Surviving environmental stress: the role of betaine aldehyde dehydrogenase in marine crustaceans

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Abstract
Betaine aldehyde dehydrogenase (BADH) belongs to the aldehyde dehydrogenases (ALDH) family, an ancestral group of enzymes responsible for aldehyde detoxification in several organisms. The BADH enzyme catalyzes the irreversible oxidation of betaine aldehyde to glycine betaine (GB) an important osmoprotector and osmoregulator accumulated in response to cellular osmotic stress. The BADH enzymes have been extensively described in terrestrial organisms, but information in marine crustaceans remains scarce. Research on crustacean stress-adaptive capacity to environmental stressors relates GB accumulation in response to salinity variations. Although GB de novo synthesis is confirmed on crustaceans, its metabolic pathways and regulation mechanism are unexplored. In this work, the state of the knowledge of betaine aldehyde dehydrogenase enzymes in marine crustaceans is summarized, as a mechanism to overcome the deleterious effects of changes in temperature, salinity and dissolved oxygen concentration in seawater. The purpose of this review is to provide a more comprehensive overview to set the basis for exploring novel functions and properties of BADHs on the response of crustaceans to environmental stress.

Key Words: betaine aldehyde dehydrogenase; stress response; osmoregulation; glycine betaine

Introduction
Besides being the largest and most dynamic reservoir of biomass on Earth, oceans are the ultimate repository for a vast amount of discharged compounds via human activities (Kennish, 1996). In addition, marine dynamics promote continuous changes in environmental factors such as temperature, salinity and dissolved oxygen concentration, which, in addition to the presence of marine pollutants, significantly affects marine wildlife. The deterioration of the environmental conditions in the oceans may eventually lead to warming, eutrophication, acidification, oxygen depletion (hypoxia) and the accumulation of toxic compounds as ammonia and sulfides (Fabry et al., 2008; Hoegh-Guldberg and Bruno 2010; Stiti et al., 2011).

A long list of toxic contaminants has been found affecting the marine ecosystem. Oil, gas and agricultural wastes are among the most studied pollutants affecting the marine environment. In addition, bioactive metabolites synthesized by microalgae in marine ecosystems, as various unsaturated aldehydes, are among the most toxic compounds(Leftlave and Ten Hage, 2009 The interaction between the aldehyde functional group of such secondary microalgal metabolites with biological molecules increases its toxicity. Nucleophile varieties of primary amines and sulphhydril or hydroxyl containing molecules are the main class of compounds that can be targeted by aldehydes, and such interactions disrupt biological function and compromise cell integrity (Taylor et al.,
Thus, it is known that aldehyde accumulation causes cellular dysfunction through protein/DNA damage, membrane destruction, and oxidative stress.

Detoxifying enzymes belong to the second line of cellular defense on aquatic organisms and contribute to their survival. Aldehyde dehydrogenases (ALDH) are an extensive group of enzymes involved in the oxidation of environmental aliphatic and aromatic aldehydes, endobiotic or xenobiotic metabolism and lipids peroxidation (Jackson et al., 2011). Oxidation of reactive aldehydes to their corresponding carboxylic acids, a major detoxifying pathway of aldehydes, occurs through the reaction catalyzed by ALDHs (Stitti et al., 2011). The resulting carboxylic acids, as glycine betaine (GB), are subsequently involved in cell growth and osmoregulation. Furthermore, the ALDH enzymes have a non-catalytic function as antioxidant, UV radiation absorption and direct binding to protect molecules from cellular stressors (Vasiloiu et al., 2013).

Betaine aldehyde dehydrogenase (BADH) belongs to the ALDH9 family and catalyzes the irreversible oxidation of betaine aldehyde to glycine betaine (GB, or N, N, N-trimethyl amine) which is an essential organic osmolyte accumulated in cells under hypertonic stress for cell volume and turgor control (Burg et al., 2007; Burg and Ferraris, 2008; Chen and Murata, 2011). GB plays a key role in animals as an osmolyte and osmoproteant to counteract cellular osmotic stress (Perozich et al., 1999; Julián-Sánchez et al., 2007). Studies on GB accumulation in marine organisms have demonstrated that it participates in several biological processes including arsenic accumulation (Fujihara et al., 2003), osmotic adjustment (De Voors and Geenevasen, 2002), modulation of feeding periods (Treberg and Trischitta, 2007), and prevention of accumulation of denaturing agents (Trischitta et al., 2012). Despite the role of BADH in osmoregulation, most studies on BADH enzymes are focused on terrestrial animals, bacteria and plants, while the information regarding its mode of action in aquatic organisms remains scarce (Arakawa et al., 1987; Pan, 1988; Arakawa et al., 1990; Valenzuela-Soto and Muñoz-Clares, 1994; Guzman-Partida and Valenzuela-Soto, 1998; Chern and Pietruszko, 1999; Velasco-Garcia et al., 1999; Figueroa-Soto and Valenzuela-Soto, 2000; Muñoz-Clares and Valenzuela-Soto, 2008).

To date, several studies have been focused on understanding the biology of marine crustaceans, particularly of commercially important species such as shrimp, crabs and lobsters, whose physiological abilities to face environmental stress have been demonstrated through their permanence in the marine ecosystem. Previous evidence confirms that crustaceans under conditions of osmotic stress may display response mechanisms such as accumulation of a variety of organic solutes (osmolytes) in an effort to counteract the resulting movement of water. These osmolytes may include free amino acids, polyhydric alcohols, quaternary ammonium or tertiary sulphonium compounds (Jahn et al., 2006).

Few studies have focused on the accumulation of osmolytes in euryhaline marine invertebrates, including mollusks and crustaceans, under different osmotic stress conditions (Pierce et al., 1995). However, the key enzymes participating in such regulatory mechanism, as BADH, have not been studied, and their roles remain to be understood. The aim of the review is to summarize the current state-of-the-art on aldehyde dehydrogenases from marine invertebrates, especially those enzymes that allow crustaceans to overcome osmotic stress conditions, in order to explore new functions and properties of this fascinating group of enzymes. Also, understanding the pathways to synthesize osmoprotectants may help to increase our knowledge about the adaptive mechanisms of these marine species and their differences with some other animal models.

**Aldehyde dehydrogenases super family and betaine aldehyde dehydrogenase**

The large family of aldehyde dehydrogenases (ALDH) may play two central roles: the enzymatic detoxification of diverse aldehyde compounds, and the synthesis of molecules such as retinoic acid, which participate in central cellular processes. The diverse functions of this family of enzymes includes production of biomolecules, modulation of cell survival, osmoregulation and osmoprotection, hormone interactions, among others (Jackson et al., 2011). The number of ALDH genes is constantly increasing, encoding proteins belonging to 24 distinct ALDH families; however, the biological roles of some of these enzymes are still not elucidated (Jackson et al., 2011; Vasiloiu et al., 2013).

ALDHs are a numerous and ancestral group of enzymes classified on various families: The mitochondrial and cytosolic ALDH1 family is involved in retinoic acid signaling; mitochondrial enzymes from animals and plants (ALDH2) are particularly important enzymes for human pathologies, they are associated with acetalddehydes from alcohol metabolism, and ALDH2 dysfunction may contribute to a variety of human diseases. ALDH3 family includes dioxin-inducible enzymes from animals, metabolizing aldehydes from lipid peroxidation; ALDH4, also known as pyrroline-5-carboxylate dehydrogenases (P5C) are mitochondrial enzymes that catalyze the second step of the proline degradation pathway; the succinate-semialdehyde dehydrogenases ALDH5, which is essential for the removal of the GABA metabolite succinic semialdehyde; the methyl malonate semialdehyde dehydrogenases (ALDH6), and the animal and plant antiquitin group (ALDH7) that participates in lysine catabolism; the cytosolic (ALDH8) that participate on retinaldehyde metabolism; and betaine aldehyde dehydrogenases from animals (ALDH9) and plants (ALDH10) (Vasiloiu et al., 1999; Julian-Sánchez et al., 2007; Swenby and Picklo, 2009).

The betaine aldehyde dehydrogenases (BADHs, E.C. 1.2.1.8.), the central topic of this review, belong to the ALDH9 family and play a key role in the detoxification of aldehydes.
### Table 1 ALDH9 enzymes identified in various marine species

<table>
<thead>
<tr>
<th>Organism/Enzyme</th>
<th>Tissue/subcellular location</th>
<th>Protein Size (kDa)</th>
<th>Accession No.</th>
<th>Kinetics parameters</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horseshoe crab</td>
<td>Heart</td>
<td>-</td>
<td>-</td>
<td>KmBA: 133 M</td>
<td>Dragolovich, 1994</td>
</tr>
<tr>
<td><em>Limulus polyphemus</em></td>
<td></td>
<td></td>
<td></td>
<td>VmaxBA: 220 μmoles/min/mL</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td>KmNAD: 22 μM</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>KmNADP: 267 μM</td>
<td></td>
</tr>
<tr>
<td>Oyster/BADH</td>
<td>Gills/mitochondria</td>
<td>62.3</td>
<td>-</td>
<td>KmBA: 360 μM</td>
<td>Perrino and Pierce, 2000</td>
</tr>
<tr>
<td><em>Crassostrea virginica</em></td>
<td></td>
<td></td>
<td></td>
<td>Vmax: 1.7 μmoles/min/mL</td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td>54.8</td>
<td>XP_005919907</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td><em>Haplochromis burtoni</em></td>
<td></td>
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<td></td>
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<tr>
<td>Cod</td>
<td>Liver</td>
<td>54.3</td>
<td>P56533.1</td>
<td>KmBA: 140 μM</td>
<td>Johansson et al., 1998</td>
</tr>
<tr>
<td><em>Gadus morhua</em></td>
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<tr>
<td>Zebra fish</td>
<td></td>
<td>55.2</td>
<td>NP_958879</td>
<td>-</td>
<td>Ling et al., 2012</td>
</tr>
<tr>
<td><em>Danio rerio</em></td>
<td></td>
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<tr>
<td>Plankton</td>
<td></td>
<td>6.4</td>
<td>AEP43737.1</td>
<td>-</td>
<td>Lauritano et al., 2012</td>
</tr>
<tr>
<td><em>Calanus helgolandicus</em></td>
<td></td>
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<td></td>
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<tr>
<td>Sea hare</td>
<td>Aplysia californica</td>
<td>56.1</td>
<td>XP_005091798.1</td>
<td>-</td>
<td>Knudsen et al., 2006</td>
</tr>
<tr>
<td>Elephant shark</td>
<td>Callorhinus milli</td>
<td>56.9</td>
<td>XP_007893828.1</td>
<td>-</td>
<td>Venkatesh et al., 2014</td>
</tr>
<tr>
<td>Coelacanth</td>
<td><em>Latimeria chalumnae</em></td>
<td>Blood</td>
<td>53.4</td>
<td>XP_005994849.1</td>
<td>Zardoya and Meyer, 1997</td>
</tr>
<tr>
<td>Green sea turtle</td>
<td><em>Chelonia mydas</em></td>
<td>-</td>
<td>51.7</td>
<td>EMP34519.1</td>
<td>Wang et al., 2013</td>
</tr>
</tbody>
</table>

Role in the metabolic pathways of organisms. As previously mentioned, BADHs participate in the betaine aldehyde oxidation to glycine betaine, an osmotically active solute synthesized in cells and released into the blood stream, acting as an osmoregulator (Muñoz-Clares and Valenzuela-Soto, 2008). Despite BADHs is a source for GB accumulation for osmotic adaptation, animals have been described as γ-aminobutyraldehyde and trimethylaminobutyraldehyde dehydrogenases (TMABA-DH) because their ability to accept other aminoaldehydes as a substrate (Vaz et al., 2000). This implicates BADH in the carnitine biosynthesis, an indispensable compound for the transport of activated fatty acids in mitochondria. Moreover, high levels of L-carnitine have been reported in distinct invertebrates (Özogul et al., 2013).

To date, mammalian BADHs are by far the most studied enzyme of this family (Julián-Sánchez et al., 2007). The human ALDH9 was intensely studied during the 1980s and 90s. Pietruszko (1997) postulated various roles for the enzyme, including the conversion of betaine aldehyde to betaine and its putative function as a defense against osmotic stress; however, the enzyme structure was not solved until 1998, when the cod liver betaine aldehyde dehydrogenase was described as similar to that of other ALDHs, as ALDH2 (mitochondrial) and ALDH3 (induced by dioxins). Each subunit of this tetrameric enzyme contains a coenzyme binding domain, a catalytic domain, and an oligomerization domain; and the conserved residues Asn and Cys comprising the substrate binding pocket were identified (Johansson et al., 1998). Yoshida et al. (1998) described the human aldehyde dehydrogenase gene family. A total of twelve genes encoding the enzyme were described, including group ALDH9; this last group of genes is mainly expressed in liver, kidney, and muscle. Soon thereafter, the first mitochondrial form of the rat liver BADH was identified, and the existence of...
Fig. 1 Kinetic mechanism for choline oxidation and glycine betaine synthesis. CDH: Choline Dehydrogenase, BADH: Betaine Aldehyde Dehydrogenase, BA: Betaine Aldehyde, GB: Glycine Betaine.

The first isolated and characterized BADH from crustaceans was reported by Dragolovich (1994). The active enzyme was partially purified from mitochondria of the heart of the horseshoe crab Limulus polyphemus, and showed high specificity for betaine aldehyde with a Michaelis-Menten constant ($K_m$) of 133 μM. The end product, GB, was demonstrated to be a competitive inhibitor of BADH activity, and according to these findings, BADH activity is modulated through the increase/decrease in concentrations of inorganic ions as $K^+$ and $Na^+$.

To the best of our knowledge, at present there are no reports of any amino acid sequences of partially purified, isolated or solved structure of crustacean BADHs. Thus, there is no evidence about the ability of crustacean’s BADH to use efficiently aminoaldehydes as substrates, as is typically observed in mammalian enzymes (Muñoz-Clares and Valenzuela-Soto, 2008). In addition, there is a lack of information about the expression of BADH in crustaceans as a response to environmental stress that correlates with GB accumulation.

So far, BADH activity of a small number marine invertebrate species has been detected using isolated mitochondria, such as that reported for L. polyphemus, whose enzyme is exclusively found in mitochondria (Dragolovich, 1994). In the same way, BADH activity has been found on the mitochondrial matrix of the oyster Crassostrea virginica which controls the synthesis of GB. Interestingly, the enzyme kinetics showed variations according to the extracellular ions concentration, being active even if the organism is not osmotically stressed (Perrino, 2000).
Fig. 2 Proposed mechanism for osmotic response in crustaceans. Intracellular choline intake is controlled by membrane choline transporters in response to ion concentration variations. Short-term hyperosmotic stress is controlled through Na⁺/K⁺ ions exchanges while long-term hyperosmotic stress requires osmolyte participation to maintain cellular homeostasis. Choline is transported into the inner mitochondrial for GB synthesis and excreted to cytosol for osmoprotection and osmoregulatory process.

Betaine aldehyde dehydrogenase and the osmotic stress response

Osmotic stress is a recurrent condition for some marine species, particularly for those living in near-shore environments as estuaries where coastal rivers, streams, and the intense solar incidence and evaporation continuously change the salinity of seawater (True, 2012). Most marine crustaceans are often considered as osmoconformers, with a high capacity of tissue water regulation that relies mainly on salt transport to prevent extracellular osmotic disturbances (Foster et al., 2010).

Probably one of the more important adaptive traits of marine crustaceans is their high body fluid osmolality, similar to that of the seawater (near to 1,000 mOsm). However, it has been demonstrated that when large variations in the water salinity are produced, the osmotic stress response of crustaceans involves gill transport mechanisms, changes in membrane permeability and a reduced production of concentrated urine (Sherwood et al., 2004). Also, a series of molecules including some enzymes participating in central metabolic pathways as oxidative stress proteins, chaperones, proteases, cytoskeletal proteins and detoxification of xenobiotics have been suggested as part of the response (Tomanek, 2011).

Besides its well-known use of free amino acids as osmolytes, the synthesis of osmoprotectant quaternary bases in the mitochondrial matrix, such as GB, contributes in the adaptive response of marine organisms to high salinities. According to previous evidence, choline (N, N, N-trimethyl-β-hydroxyethanolamine), which is supplied to shrimp
species as *Penaeus monodon* via commercial feed formulations, is an essential dietary component and the precursor for the synthesis of GB (Richard et al., 2011). Previous studies have reported that in mammalian cells, choline is oxidized in kidney and liver mitochondria. A specific choline transporter in the inner mitochondrial membrane allows choline molecules to reach the inner side of the membrane, and the FAD-linked membrane bound choline dehydrogenase (CDH) (EC 1.1.99.1) converts choline to betaine aldehyde; then the NAD-linked betaine aldehyde dehydrogenase (BADH) oxidizes betaine aldehyde to GB (Porter et al., 1992; O’Donoghue et al., 2009) (Fig. 1). Both enzymes, CDH and BADH, are soluble proteins mainly found in the cytosol (Grossman, 1989; Figueroa-Soto et al., 1999), and a small fraction of BADH is also found in the mitochondria of vertebrates (Chern and Pietruzko, 1999).

In crustaceans, mitochondria isolated from gills and hepatopancreas are able to uptake choline to convert it into GB, suggesting that the mitochondrial matrix may possess the required enzymes for choline metabolism (Dragolovich, 1994). Previous evidence showed, in addition, an accumulation of GB in hepatopancreas, gills and heart of the crab *Neohelicus (Chasmagnathus)* granulata exposed to long-term hyperosmotic stress (72 h), demonstrating that GB regulation has an important role in the response to periods of prolonged stress (Jahn et al., 2006).

Although the activity of choline dehydrogenase and betaine aldehyde dehydrogenase in crustaceans have not been characterized, the evidence of *de novo* synthesis of GB from choline suggests that crustaceans may possess mechanisms for the metabolism and transport of choline and GB (Jahn et al., 2006). Based on this information, a preliminary model for crustacean’s response to osmotic stress is proposed here (Fig. 2). In this model, cellular choline, under hyperosmotic conditions, is taken up and subsequently transported to the inner mitochondrial membrane, where it is oxidized to GB and betaine aldehyde by CDH and BADH activity, respectively. The synthesis and accumulation of GB in cells in response to long-term osmotic stress is important for the regulation of cell volume and protein stabilization.

**Crustacean’s responses to hypoxia**

Another environmental factor that continuously imposes a physiological challenge to crustaceans is hypoxia. According to previous studies, oxygen concentrations may vary from normoxic conditions (6 - 7 mg/L) to hypoxia (1 - 3 mg/L) in a diurnal cycle (Puente, 2009); therefore, aquatic organisms display a set of biochemical strategies, as well as distinct metabolic pathways to ensure stress adaptation. These mechanisms involve a number of stress response proteins which are known to be highly regulated (Palacios and Racotta, 2007). According to the findings of Jiang et al. (2009), the aldehyde dehydrogenase (ALDH1 like) of the shrimp *Penneropenaeus chinensis* is differentially expressed in the hepatopancreas of shrimp exposed to hypoxia.

Although the BADH of the shrimp *Penaeus vannamei* has not been characterized, it may be involved in the response to the effects provoked by the White Spot Syndrome Virus (WSSV). This arises from the evidence of severe infection of shrimp after NAD-dependent aldehyde dehydrogenase and HSP70 genes knockdown by double-stranded RNA interference (dsRNA). A conserved domain analysis identifies this enzyme as a member of ALDH4 family, enzymes involved in pyrroline-5-carboxylate dehydrogenase activity. In addition, a beneficial interaction between these proteins, by promoting the inhibition of the virus replication process, was suggested. It was further proposed that ALDH activity reduces the generation of toxic by-products, as aldehydes, during the oxidative stress caused by the observed hyperthermic protection of shrimp against WSSV (Lin et al., 2011). These results encourage the necessity to study other ALDHs, such as ALDH9 enzymes to elucidate their participation on stress response mechanisms in crustaceans.

**Concluding remarks and future directions**

Salinity oscillations, as other physical factors, including temperature and oxygen, affect the behavior, distribution, growth, reproduction, and survival of organisms inhabiting the oceans. Such disturbances have shown to elicit adaptive responses that are crucial to protect the organism against subsequent exposure to similar environmental insult. When water salinity fluctuations occur, the osmotic pressure of the celomic fluid of marine invertebrates changes, which commonly leads to osmotic stress (Torres et al., 2007; Rhodes-Onidi and Turner, 2009). Osmotic stress induces adverse effects over cellular ion regulation, which may lead to disruption of protein synthesis and protein damage (Yancey et al., 1982; Kültz, 2005; Fiol and Kültz, 2007). Thus, osmoregulation is a central physiological mechanism intended to conserve the homeostasis of marine animals.

The accumulation of GB is critical for crustaceans to achieve a full response against environmental stress, which requires a functional biosynthetic pathway for GB. Enzymes catalyzing irreversible reactions are often considered as control sites of metabolic pathways, and the importance of BADHs is evident from the fact that its principal substrate, betaine aldehyde, is exclusively synthesized from choline and seems to play a central role in controlling the abilities of marine crustaceans to deal with osmotic and environmental stress. The BADHs investigated to date represent a heterogeneous group in accordance to their structure, localization and kinetic mechanism, relating these characteristics with enzyme participation at distinct metabolic levels. Further investigations focused on BADH biochemical and kinetic characterization is needed to increase the knowledge of stress regulation in crustaceans.
Despite its fundamental osmoregulatory and detoxifying roles in marine crustaceans, betaine aldehyde dehydrogenase (BADH) is still poorly studied. It is precisely this lack of knowledge what should provide a stimulus for further promising research efforts. As our scientific knowledge of their structure and function improves and we reach a deeper understanding of its catalytic mechanisms and evolution, BADHs from crustaceans may yield as a potential source for biotechnological applications. Thus, it seems likely that the biological importance of BADHs might continue to expand. Furthermore, the important role of BADH should be emphasized as a tangible application to improve shrimp farming production by reducing stress in crustaceans.

References


