Hypoxia-induced oxidative stress and transcriptome changes in the mud crab (Scylla paramamosain)

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Abstract

Mud crab (*Scylla paramamosain*) is an economically important cultured species in China. Hypoxia is a major environmental stressor during mud crab culture. In the present study, we investigated the antioxidant system response and molecular mechanism of mud crab after intermediate hypoxia stress with dissolved oxygen (DO) 3.0 ± 0.2 mg/L (named as "DO3") and acute hypoxia stress with DO 1.0 ± 0.2 mg/L (named as "DO1") for 0, 3, 6, 12 and 24 h. The superoxide dismutase (SOD) activity of DO1 increased significantly at 3, 6 and 24 h after hypoxia stress, while SOD activity of DO3 increased significantly at 6 and 24 h. The total antioxidant capacity (T-AOC) increased significantly at 6, 12 and 24 h after hypoxia stress. The malondialdehyde (MDA) concentration of DO1 increased significantly at 6, 12 and 24 h after hypoxia stress, while MDA concentration of DO3 only increased significantly at 6 h. Transcriptomic analysis was conducted at 24 h of gill tissues after hypoxia stress. A total of 1052 differentially expressed genes (DEGs) were obtained, including 394 DEGs between DO1 and DO3, 481 DEGs between DO1 and controlled, 177 DEGs between DO3 and control group. DEGs were enriched in the pathways related to oxidative stress response, metabolism, immune functions, ion transport, and signal transduction. Transcriptional analysis showed that glycolysis and tricarboxylic acid cycle genes were the key factors in regulating the adaptation of mud crab to hypoxia stress.

Materials & Methods

The DO of hypoxic treatment was achieved within 10 minutes by injecting nitrogen gas into the water and measured using a DO meter (JPB-607A, China). Gill tissues collected from 3 mud crabs at 0, 3, 6, 12, 24 h after hypoxia challenge were mixed as one sample, and each treatment was conducted in triplicate.

- The activities of SOD, T-AOC and MDA content were measured using commercially available kits (Nanjing Jiancheng Chemical Industries, Nanjing, Jiangsu, China) following the instructions of the manufacturer.
- Gill samples collected at 24 h of each group were used for transcriptional analysis, including total RNA extraction, cDNA library construction, and sequencing.

Results & Discussion

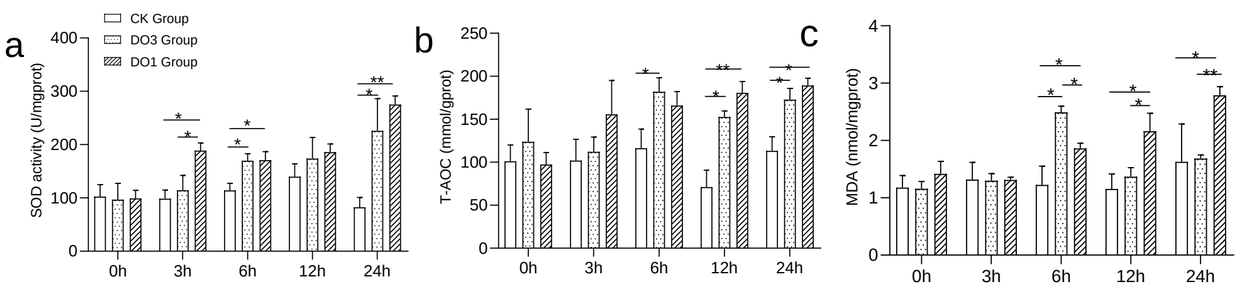


Fig.1 SOD activity (a), T-AOC (b), MDA production (c) and LDH activity (d) gills after hypoxia stress.

In the present study, the activities of SOD (a), T-AOC (b) and MDA (c) level in the gills of mud crabs significantly increased after hypoxia stress, which indicated that the mud crabs activated anti-oxide abilities to tolerate hypoxia stress. The enhancement of antioxidant enzymes under hypoxic conditions may serves as a preparatory mechanism to respond to the physiological oxidative stress that occurs rapidly in the early recovery period during reoxygenation.

With the DO concentration gap widened, the number of DEGs (differentially expressed unigenes) increased, suggesting that different levels of hypoxia may inhibit the expression of specific genes(Fig. 2).

Many genes related to glycolysis were up-regulated after hypoxia stress base on our DEGs analysis, such as hexokinase, aldolase, dihydroxyacetone kinase, glyceraldehyde-3-phosphate dehydrogenase, alcohol dehydrogenase, and lactate dehydrogenase. On the contrast, many key enzyme genes in the TCA cycle have down-regulated, including citrate synthase, aconitase, isocitrate dehydrogenase, and succinate dehydrogenase. These results indicated that environmental hypoxia caused in a shift of mud crab from aerobic to anaerobic metabolism, which may be the main energy source of mud crab under acute hypoxia stress.

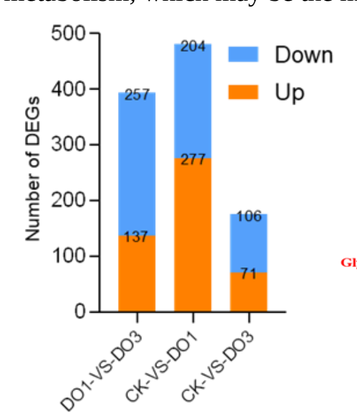
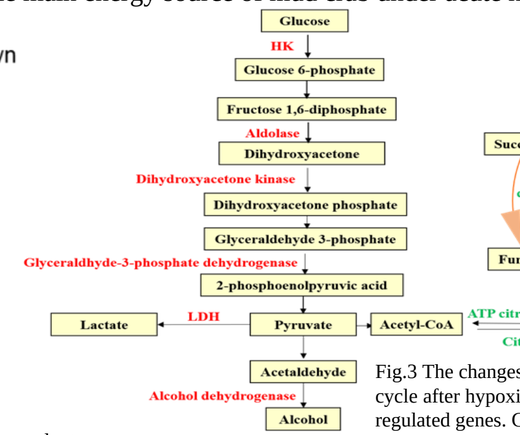


Fig.2 Statistic of differentially expressed genes (DEGs) between different groups under hypoxia.



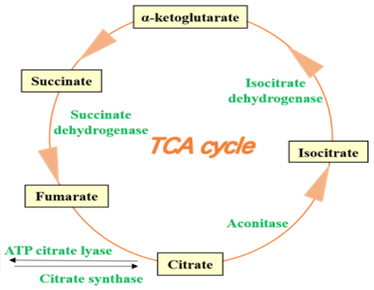


Fig.3 The changes in the glycolysis pathway and TCA cycle after hypoxia exposure. Red represents the up-regulated genes. Green represents the down-regulated genes.

HIF-1α is one of the most critical factors in the identified hypoxia signal transduction pathway. In the present study, we found that the expression of Elongin B in the HIF pathway was down-regulated and p300/CBP was up-regulated after hypoxia stress. Interestingly, we did not observe a change in the expression level of the HIF-1α gene after 24 h hypoxia stress. One possible explanation for the seemingly surprising results that the expression level of HIF-1α gene did not change after 24 h of hypoxia stress may be associated with the timing of regulation between HIF-1α mRNA and HIF-1α protein levels, rather than a complete lack of response to hypoxia stress. On the other hand, we found that HSP90 levels decreased after hypoxia stress. HSP90 can modulate the stabilization of HIF-1α to regulate the expression of cytoprotective genes under hypoxia stress. In addition, transcriptome analysis indicated that hypoxia stress changed the expression of genes related to immune functions, cell death, stress response and ion transport. These results provided valuable information for understanding the molecular regulation mechanism of mud crab under hypoxia stress.

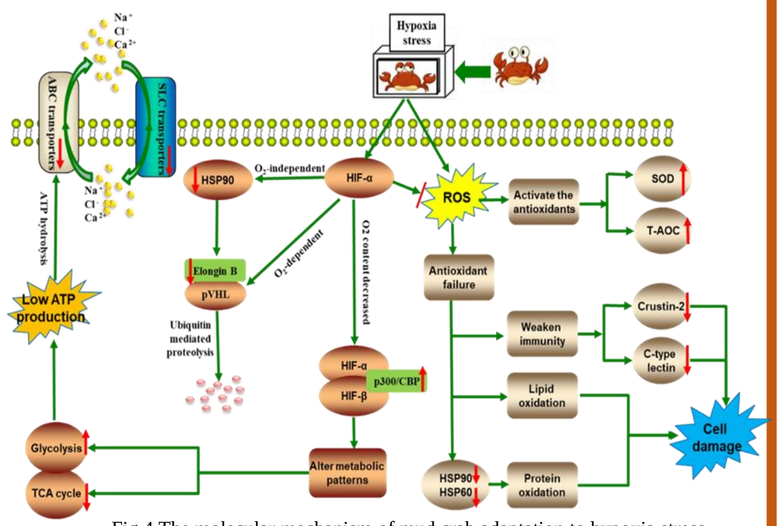


Fig.4 The molecular mechanism of mud crab adaptation to hypoxia stress.