible fish of India. Metabolically active tissues from liver, adrenal gland and gills were carefully dissected out from five fishes of both species and used for the determination of total SOD activities. The observed SOD activities of all three tissues are shown in Table 2 and Table 3 for Clarias batrachus and Catla catla, respectively.

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Table 2: Observed total SOD activities (units. mg^{-1} protein)* in the liver, adrenal gland and gill tissues of Clarias batrachus collected from four different sites of river Ganga.

Experimental site	Liver tissue	Adrenal gland tissue	Gill tissue
Near Buxar	7.6 ± 0.410	4.0 ± 0.508	2.4 ± 0.381
Near Patna	7.9 ± 0.487	4.4 ± 0.374	2.9 ± 0.446
Near Mokama	9.9 ± 0.412	7.6 ± 0.514	4.5 ± 0.446
Near Barh	10.4 ± 0.449	8.4 ± 0.370	5.4 ± 0.525

* Value indicate mean ± standard deviation of 5 measurement

Observation and results shows significant differences in the total SOD activities among the three tissues of Clarias batrachus across four different experimental sites near river Ganga. The total SOD activities were not much different between samples collected near Buxar and Patna, particularly in liver and adrenal gland tissues. The highest total SOD activity (10.4 (± 0.449) units. mg^{-1} protein) was observed in the liver tissues of sample collected near Barh, while lowest activity (2.4 (± 0.381) units. mg^{-1} protein) was observed in the gill tissue collected near Buxar.

Table 2: Observed total SOD activities (units. mg^{-1} protein)* in the liver, adrenal gland and gill tissues of Catla catla collected from four different sites of river Ganga.

Experimental site	Liver tissue	Adrenal gland tissue	Gill tissue
Near Buxar	6.9 ± 0.316	3.9 ± 0.370	2.3 ± 0.412
Near Patna	6.9 ± 0.423	4.3 ± 0.358	2.8 ± 0.370
Near Mokama	9.9 ± 0.546	7.5 ± 0.531	3.6 ± 0.370
Near Barh	9.9 ± 0.381	8.2 ± 0.412	5.2 ± 0.511

* Value indicate mean ± standard deviation of 5 measurement

Similar pattern also observed in Catla catla. However, the estimated total SOD activities were nearly identical between samples collected near Buxar and Patna, particularly in liver tissues. The highest total SOD activities were observed around 9.9 units. mg^{-1} protein in the liver tissues of sample collected near Barh and Mokama, while lowest activity (2.3 (± 0.412) units. mg^{-1} protein) was observed in the gill tissue collected near Buxar.

Overall the data and results indicate that the SOD activities are much difference between different tissues in both Clarias batrachus and Catla catla among four different sites of river Ganga. The measured data indicate that air breathing fishes have higher SOD activities than the non-air breathing fishes. This might be due to high metabolism in air breathing fishes. Interestingly, there is almost no difference in SOD activities in the liver tissues of Catla catla near Mokama and Barh. Further, the observed data suggest that SOD activities positively correlates with the DO content among the four experimental sites. Effect of freely available oxygen, temperature and pH changes have a direct influence on the SOD activities. It has been also shown that the species which are incapable of synthesizing Vitamin C and other anti-oxidant systems may have enhanced SOD activity in the metabolically active tissues. The presence of xenobiotics may also results into increase in SOD activities.

IV. CONCLUSIONS

The present study revealed that the SOD activities vary significantly among the different tissues of Clarias batrachus and Catla catla from different sites of river Ganga. The SOD activities are relatively higher in air breathing fishes than the non-air breathing fishes The SOD activities are more in liver tissues than in the gill tissues in both Clarias batrachus and Catla catla. Further, the total SOD activity positively correlates with the content of DO in the water samples.

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