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Research Article

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Effect of heavy metals and organic pollutants on antioxidant enzyme activities in Zebrafish by polyacrylamide gel electrophoresis

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ABSTRACT

The potential utility of antioxidant enzyme activity was evaluated as indicators of exposure to heavy metals and organic pollutants in aquatic organisms. The effect of heavy metals and organic pollutants on antioxidant enzyme activity of zebrafish was studied. The trends of superoxide dismutase (SOD) and catafase (CAT) activity in zebrafish under after exposure to heavy metals and organic pollutants for different time were analyzed by polyacrylamide gel electrophoresis (PAGE) technique. The brightness of bands in the polyacrylamide gel electrophoresis is almost consistent with the trends of antioxidant enzymes (SOD and CAT) activity in zebrafish, namely, the greater brightness of the bands was, the stronger activity of SOD and CAT was, indicating PAGE is an effective technique to evaluate the antioxidant enzyme activity. Moreover, the results showed that the exposure time and concentrations of pollutants have a great effect on the SOD and CAT activity in zebrafish, and the dose-response relationship between SOD/CAT activity and heavy metals or organic pollutants was established.

Keywords: Zebrafish; Heavy Metals; Organic Pollutants; Superoxide Dismutase(SOD); Catalase(CAT); Polyacrylamide Gel Electrophoresis(PAGE)

INTRODUCTION

As the urbanization and industrialization process accelerating, more and more heavy metals and organic pollutants is directly or indirectly discharged into natural water, causing serious water pollution. Aquatic organism's behavior, physiological and biochemical have reacted in contaminated water before causing death. Consequently, Biomarkers at the cellular or molecular level can be used as early warning indicators of contaminant exposure and toxicity [1]. Antioxidant enzymes including superoxide dismutase (Superoxide Dismutase, SOD), catalase (Catafase, CAT) are used as poison toxicity biomarkers and have been widely used in toxicological studies [2-4].

Gel electrophoresis has become an important method for separation, purification and identification of nucleic acids and proteins due to its high selectivity, simplicity, negligible consumption of organic solvent with respect to gas chromatography, HPLC, ion-phase spectrum, membrane separation technology. Polyacrylamide gel electrophoresis (PAGE). Based on polyacrylamide gel as a support medium zone electrophoresis, exhibits high resolution and flexibility, thus it has been becoming a common biological separation technology in recent years [5]. In this study, polyacrylamide gel electrophoresis technology was adopted to analyze the effect of heavy metals pollutants (Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}) and organic contaminants (phenol, bisphenol A) on SOD and CAT activity trends in zebrafish at different concentrations and different time, which could provide a pathway for the research of toxicology biochemical indicator.

EXPERIMENTAL SECTION

2.1 Materials

Live zebrafish (Danio rerio), whose body length was 30.25 ± 3.12 mm, purchased in the Qianhuaban market of

Nanchang. Polyacrylamide, Bis-acrylamide, sodium dodecyl sulfate (SDS), aminoacetic acid, ammonium persulfate, N,N,N',N'-tetramethylethylenediamine (TEMED), bromophenol blue (AR), $FeC1_3$ (AR), $K_3Fe(CN)_6 \cdot 6H_2O$ (AR), Nitrotetrazolium Blue chloride (NBT), Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), riboflavin, Tris- HCl buffer, 45 mmol/L pyrogallol solution, sulfuric acid (H_2SO_4), 30% gel stock solution, ammonium persulfate, electrophoresis buffer, bromophenol blue, hydrogen peroxide(H_2O_2), ferric chloride ($FeC1_3$**)**, 1% $K_3Fe(CN)_6$, 2.45×10^{-4} mol/L NBT and phosphate buffer were supplied by Shantou Xilong Chemical Co., Ltd (Shantou, China). All the reagents were of analytical grade and used as received without further purification. Water was purified using a Milli-Q water system (Bedford, USA).

2.2 Main instruments

Polyacrylamide gel electrophoresis was conducted on vertical electrophoresis device (Mini-PROTEAN) and gel imaging system (JS-680S).

2.3 Experimental Methods

2.3.1 Preparation of 10% separation gel

1.3 mL 30% polyacrylamide, 1.0 mL1.5 mol/L Tris-HCl buffer (pH 8.8), 1.5 mL MillliQ water, 0.04 mL 10% ammonium persulphate and 0.002 mL TEMED were quickly mixed at a low temperature, and then was injected into the middle of a glass plate with water sealed. After several minutes, the mixture was completely frozen.

2.3.2 Preparation of tacking gel

0.13 mL 30 % polyacrylamide, 0.25 mL 1.0 mol/L Tris-HCl buffer (pH 6.8), MillliQ water, 0.01 mL 10% ammonium persulfate and 0.001 mL TEMED were rapidly mixed at a low temperature, and then was injected into the separation gel. The combs was inserted, and waited until the mixture was completely frozen.

2.3.3 Protein electrophoresis

0.05 mol/L pH 6.8Tris-HCl buffer, 0.1 mol/L dithiothreitol, 0.1% bromophenol blue and 10% glycerol were mixed to form a gel buffer. An equal volume of gel sample buffer and protein extract were sufficiently mixed to prepare the electrophoresis fluid. 10 μL solution with bromophenol blue as an indicator was injected into each bore, and then electrophoresis began in the 1× electrophoresis buffer system. The electric current of stacking gel was 5 mA, and the electric current of separating gel was 10 mA.

2.3.4 Superoxide dismutase staining

First, polyacrylamide gel was washed for 2-3 times with MillliQ water after the electrophoresis, and soaked in 2.45×10^{-4} mol/L nitrotetrazolium blue chloride (NBT) at 4 ^{o}C for 20 min. Second, the polyacrylamide gel was washed for 1-2 times with MillliQ water, and immersed it in pH 7.8, 0.036 mol/L phosphate buffers (containing 2.8×10^{-5} mol/L riboflavin and 2.8×10^{-2} mol/L TEMED) and shocked for 15 min in a low-speed shaker. And then polyacrylamide gel was washed for 2-3 times with MillliQ water after soak concussion. Then it was slowly shocked in the shaker under light irradiation, and stopped until polyacrylamide gel film appeared relatively clear bands on a blue background. Finally, polyacrylamide gel film was slightly washed with MillliQ water again and taken pictures by placing the film in gel imaging system, and then immersed into 95% ethanol for a few minutes.

2.3.5 Hydrogen peroxidase staining

Washed polyacrylamide gel 2-3 times repeatedly by using MillliQ water after the electrophoresis, and then soaked into a mass concentration of 0.3% H_2O_2 , shocked for 20 min in the low-speed shaker.

To wash polyacrylamide gel 2-3 times repeatedly with water MillliQ after soak concussion. The washed polyacrylamide gel , which was transferred to a mixture with the volume ratio of 1:3 1% $FeC1_3$ and 1% $K_3Fe(CN)_6$, was slowly shocked again in the shaker and stopped until polyacrylamide gel film appeared relatively clear bands on a blue background. Next polyacrylamide gel film was slightly washed with MillliQ water to take pictures by placing the film in gel imaging system.

2.3.6 Electrophoresis injection

Different concentrations of heavy metals and organic pollutants were $1/20LC_{50}$, $1/10LC_{50}$, $1/5\ LC_{50}$, were then injected.

RESULTS AND DISCUSSION

3.1 Effect of Cu^{2+} on the activity trends of SOD and CAT

Gel electrophoresis of SOD and CAT activity trends in presence of Cu^{2+} were shown in Fig.1. The inhibition rate of SOD and CAT activity in zebrafish under Cu^{2+} stimulated condition was shown in Table 1.

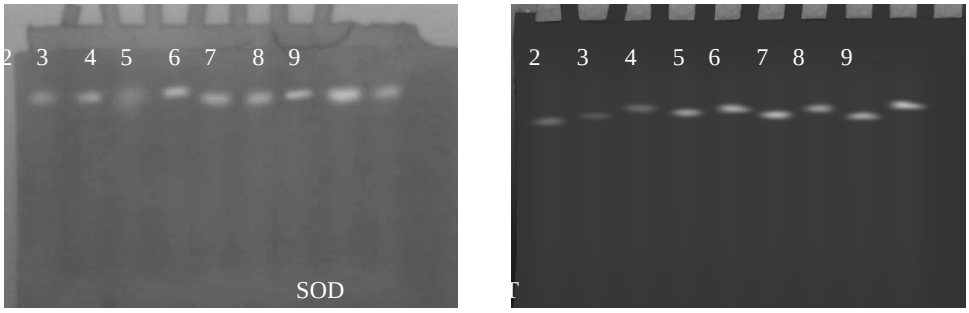

Fig.1 SOD and CAT banding pattern in presence of Cu^{2+}

Table 1 SOD and CAT activity inhibition rate after Cu^{2+} exposure

Enzymes	1/20 LC_{50}			1/10 LC_{50}			1/5LC_{50}		
	1 d	7 d	14 d	1 d	7 d	14 d	1 d	7 d	14 d
SOD	0.211	0.173	0.116	0.196	0.182	0.156	0.253	0.197	0.233
CAT	1.183	0.700	0.719	1.385	0.600	0.603	1.502	0.552	0.516

It can be seen from Fig.1 and Table 1 that the trends of bands brightness were consistent with enzyme activity, namely, the stronger enzyme activity was, the greater brightness of bands. The above results indicated that SOD and CAT activity in zebrafish decreased with time in the presence of Cu^{2+}, and the activity trends of SOD and CAT increased with the increasing concentration.

3.2 Effect of Cd^{2+} on the activity trends of SOD and CAT

Gel electrophoresis of SOD and CAT activity trends in presence of Cd^{2+} were shown in Fig.2. SOD and CAT activity inhibition rate in zebrafish under Cd^{2+}-stimulated condition was shown in Table 2.

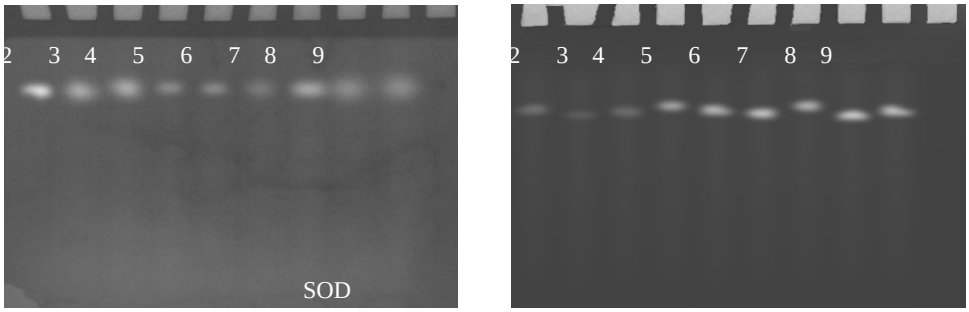

Fig. 2 SOD and CAT banding pattern in presence of Cd^{2+}

Table 2 SOD and CAT activity inhibition rate on Cd^{2+} exposure

Enzymes	1/20 LC_{50}			1/10 LC_{50}			1/5LC_{50}		
	1 d	7 d	14 d	1 d	7 d	14 d	1 d	7 d	14 d
SOD	0.208	0.211	0.244	0.173	0.238	0.314	0.228	0.269	0.342
CAT	1.502	0.698	0.707	1.607	0.617	0.676	2.085	0.540	0.608

The trend of the bands brightness was consistent with enzyme activity, namely the stronger enzyme activity was, and the greater brightness of bands was. The above results indicated that activity growth of SOD weakened with time extending in the Cd^{2+}-containing water, while CAT activity increased at a low concentration, whereas when the concentration increased, whose activity appeared to the first decrease and then increase.

3.3 Effect of Zn^{2+} on the activity trends of SOD and CAT

Gel electrophoresis of SOD and CAT activity trends in presence of Zn^{2+} were shown in Fig.3. SOD and CAT enzyme activity inhibition rate in zebrafish under Zn^{2+}-stimulied condition was shown in Table 3.

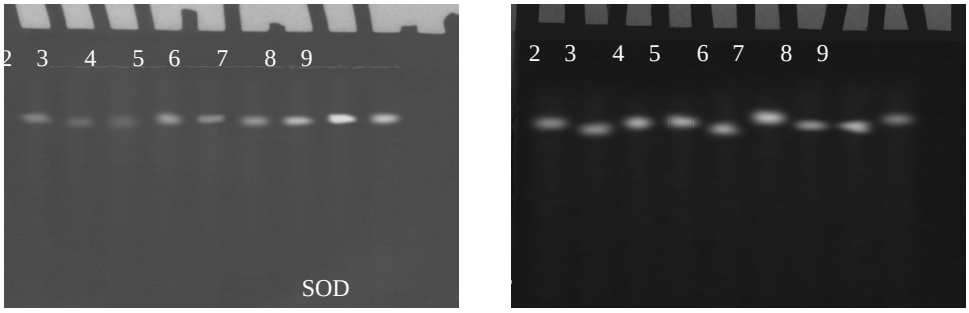

Fig. 3 SOD and CAT banding pattern in presence of Zn^{2+}

Table 3 SOD and CAT enzyme activity inhibition rate on Zn^{2+} exposure

Enzymes	1/20 LC50			1/10 LC50			1/5LC50		
	1 d	7 d	14 d	1 d	7 d	14 d	1 d	7 d	14 d
SOD	0.220	0.147	0.090	0.163	0.204	0.072	0.180	0.136	0.104
CAT	0.766	0.785	0.538	0.569	0.755	0.686	0.536	0.763	0.523

It can be seen from Fig. 3 and Table 3 that the brightness of bands was consistent with the variation trends of enzyme activity, namely the stronger enzyme activity was, the greater brightness of bands was. The experiment result showed that the SOD activity first decrease with extending time and then increased in Zn^{2+}-containing water. Moreover, upward trend of SOD activity reduce with the increase of concentration. CAT activity increased in the first time stage, and then decreased in the second time stage.

3.4 Effect of Pb^{2+} on the activity trends of SOD and CAT

Gel electrophoresis of SOD and CAT activity trends in the presence of Pb^{2+} were shown in Fig.4. SOD and CAT activity inhibition rate in zebrafish under Pb^{2+}-stimulated condition was shown in Table 4.

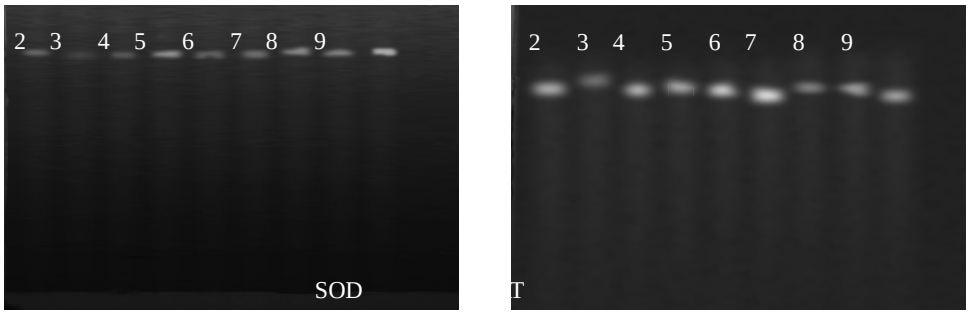

Fig. 4 SOD and CAT banding pattern in presence of Pb^{2+}

Table 4 SOD and CAT enzyme activity inhibition rate on Pb^{2+} exposure

Enzymes	1/20 LC_{50}			1/10 LC_{50}			1/5LC_{50}		
	1 d	7 d	14 d	1 d	7 d	14 d	1 d	7 d	14 d
SOD	0.178	0.150	0.087	0.199	0.160	0.112	0.156	0.101	0.107
CAT	0.752	0.961	0.481	0.375	0.962	0.541	0.682	0.709	0.717

The trends of bands brightness were consistent with enzyme activity, namely the stronger enzyme activity was, the greater brightness of bands was,. The results indicated that the SOD activity enhanced with exposure time in the Pb^{2+}-containing water, and SOD activity weakened with increasing concentration of Pb^{2+} ions. While CAT activity initially increased during the first time stage, goes through a maximum CAT activity, and then decreases obviously. First, SOD respond to the stimulation of heavy metals and decomposed free radical into H_2O_2 and O_2, and then CAT decompose H_2O_2 into H_2O and O_2 [6] . Extremely low concentration of heavy metals in water would cause the response of antioxidant enzymes in organisms, which was particularly sensitive to the pollution of heavy metals. Therefore it could be used as an important marker for early warning of heavy metals pollution in the environment [7].

The toxicity mechanism to zebrafish was likely to be that SOD and CAT were damaged under the exposure of heavy metals such as Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+} , leading to the loss of the redox capacity of SOD and CAT to convert peroxide into less toxic or harmless substances, thereby cause harm to the zebrafish [8,9].

3.5 Effect of phenol on the activity trends of SOD and CAT

Gel electrophoresis of SOD and CAT activity trends under the influence of phenol were shown in Fig.5. SOD and CAT activity inhibition rate in zebrafish under phenol-stimulated condition was shown in Table 5.

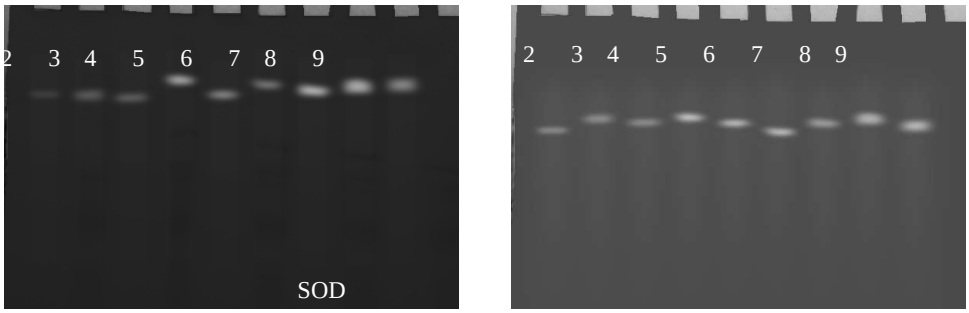

Fig. 5 SOD and CAT banding pattern in presence of phenol

Table 5 SOD and CAT enzyme activity inhibition rate on phenol exposure

Enzymes	1/20 LC_{50}			1/10 LC_{50}			1/5LC_{50}		
	1 d	7 d	14 d	1 d	7 d	14 d	1 d	7 d	14 d
SOD	0.090	0.066	0.062	0.094	0.057	0.049	0.116	0.045	0.042
CAT	0.410	0.649	0.706	0.350	0.569	0.614	0.323	0.454	0.497

The trend of bands brightness was consistent with enzyme activity, namely the stronger enzyme activity was, and the greater brightness of bands was. The above results indicated that SOD and CAT activity growth enhanced with extending time in the phenol-containing water, and the variation trends were enhanced as the increasing concentration.

3.6 Activity variation trends of SOD and CAT under the influence of bisphenol A

Gel electrophoresis of SOD and CAT activity trends under the influence of bisphenol A was shown in Fig. 6. SOD and CAT activity inhibition rate in zebra fish in presence of bisphenol A was shown in Table 6.

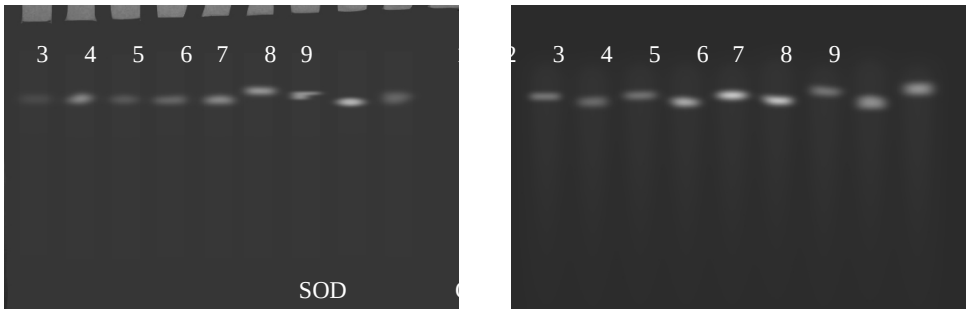

Fig. 6 SOD and CAT banding pattern in presence of bisphenol A

Table 6 SOD and CAT enzyme activity inhibition rate under bisphenol A exposure

Enzymes	1/20 LC_{50}			1/10 LC_{50}			1/5LC_{50}		
	1 d	7 d	14 d	1 d	7 d	14 d	1 d	7 d	14 d
SOD	0.064	0.055	0.057	0.041	0.070	0.057	0.116	0.045	0.042
CAT	0.323	0.454	0.497	0.308	0.381	0.401	0.323	0.454	0.497

The trend of bands brightness was consistent with enzyme activity, namely the stronger enzyme activity was, the greater brightness of bands was, and vice versa. The experimental results indicated that SOD activity decreased initially with increasing exposure time and concentration of bisphenol A, reached a minimum value, and then decreases obviously. CAT activity enhanced as time extends, which was consistent with the activity of antioxidant enzymes [10, 11].

CONCLUSION

Under the exposure of four heavy metals including Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+} and phenol bisphenol, SOD and CAT activity in zebrafish were investigated. the same under the influence of four heavy metals including Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+} and phenols.The exposure time or concentrations of pollutants have a great effect on the SOD and CAT activity

in zebrafish. The brightness of bands in the polyacrylamide gel electrophoresis figure is almost consistent with the activity trends of antioxidant enzymes (SOD and CAT) in zebrafish, namely the stronger activity of SOD and CAT was, the greater brightness of the bands was, indicating that SOD and CAT in zebrafish could be used as a biological indicator of heavy metals and organic pollutants in water, and the dose-response relationship between SOD/CAT and heavy metals or organic pollutants was established.

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