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REVIEW

# 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 osmoptrotector 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(Leflaive and Ten‐Hage, 2009 The interaction between the aldehyde functional group of such secondary microalgal metabolites with biological molecules increases its toxicity. Nucleophilic varieties of primary amines and sulphydryl 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.,*

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2005). 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 (Stiti *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 (Vasiliou *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 osmoprotectant 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 Vooys and Geenevasen, 2002), modulation of feeding periods (Treberg and Driedzic, 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-García *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; Vasiliou *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 acetaldehydes 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) (Vasiliou *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

**Table 1** ALDH9 enzymes identified in various marine species

| Organism/ Enzyme | Tissue/ subcellular location | Protein Size (kDa) | Accesion No. | Kinetics parameters | References |
|---|---|---|---|---|---|
| Horseshoe crab *Limulus polyphemus* | Heart | - | - | $Km_{BA}$: 133 M<br>$Vmax_{BA}$:220 μmoles/min/mL<br>$Km_{NAD}$: 22 μM<br>$Km_{NADP}$: 267 μM | Dragolovich, 1994 |
| Oyster/BADH *Crassostrea virginica* | Gills/ mitochondria | 62.3 | - | $Km_{BA}$: 360 μM<br>Vmax: 1.7 μmoles/min/mL<br>$Km_{NAD}$: 350 μM | Perrino and Pierce, 2000 |
| Fish *Haplochromis burtoni* | - | 54.8 | XP_005919907 | - | ND |
| Cod *Gadus morhua* | Liver | 54.3 | P56533.1 | $Km_{BA}$: 140 μM | Johansson *et al*., 1998 |
| Zebra fish *Danio rerio* | - | 55.2 | NP_958879 | - | Ling *et al.*, 2012 |
| Plankton *Calanus helgolandicus* | - | 6.4 | AEP43737.1 | - | Lauritano *et al.*, 2012 |
| Sea hare *Aplysia californica* | - | 56.1 | XP_005091798.1 | - | Knudsen *et al.*, 2006 |
| Elephant shark *Callorhinchus milii* | - | 56.9 | XP_007893828.1 | - | Venkatesh *et al.*, 2014 |
| Coelacanth *Latimeria chalumnae* | Blood | 53.4 | XP_005994849.1 | - | Zardoya and Meyer, 1997 |
| Green sea turtle *Chelonia mydas* | - | 51.7 | EMP34519.1 | - | Wang *et al.*, 2013 |

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 osmoprotectant compound (Muñoz-Clares and Valenzuela-Soto, 2008). Despite BADHs is a source for GB accumulation for osmotic adaptation, animals BADHs have been described as γ-aminobutyraldehyde and trymethylaminobutyraldehyde 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

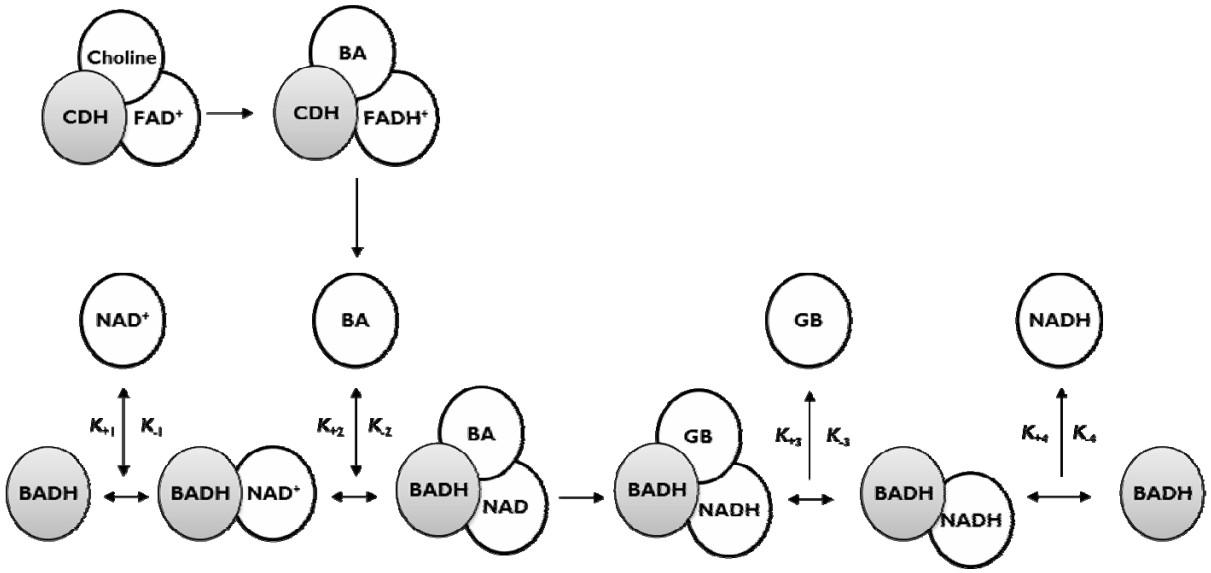


**Fig. 1** Kinetic mechanism for choline oxidation and glycine betaine synthesis. CDH: Choline Dehydrogenase, BADH: Betaine Aldehyde Dehydrogenase, BA: Betaine Aldehyde, GB: Glycine Betaine.

two isoenzyme forms (cytosolic and the mitochondrial), encoded by the same gene, was suggested (Chern and Pietruszko, 1999; Pietruszko and Chern, 2001).

In recent years, developments in the field have led to an increase in the amount of information on ALDH9 and BADH genes, and on proteins from several species, with approximately 16,765 entries from Pfam database (Jackson *et al.,* 2011). However, only a limited number of these enzymes have been biochemically and kinetically characterized. Even though BADHs play a key role in animal adaptive mechanisms, information about these enzymes in marine species is scarce (Table 1).

*The crustacean BADHs*

An increasing number of studies about ALDHs from marine species has emerged from recent research developments, mainly as an effort to better understand their function, which is closely related to the ability of these animals to face stress conditions in their environment.

The main function attributed to invertebrate BADHs is their ability to synthesize glycine betaine (GB), an organic compound that can be produced in tissues and extracellular fluids of marine animals to balance their internal osmolarity with respect to the external environment (Burg and Ferraris, 2008). Jahn *et al.* (2006) demonstrated for the first time that GB is accumulated in crustaceans as an organic solute involved in the osmotic adjustment during hyperosmotic stress and that metabolic responses occur mainly in the hepatopancreas of these species.

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).

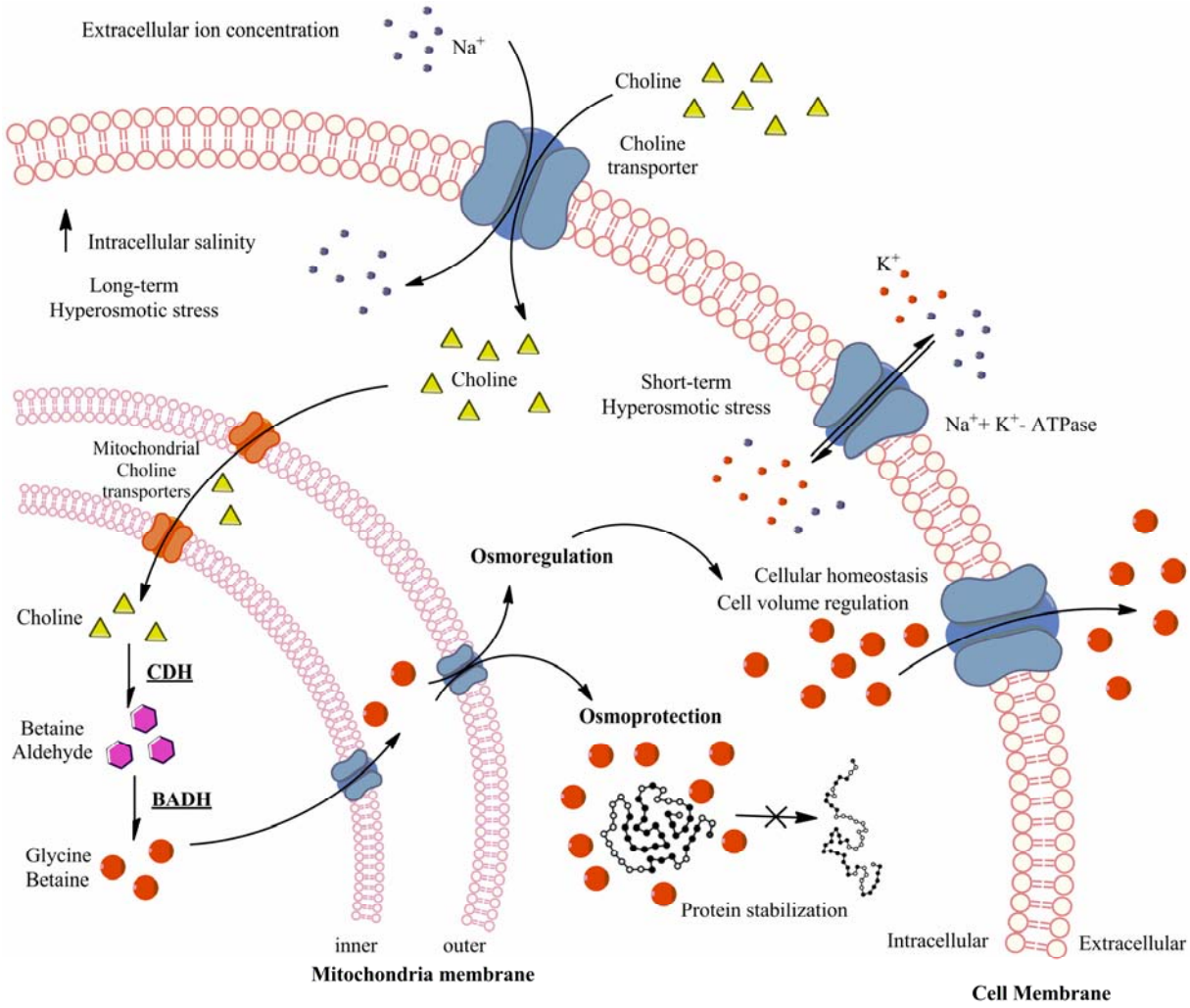


**Fig. 2** Proposed mechanism for osmotic response in crustaceans. Intracellular choline intake is controlled by membrane choline transporters in response to ion concentration variations. Short-term hyperosmotic stress is controlled through $Na^+/K^+$ ions exchanges while long-term hyperosmotic stress 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 sea water (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 a*l., 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 *Neohelice (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 *Fenneropenaeus 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-Ondi 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**


Arakawa K, Katayama M, Takabe T. Levels of betaine and betaine aldehyde dehydrogenase activity in the green leaves, and etiolated leaves and roots of barley. Plant Cell Physiol. 31: 797-803, 1990.

Arakawa K, Takabe T, Sugiyama T, Akazawa T. Purification of betaine aldehyde dehydrogenase from spinach leaves and preparation of its antibody. J. Biochem. 101: 1485-1488, 1987.

Burg MB, Ferraris JD. Intracellular organic osmolytes: function and regulation. J. Biol. Chem. 283: 7309-7313, 2008.

Burg MB, Ferraris JD, Dmitrieva NI. Cellular response to hyperosmotic stresses. Physiol. Rev. 87: 1441-1474, 2007.

Chen THH, Murata N. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. Plant Cell Environ. 34: 1-20, 2011.

Chern MK, Pietruszko R. Evidence for mitochondrial localization of betaine aldehyde dehydrogenase in rat liver: purification, characterization, and comparison with human cytoplasmic E3 isozyme. Biochem. Cell Biol. 77: 179-187, 1999.

Chern MK, Pietruzko R. Evidence for mitochondrial localization of betaine aldehyde dehydrogenase in rat liver: purification, characterization, and comparison with human cytoplasmic E3 isozyme. Biochem. Cell Biol. 77: 179-187, 1999.

De Vooys C, Geenevasen J. Biosynthesis and role in osmoregulation of glycine-betaine in the Mediterranean mussel *Mytilus galloprovincialis* LMK. Comp. Biochem. Physiol. 132B: 409-414, 2002.

Dragolovich J. Characterization of partially purified betaine aldehyde dehydrogenase from horseshoe crab (*Limulus polyphemus*) cardiac mitochondria. J. Exp. Zool. 270: 417-425., 1994.

Fabry VJ, Seibel BA, Feely RA, Orr JC. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J. Mar. Sci.: Journal du Conseil 65: 414-432, 2008.

Figueroa-Soto CG, López-Cervantes G, Valenzuela-Soto EM. Immunolocalization of betaine aldehyde dehydrogenase in porcine kidney. Biochem. Biophys. Res. Commun. 258: 732-736, 1999.

Figueroa-Soto CG, Valenzuela-Soto EM. Kinetic study of porcine kidney betaine aldehyde dehydrogenase. Biochem. Biophys. Res. Commun. 269: 596-603, 2000.

Fiol DF, Kültz D. Osmotic stress sensing and signaling in fishes. FEBS J. 274: 5790-5798, 2007.

Foster C, Amado EM, Souza MM, Freire CA. Do osmoregulators have lower capacity of muscle water regulation than osmoconformers? A study on decapod crustaceans. J. Exp. Zool. Part A 313: 80-94, 2010.

Fujihara J, Kunito T, Kubota R, Tanabe S. Arsenic accumulation in livers of pinnipeds, seabirds and sea turtles: subcellular distribution and interaction between arsenobetaine and glycine betaine. Comp. Biochem. Physiol. 136C: 287-296, 2003.

Grossman EB, Hebert SC. Renal inner medullary choline dehydrogenase activity: characterization and modulation. Am. J. Physiol. 256 (Renal Fluid Electrolyte Physiol. 25): F107-F 112, 1989.

Guzman-Partida AM, Valenzuela-Soto EM. Porcine kidney betaine aldehyde dehydrogenase: purification and properties. Comp. Biochem. Physiol. 119B: 485-491, 1998.

Hoegh-Guldberg O, Bruno JF. The impact of climate change on the world's marine ecosystems. Science 328: 1523-1528, 2010.

Jackson B, Brocker C, Thompson DC, Black W, Vasiliou K, Nebert DW, *et al.* Update on the aldehyde dehydrogenase gene (ALDH) superfamily. Hum. Genomics 5: 283-303, 2011.

Jahn MP, Cavagni GM, Kaiser D, Kucharski LC. Osmotic effect of choline and glycine betaine on the gills and hepatopancreas of the *Chasmagnathus granulata* crab submitted to hyperosmotic stress. J. Exp. Mar. Biol. Ecol. 334: 1-9, 2006.

Jiang H, Li F, Xie Y, Huang B, Zhang J, Zhang C, *et al.* Comparative proteomic profiles of the hepatopancreas in *Fenneropenaeus chinensis* response to hypoxic stress. Proteomics 9: 3353-3367, 2009.

Johansson K, El-Ahmad M, Ramaswamy S, Hjelmqvist L, Jörnnvall H, Eklund H. Structure of betaine aldehyde dehydrogenase at 2.1 A resolution. Protein Sci. 7: 2106-2117, 1998.

Julián-Sánchez A, Riveros-Rosas H, Martínez-Castilla LP, Velasco-García R, Muñoz-Clares RA. Phylogenetic and structural relationships of the betaine aldehyde dehydrogenases. Enzymology and Molecular Biology of Carbonyl Metabolism. In: Weiner H, Plapp B, Lindahl R, Maser E (eds), West Lafayette, Purdue University Press, 13: 64-76, 2007.

Kennish MJ. Practical handbook of estuarine and marine pollution, CRC press, 1996.

Knudsen B, Kohn AB, Nahir B, McFadden, CS, Moroz LL. Complete DNA sequence of the mitochondrial genome of the sea-slug, Aplysia californica: Conservation of the gene order in Euthyneura. Mol. Phylogen. Evol. 38: 459-469, 2006.

Kültz D. Molecular and evolutionary basis of the cellular stress response. Annu. Rev. Physiol. 67: 225-257, 2005.

Lauritano C, Procaccini G, Ianora A. Gene expression patterns and stress response in marine copepods. Mar. Environ. Res. 76: 22-31, 2012.

(Leflaive and Ten ‐ Hage, 2009diatoms: role of polyunsaturated aldehydes and precursors. New Phytologist 184: 794-805, 2009.

Lin Y-R, Hung H-C, Leu J-H, Wang H-C, Kou G-H, Lo C-F. The role of aldehyde dehydrogenase and hsp70 in suppression of white spot syndrome virus replication at high temperature. J. Virol. 85: 3517-3525, 2011.

Ling X.-P, Lu Y-H, Huang H-Q. Differential protein profile in zebrafish (*Danio rerio*) brain under the joint exposure of methyl parathion and cadmium. Environ. Sci. Poll. Res. 19: 3925-3941, 2012.

Muñoz-Clares RA, Valenzuela-Soto EM. Betaine aldehyde dehydrogenases: evolution, physiological functions, mechanism, kinetics, regulation, structure, and stability. In: García-Hernández E, DA Fernández-Velasco DA (eds), Adv. Protein Phys. Chem., Kerala, India, Res. Sign Post, pp 279-302, 2008.

O'Donoghue N, Sweeney T, Donagh R, Clarke KJ, Porter RK. Control of choline oxidation in rat kidney mitochondria. Biochim. Biophys. Acta Bioenergetics 1787: 1135-1139, 2009.

Özogul Y, Kuley Boga E, Özogul F, Ayas D. L ‐ Carnitine Contents in Seafoods Commonly Eaten in Middle Eastern Countries. J. Food Biochem. 37: 702-707. 2013

Palacios E, Racotta IS. Salinity stress test and its relation to future performance and different physiological responses in shrimp postlarvae. Aquaculture 268: 123-135, 2007.

Pan SM. Betaine aldehyde dehydrogenase in spinach. Bot. Bull. Acad. Sinica 29: 255-263, 1988.

Perozich J, Nicholas H, Wang BC, Lindahl R, Hempel J. Relationships within the aldehyde dehydrogenase extended family. Protein Sci. 8: 137-146, 1999.

Perrino L. Betaine aldehyde dehydrogenase kinetics partially account for oyster population differences in glycine betaine synthesis. J. Exp. Zool. 286: 238-249., 2000.

Pietruszko R, Chern MK. Betaine Aldehyde dehydrogenase from rat liver mitochondrial matriz. Chem.-Biol. Interact. 130-132: 193-199, 2001.

Pietruszko R, Chern MK, Izaguirre G. Human aldehyde dehydrogenase E3. Further characterization. Adv. Exp. Med. Biol. 414: 243-252., 1997.

Porter RK, Scott JM, Brand M. Choline transport into rat liver mitochondria. Characterization and kinetics of a specific transporter. J. Biol. Chem. 267: 14637-14646, 1992.

Richard L, Vachot C, Surget A, Rigolet V, Kaushik SJ, Geurden I. The effect of choline and cystine on the utilisation of methionine for protein accretion, remethylation and trans-sulfuration in juvenile shrimp *Penaeus monodon*. Brit. J. Nutr. 106: 825-835, 2011.

Rhodes-Ondi SE, Turner RL. Salinity tolerance and osmotic response of the estuarine hermit crab *Pagurus maclaughlinae* in the Indian River Lagoon, Florida. Estuar. Coast. Shelf Sci. 86: 189-196, 2009.

Stiti N, Adewale I, Petersen J, Bartels D, Kirch H. Engineering the nucleotide coenzyme specificity and sulfhydryl redox sensitivity of two stress-responsive aldehyde dehydrogenase isoenzymes of *Arabidopsis thaliana*. Biochem. J. 434: 459-471, 2011.

Swenby NP, Picklo MJ. The conserved R166 residue of ALDH5A (succinic semialdehyde dehydrogenase) has multiple functional roles. Chem. Biol. Inter. 178: 70-74, 2009.

Taylor RL, Caldwell GS, Bentley MG. Toxicity of algal-derived aldehydes to two invertebrate species: Do heavy metal pollutants have a synergistic effect? Aquat. toxicol. 74: 20-31, 2005.

Taylor RL, Caldwell GS, Bentley MG. Toxicity of algal-derived aldehydes to two invertebrate species: Do heavy metal pollutants have a synergistic effect? Aquat. Toxicol. 74: 20-31, 2005.

Tomanek L. Environmental proteomics: changes in the proteome of marine organisms in response to environmental stress, pollutants, infection, symbiosis, and development. Annu. Rev. Mar. Sci. 3: 373-399, 2011.

Torres G, Giménez L, Anger K. Effects of osmotic stress on crustacean larval growth and protein and lipid levels are related to life-histories: The genus Armases as a model. Comp. Biochem. Physiol. 148B: 209-224, 2007.

Treberg JR, Driedzic WR. The accumulatio and synthesis of betaine in winter skate (*Leucoraja ocellata*). Comp. Biochem. Physiol. 147A: 475-483, 2007.

Trischitta F, Faggio C, Torre A. Living with high concentrations of urea: They can! Open J. Anim. Sci. 2: 32-40, 2012.

True JD. Salinity as a structuring force for near shore coral communities. Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia, 2012.

Valenzuela-Soto EM, Muñoz-Clares RA. Purification and properties of betaine aldehyde dehydrogenase extracted from detached leaves of *Amaranthus hypocondriacus* L. Subjected to water deficit. J. Plant Physiol. 143: 145-152, 1994.

Vasiliou V, Bairoch A, Tipton KF, Nebert DW. Eukaryotic aldehyde dehydrogenase (ALDH) genes: human polymorphisms, and recommended nomenclature based on divergent evolution and chromosomal mapping. Pharmacogenet. Genomics 9: 421-434, 1999.

Vasiliou V, Thompson DC, Smith C, Fujita M, Chen Y. Aldehyde dehydrogenases: from eye crystallins to metabolic disease and cancer stem cells. Chem. Biol. Inter. 202: 2-10, 2013.

Vaz FM, Fouchier SW, Ofman R, Sommer M, Wanders RJ. Molecular and biochemical characterization of rat γ-trimethylamino-butyraldehyde dehydrogenase and evidence for the involvement of human aldehyde dehydrogenase 9 in carnitine biosynthesis. J. Biol. Chem. 275: 7390-7394, 2000

Velasco-García R, Mújica-Jiménez C, Mendoza-Hernández G, Muñóz-Clares R. Rapid purification and properties of betaine aldehyde dehydrogenase from *Pseudomonas aeruginosa*. J. Bacteriol. 181: 1292-1300, 1999.

Venkatesh B, Lee AP, Ravi V, Maurya AK, Lian MM, Swann JB, *et al*. Elephant shark genome provides unique insights into gnathostome evolution. Nature 505: 174-179, 2014.

Wang Z, Pascual-Anaya J, Zadissa A, Li, W, Niimura Y, Huang Z, *et al*. The draft genomes of soft-shell turtle and green sea turtle yield insights into the development and evolution of the turtle-specific body plan. Nat. Genetics 45: 701-706, 2013.

Yancey P, Clark M, Hand S, Bowlus R, Somero G. Living with water stress: evolution of osmolyte systems. Science 217: 1214-1222, 1982.

Yoshida A, Rzhetsky A, Hsu LC, Chang C. Human aldehyde dehydrogenase gene family. Eur. J. Biochem. 251: 549–557, 1998.

Zardoya R, Meyer A. The complete DNA sequence of the mitochondrial genome of a “living fossil,” the coelacanth (*Latimeria chalumnae*). Genetics 146: 995-1010, 1997.