The cytochrome c oxidase and its mitochondrial function in the whiteleg shrimp *Litopenaeus vannamei* during hypoxia

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**Abstract** Cytochrome c oxidase (COX), which is located in the inner membrane of mitochondria, is a key constituent of the electron transport chain that catalyzes the reduction of oxygen. The Pacific whiteleg shrimp *Litopenaeus vannamei* is constantly exposed to hypoxic conditions, which affects both the central metabolism and the mitochondrial function. The purpose of this study was to isolate shrimp mitochondria, identify the COX complex and to evaluate the effect of hypoxia on the mitochondrial function and in the COX activity. A 190 kDa protein was identified as COX by immunodetection techniques. The effect of hypoxia was confirmed by an increase in the shrimp plasma L-lactate concentration. COX activity, mitochondrial oxygen uptake and protein content were reduced under hypoxic conditions, and gradually restored as hypoxia continued, this suggests an adaptive mitochondrial response and a highly effective COX enzyme. Both mitochondrial oxygen uptake and COX activity were completely inhibited by KCN and sodium azide, suggesting that COX is the unique oxidase in *L. vannamei* mitochondria.

**Keywords** COX activity · Hypoxia · Inhibition · Mitochondrial oxygen uptake · Shrimp

**Introduction**

The synthesis of chemical energy and respiration in eukaryotic cells is carried out in the mitochondrion (Mayevsky and Rogatsky 2007). Cytochrome c oxidase (COX) is a multimeric complex which catalyzes the reduction of dioxygen (O₂) to water, being the terminal enzyme of the electron transport chain (Vijayasarthathy et al. 2003; Dukkina et al. 2008; Fontanesi et al. 2008).

In the marine environment, the concentration of dissolved oxygen varies cyclically reaching levels considered as hypoxic, which affects the energy metabolism of marine invertebrates (Abele et al. 2007). Since oxygen is the final electron acceptor, the respiratory process of mitochondria is not completed during hypoxia, this promotes a failure on the oxidative phosphorylation process and the inhibition of ATP synthesis (Hochachka and Somero 2002).

In aerial breathers, the prolonged effect of hypoxia produces a failure in the activity of mitochondrial enzymes as COX, which responds to hypoxia by altering its subunits composition and reducing its activity (Prabu et al. 2006; Fukuda et al. 2007).

The Pacific whiteleg shrimp *Litopenaeus vannamei* is one of the most studied species around the world due its commercial importance (Paez-Osuna et al. 2003); however, scarce information concerning crustacean mitochondrial enzymes and their response to hypoxia is available.

In our knowledge only three studies have dealt with the response of COX to hypoxia in crustaceans, these include the quiescent embryos of *Artemia franciscana* (Hofmann and Hand 1990), the mud crab *Scylla serrata* (Patial and Chainy 2012), and the white shrimp *Litopenaeus vannamei* (Jimenez-Gutierrez et al. 2013), but many questions are open to