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Comparative study of superoxide dismutases activities in response to change of the total dissolved oxygen content

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ABSTRACT: Superoxide dismutases (SODs) are metal-containing scavenging enzymes that catalyze the dismutation of superoxide radicals to oxygen and hydrogen peroxide. SODshave been reported in almost all aerobic organisms to play a major role in the defense against toxicreduced oxygen species. A comparative study of SOD activities in air breathing fish *Channa punctatus*shows diversity among different sites of river Ganga. Result shows significant differences in the SOD activity among different tissues in response to change in the content of dissolved oxygen. Further, results also suggest that the total SOD activities positively correlated with the content of dissolved oxygen in the water samples.

KEYWORDS: Superoxide dismutases, dissolved oxygen, Ganga, *Channa punctatus*

I. INTRODUCTION

Reactive oxygen species (ROS) are produced as bye products of metabolism, such as formation of superoxide anion and hydrogen peroxide (H_2O_2) during metabolism of xenobiotics. These ROS are threat to cellular integrity and functions. To combat such menaces, organisms have developed antioxidant defense systems. 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). Several forms and varieties of SOD have been reported. Largely SOD may be classified in three groups based on the type of metal found as their prosthetic group. These are manganese superoxide dismutase (Mn-SOD), iron superoxide dismutase (Fe-SOD) and copper – zinc superoxide dismutase (Cu-Zn SOD). The Mn-SOD and Fe-SOD show extensive amino acid sequence homology, while Cu-Zn SOD represents an independently evolved group. The Cu-Zn SOD is a remarkable conserved family of protein in context of gross structural properties (Fridovich 1974 and 1978).

SOD control and maintain the levels of a variety of reactive oxygen species and reactive nitrogen species that produced through several metabolic processes and signaling functions(Crapo and Tierney, 1974; Fridovich 1978, 1986). Oxygen is toxic and mutagenic entities (Fridovich 1986). The degree of toxicity depends on the concentration of oxygen. A prolonged exposure of pure oxygen may result in to damage of central nervous system. The absence of SOD2 may be lethal. The lack of SOD2 may results in death due to massive oxidative stress (Li et al., 1995).Evidence suggest that the absence of SOD1 may results in a wide range of pathologies, like hepatocellular carcinoma (Elchuri et al., 2005), age-related muscle mass loss (Muller et al., 2006), earlier incidence of cataracts, and reduced lifespan. Further, it has been shown that the absence of SOD3 may results intoincreased sensitive to hyperoxic injury(Sentman et al., 2006).

The SOD activities also vary among different part of body and tissues. Fried and Mandel (1975) observedhigh activity ofSOD in liver, while intermediate level of activities in adrenal tissues and RBCs. However, the low activities of SODs were reported in most of the other tissues including brain. Thomas et al., (1976) showed relatively homogenous distribution of SOD in brain; however it was about two fold ranges from the highest area to the lowest

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area (cortex). In the present work, an attempt has been made to study the activities of SOD among different active tissues in air breathing fish *Channa punctatus*from different sites of river Ganga.

II. MATERIAL AND METHODS

Four different point of river Ganga: Buxar, Patna, Mokama and Barh, were chosen as experimental site for the study. The total dissolved oxygen (DO) in thecollected water was estimated byWinkler's modified azide method. The DO is measured by precipitating as manganese basic oxide which is dissolved by concentrated sulphuric acid forming manganese sulphate. Further it reacts with potassium iodide, that liberate iodine which is determined by titration with sodium thiosulphate (0.025 N).

Channa punctatus were sacrificed by decapitation, and tissues (viz, liver, adrenal gland and gill) were taken out. Tissues were thoroughly washed and cleaned in chilled saline water. Blood and adhering tissues were removed properly before biochemical studies.Using potassium phosphate buffer (0.05M; pH 7.0), 10% (w/v) homogenate of tissues (mentioned above) were prepared with the help of Yorks homogenizer fitted with Teflon plunger.Protein estimation was carried out in post nuclear fractions and cytosolic supernatant by using method of Lowry et. al.,(1951). For standard protein solution, 10mg of crystalline bovine serum was dissolved in 100 ml of deionized water. Standard solution (BSA, 20-100 µg) and blank were run simultaneously.

SOD was estimated and calculated by using method of McCord and Fridovich (1969). The nitroblue tetrazolium (NBT) get reduced by generated superoxide anion to form a blue formazan which is measured at 560 nm. SOD inhibited the reduction of NBT, and thus enzyme activity is measured by calculating the rate of decrease in optical density at 560 nm.The tissue homogenate were diluted 1:9 for gills and adrenal gland, 1:4 for other tissues. 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.

III. RESULTS AND DISCUSSION

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

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

Experimental site	DO (mg/lit)*
Near Buxar	7.6 ± 0.98
Near Patna	11.6 ± 0.93
Near Mokama	11.6 ± 0.53
Near Barh	12.0 ± 1.97

* Value indicate mean ± standard deviation of 20 measurement

Channapunctatus is common edible fish of Indian subcontinent widely distributed in the lakes, ponds and rivers. Metabolically active tissues such as from liver, adrenal gland and gillswere carefully dissected out and used for the determination of total SOD activities.The estimated SOD activities in the different tissues are shown in Table 2.

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

Experimental site	Liver tissue	Adrenal gland tissue	Gill tissue
Near Buxar	7.2 ± 0.370	4.9 ± 0.345	2.6 ± 0.444
Near Patna	7.7 ± 0.316	5.0 ± 0.287	3.1 ± 0.309
Near Mokama	9.8 ± 0.503	7. 7 ± 0.316	4.3 ± 0.485
Near Barh	10.1 ± 0.431	8.2 ± 0.525	5.3 ± 0.449

* Value indicate mean ± standard deviation of 5 measurement

Observed data and results shows significant differences in the total SOD activities among the three tissues of *Channapunctatus*across four different experimental sites near river Ganga. The total SOD activities were not much different between samples collected near Mokama and Barh, and between samples collected near Buxar and Patna, particularly in liver and adrenal gland tissues. The highest total SOD activity (10.1(± 0.431) units. mg^{-1} protein) was

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observed in the liver tissues of collected near Barh, while lowest activity (2.6(± 0.444) 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 of*Channapunctatus*among four different sites of river Ganga. 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. The presence of xenobiotics may also results into increase in 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.

IV. CONCLUSIONS

The present study revealed that the SOD activities vary significantly among the different tissues of *Channa punctatus*from different sites of river Ganga.The SOD activities are more in liver tissues than in the gill tissues. Further, the total SOD activity positively correlates with the content of DO in the water samples.

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