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Study on tolerating mechanism of sulfide of *Urechis unicinctus*
I . The function of coelomic fluid during tolerance of toxicity sulfide

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Introduction

Echuria is a familiar benthos. It is often founded in the rich H_2S sea area while majority animals can not live and can tolerate the strong toxicity of H_2S. Arp et al. indicated that in *Urechis caupo*, H_2S was tolerated by resisting mechanism and oxidizing mechanism (Arp *et al.* 1995). It was thought that the relation between the oxidizing activity and the content of free hematin was direct ratio (Mark. and Arp, 1989).

At present it did not report on its tolerating mechanism for H_2S. In this paper, we reported the continue changes of hemoglobin, Fe^{++}-heme and hematin from coelomic fluid in rich H_2S condition and discussed its tolerating mechanism for H_2S.

Materials and Methods

1.1 Materials and the test condition: The coelomic fluid was taken out by syringe penetrating the body wall directly. The germ cells were gained by centrifugation. Mature oocyte was gained from store duct by dissecting animal.

The health individuals (15-20cm) were selected. They were used to test after cultured about 2 weeks in the lab. 5 individuals in every group were cultured in the container filled 1.5 ℓ filter sea water separately. The concentration of H_2S in the different group was 0(control), 60 μM, 160 μM, and 640 μM separately. The container was covered in order to reduce the transgression of H_2S, but a aerate hole was opened. During culturing, water was changed one time per 12 hours and the concentration of H_2S was renewed. Meanwhile the coelomic fluid was taken out and checked. The test was repeated 4 times.

1.2 Determining the concentration of H_2S and protracting the Transgression curve of H_2S

H_2S was added into the water in the store liquid form (100 mM Na2S · 9H2O,

125mM
imidazole, pH 6.8). Its concentration was determined by methylene blue demarcatemethod. The practice concentration was educed according to the standard curve.

1.3 Various composition of coelomic fluid determined and calculated

Heme in the coelomic fluid was taken out by acetone extract method. It wascentrifugated at 5000 g, 10 mins. The supernatant was colorimeteric analysis at 635 nm by spectrophotometer. The heme content was looked up in the standard curve according to the absorbing value.

The absorbing value was determined by TMB method. The content of hemoglobin was calculated as follow: hemoglobin (μM) = (tested sample value control value) / (standard sample value control value) × 30 × 100 × 0.155.

Hematin = total heme - 4 × hemoglobin.

The percent of hematin = hematin / total heme × 100%.

1.4 Data processing

In order to increase the data comparative reliability and decrease the effect bought by individual difference, the initial value was the first test value of some index in every individual. The relative value of some index was gained by the ratio of the test value and initial value.

Results and Disscussion

The changes of hemoglobin, Fe^{++}-hemoglobin and hematin in the coelomic fluid of *Urechis unicinctus* were investigated by biochemical technique in the continuance different concentration of sulfide. The result showed that various kinds component, total heme, hemoglobin and hematin, changed in the regular pattern accompanying the different sulfureted hydrogen environment. It was illuminated that the coelome liquid, especially component correlation with hemoglobin, had an important function in the physiological process of tolerating sulfide toxicity.

References

Arp et al., 1995. Multiple mechanisms provide tolerance to environmental sulfide in *Urechis caupo*. American Zoology, 35(2): 132-144.

Arp A.J., Hansen B.M. and D. Julian, 1992. Burrow environment and coelomic luid chareacteristics of the echiuran worm *Urechis caupo* from populations at three sites in northern California. Marine biology, 113(4): 613-623.