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Short communication

Proteomic responses reveal the differential effects induced by cadmium in mussels *Mytilus galloprovincialis* at early life stages

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ABSTRACT

Cadmium (Cd) has become an important metal contaminant and posed severe risk on the organisms in the coastal environments of the Bohai Sea. Marine mussel *Mytilus galloprovincialis* is widely distributed along the Bohai coast and consumed as seafood by local residents. Evidences indicate that the early stages of marine organisms are more sensitive to metal contaminants. In this study, we applied two-dimensional electrophoresis-based proteomics to characterize the biological effects of Cd (50 μg L^{-1}) in the early life stages (D-shape larval and juvenile) of mussels. The different proteomic responses demonstrated the differential responsive mechanisms to Cd exposure in these two early life stages of mussels. In details, results indicated that Cd mainly induced immune and oxidative stresses in both D-shape larval and juvenile mussels via different pathways. In addition, the significant up-regulation of triosephosphate isomerase and metallothionein confirmed the enhanced energy demand and mobilized detoxification mechanism in D-shape larval mussels exposed to Cd. In juvenile mussels, Cd exposure also induced clear apoptosis. Overall, this work suggests that Cd is a potential immune toxicant to mussel *M. galloprovincialis* at early life stages.

1. Introduction

Because of the industrial discharge from numerous metal smelteries, cadmium (Cd) has become an important metal contaminant in the marine and coastal environments along the Bohai Sea. Sun et al. reported that Cd posed prominent risk on the marine organisms and ecosystem due to the high concentrations of Cd in the sediments from the Bohai coast [1]. Our recent research indicated that the dominant species, shrimp *Crangon affinis*, was contaminated by Cd from the Yellow River Estuary along the Bohai Sea [2]. As it is known, Cd can induce multiple adverse effects in organisms. It may induce oxidative stress in organisms by producing excessive reactive oxygen species [3]. Additionally, Cd can disturb the energy metabolism in clam *Ruditapes philippinarum* indicated by the enhanced anaerobic metabolism [4]. Since Cd has become one of the most severe metal contaminants in the Bohai Sea, it is necessary to characterize the biological effects of Cd in marine animals.

The traditional biological approaches basically focus on the test of specific responses, such as the selected gene expression patterns or enzyme activities, to characterize the biological effects of environmental stressors in organisms [5,6]. In recent years, the "—omic" approaches including genomics, transcriptomics, proteomics and metabolomics have been widely used in environmental biology [7–11]. Among these approaches, two-dimensional electrophoresis (2DE)-based proteomics is useful to present complex biologically functional protein networks [12]. Not only is proteomics a powerful tool for describing complete proteomes at organelle, cell, organ or tissue levels, but it can also be used to detect the minor proteomic responses in biological samples under different conditions [13].

Marine mussels are ubiquitous, sedentary filter-feeders that play critical roles in maintaining marine ecosystem health. This species can accumulate high amounts of contaminants from marine

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and coastal environments and therefore is used to monitor marine and coastal environmental contaminants [14]. The mussel *Mytilus galloprovincialis* is widely distributed along the Bohai coast and popularly consumed as seafood by local residents. In this study, mussel *M. galloprovincialis* was used as the experimental animal to investigate the biological effects of Cd. However, evidences indicate that the earlier life stages are more sensitive than the adults to environmental stressors [15]. To compare the differential proteomic responses in the early life stages of mussels *M. galloprovincialis*, the D-shape larval and juvenile mussels were exposed to Cd with an environmentally relevant concentration (50 μg L^{-1}) for 48 h. The 2DE-based proteomics was conducted on the mussel samples to elucidate the differential biological effects of Cd in these two early life stages of mussels *M. galloprovincialis*.

2. Materials and methods

2.1. Larvae breeding and experimental design

Adult mussels *M. galloprovincialis* reached sexual maturity were collected in April 2015 from a pristine site (Yantai, China). All mussels were transported to the laboratory and acclimatized in aerated natural seawater (salinity 31 psu) at 21 °C for 7 days. After acclimatization, these mussels were kept in air in dark place for 2 h. Then each mussel was put into a beaker containing warmer seawater (24 °C). After 2 h, the majority of mussels came to ovulation and spermiation. The 500 mesh sieve screen was used to filter impurities. Then the sperms and eggs were quickly collected, respectively, to fertilize. All the fertilized eggs were then transferred into 50 L of normal filtered seawater (FSW) in a bucket. Continuous aeration was conducted during incubation. After approximately 48 h, the majority of fertilized eggs developed into D-shape larvae (Fig. 1A). Then the D-shape larval mussels were divided into two groups (control and Cd exposure) each containing 6 buckets (30 L) with a density of ~25 D-shape larval mussels per milliliter. The environmentally relevant concentration (50 μg L^{-1}) of Cd was selected for the exposures of D-shape larval mussels *M. galloprovincialis*. During the acclimatization and exposure periods, all the larvae were kept under a photoperiod of 12 h light and 12 h dark, and fed with the *Chlorella vulgaris* daily. After exposure for 48 h, all the larvae (~6.0–7.5 × 10^5 individuals) from each two bucket were immediately filtered out by 500 mesh sieve screen and collected into one D-shape larval mussel sample. These D-shape larval mussel samples were quickly snap-frozen in liquid nitrogen and stored at −80 °C.

Thirty juvenile mussels (length: ~1.0 cm, Fig. 1B) were purchased from local culturing farm in Yantai, China. After transported to the culture laboratory, the juvenile mussels were allowed to acclimate in aerated seawater (21 °C, 31 psu, collected from pristine environment) in the laboratory for 7 d and fed with the *Chlorella vulgaris Beij* at a ration of 2% tissue dry weight daily. After acclimatization, the mussels were randomly divided into two groups (control and Cd exposure) containing fifteen individuals in 20 L of aerated seawater. The same concentration (50 μg L^{-1}) of Cd was used for the exposure. After exposure for 48 h, the whole soft tissues from juvenile mussels were dissected quickly. The samples of whole soft tissues were flash-frozen in liquid nitrogen and stored at −80 °C before further procedures. During the exposure periods, all the experimental conditions for juvenile mussels were completely identical to those for D-shape larval mussels.

2.2. Protein extraction of mussel samples

Total protein extraction was performed based on previous studies with some modifications [16,17]. Briefly, the mussel

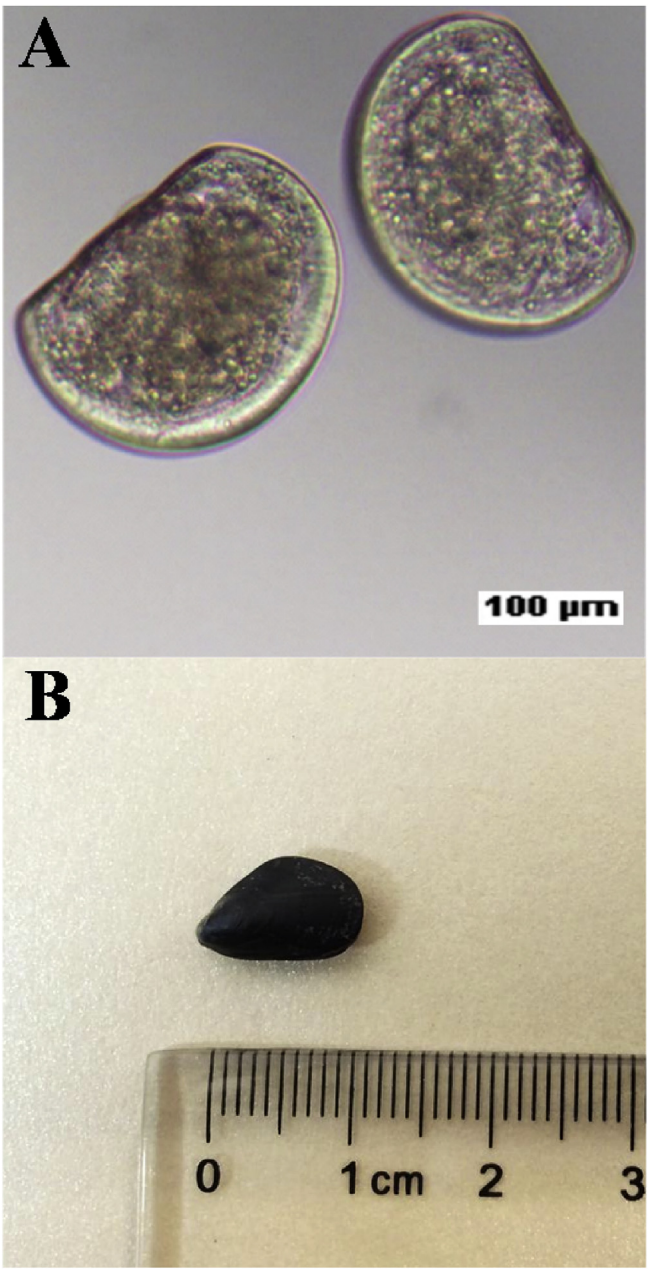

Fig. 1. The mussels *M. galloprovincialis* at early life stages used in this study, (A) D-shape larval and (B) juvenile.

samples were homogenized quickly on ice with 1 mL of TRIzol reagent and centrifuged at 12000 *g* for 5 min at 4 °C. The supernatant was added with 200 μL of chloroform before shaking vigorously for 3 min and precipitating for 3 min. The mixture was centrifuged at 12000 *g* for 15 min at 4 °C, and its upper aqueous layer was discarded. A volume of 300 μL of absolute ethyl alcohol was added and the mixture was allowed to stand for 3 min at room temperature before being centrifuged at 2000 *g* for 5 min at 4 °C. The phenol/ethanol supernatant was precipitated for 30 min at room temperature by the addition of 750 μL of isopropanol prior to centrifugation at 14000*g* for 10 min at 4 °C. The pellets were washed with 1 mL of ethanol (v/v 95%) and centrifuged at 14000 *g* for 10 min at 4 °C. This procedure was repeated twice. The pellets were solubilized in the lysis buffer (7 M urea; 2 M thiourea; 4% m/V CHAPS; 65 mM DTT and 0.2%W/V Bio-lyte buffer) and then incubated for