Comparative Biochemistry and Physiology, Part A 161 (2012) 327–336

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Effect of air exposure on lysosomal tissues of *Mytilus edulis* L. from natural intertidal wild beds and submerged culture ropes

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ARTICLE INFO

Article history:
Received 7 June 2011
Received in revised form 1 December 2011
Accepted 1 December 2011
Available online 8 December 2011

Keywords:
Autophagy
Blue mussel
Hypoxia
Lysosomal membrane stability
Stress response

ABSTRACT

Blue mussels collected from suspended culture ropes and from three natural intertidal wild beds from different areas of the German Bight were tested for their ability to cope with hypoxic conditions. During the experiment mussels were exposed to air from 0 to 72 h. Mussels from all sampling sites displayed high tolerance to aerial exposure with moderate levels of mortality after 12 to 48 h of exposure. Lysosomal membrane stability (LMS), a biomarker of general stress, changed notably between minimum values after 12 h and maximum values after 24 h of aerial exposure in intertidal mussels. In contrast, labilization times of mussels from the hanging culture increased continuously up to 48 h of exposure. Intertidal mussels from the island of Heligoland exhibited significantly decreased membrane stability after 72 h of air exposure, correlating to higher mortality rates. Intertidal mussels, although adapted to daily aerial exposure in their natural environment, showed a similar pattern of mortality and lower LMS values during the experiment than mussels from the suspended culture site. The increase of LMS values of mussels under hypoxic conditions at the beginning of the experiment at all sites was tested for the influence of macro-autophagic processes using immune labelling techniques. With this approach it could be demonstrated that high LMS values significantly correlate with low autophagic activity. However, hypoxic conditions do not enhance autophagic processes during the early periods of aerial exposure. Only at the end of the experiment, high values for autophagy were measured in mussels from an intertidal site accompanied with high mortalities. The results indicate that autophagic processes are not involved in the early adaptive processes that enable the mussel to cope with periods of aerial exposure.

1. Introduction

Since the early 1970s when the first biomonitoring programmes were initiated, blue mussels have been the internationally accepted indicator species for the assessment of contamination levels and their harmful effects on marine and estuarine biota (Goldberg, 1975; UNEP, 2007). In addition to having a wide geographic distribution, blue mussels are abundant, stationary, and easy to sample. Further, mussels respond to natural abiotic changes, such as e.g. salinity, tidal or temperature variations (Bayne et al., 1985; Shakhmatova et al., 1991; Zaldibar et al., 2004) and biotic stress caused by parasites, changing food availability or the presence of pathogens (Robledo et al., 1995; Sarà et al., 1998; Galimany et al., 2008). Thus, mussels are able to reflect integrative responses to various stress factors (e.g. Robinson and Langton, 1980; Labarta et al., 1997; Lòpez et al., 2001). As intertidal sessile organisms, mussels have to cope with harsh and rapidly changing physical conditions during tidal cycles, which include thermal stress (Helmuth and Hofmann, 2001), desiccation (Moore et al., 1979), anoxia (Hole et al., 1995), and reduced food availability during periods of emersion. In contrast, subtidal mussels and mussels cultured on submerged ropes inhabit a more stable environment (Hunt and Scheibling, 2001) although differences between high and low tide impact factors such as food availability which could affect their physiology. As a result, some intertidal bivalve species such as the ripped mussel *Geukensia demissa* (Dillwyn) exhibit a higher capacity to adapt to environmental changes more rapidly than subtidal organisms (Charles and Newell, 1997). According to Moore et al. (2006a) animals living in a fluctuating environment are more tolerant towards pollutants since changes in salinities and food availability as well as periodical hypoxia enhance the autophagic processes.

Macroautophagy, usually referred to as autophagy, is a major pathway for bulk degradation of cytoplasmic constituents and organelles (Munafó and Colombo, 2001). In this process, portions of the cytoplasm are sequestered into double membrane vesicles, the autophagosomes, and subsequently delivered to the lysosome for

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doi:10.1016/j.cbpa.2011.12.001

degradation and recycling (Klionsky and Emr, 2000; Kuma et al., 2004). The process is a highly conserved evolutionary mechanism found in diverse groups of animals including nematodes, flies and mammals (Cuervo, 2004; Moore et al., 2006a). Autophagy is up-regulated under stress-inducing situations including hypothermia, hypoxia, environmental factors such as an increase in salinity (Moore et al., 2007) or physiological changes in the mussel; allowing cells to temporarily self-sustain in periods when nutrients are restricted (Bergamini et al., 2003). The energy required is produced by the recycling of proteins, lipids and cell organelles (Levine, 2005). In addition to the role of autophagy as a survival strategy (Moore et al., 2006b) it is also discussed as an important response to remove oxidatively damaged proteins and organelles (Bergamini et al., 2003; Cuervo, 2004).

Various genes encoding for autophagy-related proteins are required for the formation of autophagosomes. These genes were identified by genetic screens in yeast, but in recent years related homologs have been found in many different organisms including mammals (Klionsky et al., 2003). One homolog of the autophagy-related proteins in yeast is the microtubule-associated protein light chain 3 (LC3). This protein is essential for the formation of autophagosomes (Tanida et al., 2004). LC3 exists in a cytoplasmic form (LC3-I) and associated with the autophagosome membrane (LC3-II). The amount of LC3-II correlates with the extent of autophagosome formation and is therefore used as an autophagosomal marker (Kabeya et al., 2000; Tanida et al., 2004; Wu et al., 2006).

A well-established tool to assess the health status of mussels from different sites is the test for lysosomal membrane stability (LMS). The lysosomal responses in molluscan digestive cells constitute one of the most accepted biomarkers world-wide for assessing the health status of the species and its environment (Marigómez et al., 1996; ICES, 2006, 2007, 2008; UNEP, 2007). These tools, integrating the effects of various classes of pollutants and indicating toxicant-induced cell pathologies, can provide evidence of the degree of stress or disease affecting the organism (Allen and Moore, 2004). Lysosomes respond to a broad range of pollutants with a significant increase in size (e.g. Moore et al., 1978; Lowe et al., 1981; Moore, 1988; Cajaraville et al., 1989; Viarengo et al., 1992) and labilisation of their membranes (Moore et al., 1978; Viarengo et al., 1992; Koehler et al., 2002; Marigómez and Baybay-Villacorta, 2003). It has been shown that this response is correlated to site-specific contamination levels (Broeg et al., 1999, 2002; Kagley et al., 2003; Sturve et al., 2005; Schiedek et al., 2006). In addition to chemical pollutants, natural stress factors such as temperature, salinity, availability and quality of food, tidal cycle, and habitat conditions may also influence the stability of lysosomal membranes (e.g. Marigómez et al., 1991; Tremblay and Pellerin-Massicotte, 1997; Abele et al., 1998; McVeigh et al., 2006). A decrease of the labilisation period and lysosomal enlargement in mussels has also been shown to occur under combined hypoxic and hyperthermic conditions (Tremblay and Pellerin-Massicotte, 1997). In addition to anthropogenic and natural stressors also metabolic processes such as reproductive status, infection or disease may influence lysosomal properties (Bayne et al., 1985; Bignell et al., 2008; Broeg, 2010). Izagirre (2007) showed changes of lysosomal membrane stability in mussels from the high intertidal, beginning with aerial exposure, and discussed these results as a response to digestion processes. Since numerous factors may affect size and membrane stability of lysosomes, appropriate experimental designs and a fundamental knowledge of basic responses are necessary to facilitate interpretations of results with respect to the anthropogenic or natural causes of the observed effects.

Many bivalve species have developed strategies to cope with oxygen depletion in their natural environments. Intertidal organisms are exposed to air every tidal cycle during low tide, whereas animals living in subtidal areas may have to cope with periodic oxygen depletion in their habitat due to hydrographic (e.g. reduced water exchange) or anthropogenic influences (e.g. increased oxygen demanding degradation processes due to euthrophication) (Oeschger, 1990). The species *Arctica islandica*, inhabiting the muddy bottoms of the Baltic Sea, for example, undergo self-induced anaerobiosis, since they dig burrows in oxygen depleted sediments. In experimental trials *A. islandica* survived for 55 days in oxygen deficient sea water (Theede et al., 1969). Some intertidal acclimatized bivalves close their valves, maintaining a small gap through which oxygen can diffuse into the mantle cavity. Species like *Mytilus californicus* reach values up to 80% of the immersed rate of oxygen uptake by gaping under air exposure, whereas *Mytilus edulis* generally close the valves during time of aerial exposure (Widdows et al., 1979).

Closing valves lead to oxygen deprivation in the tissues, followed by a profound depression of energy demand and a change from aerobic to anaerobic metabolism (Eertman et al., 1993; Oeschger and Storey, 1993). Under anaerobic conditions, the fermentation of carbohydrates such as glycogen becomes the main source for energy in bivalves (de Zwaan and Wijsman, 1976). This anaerobic fermentation process is less efficient than aerobic oxidation but may still exceed 12% of aerobic ATP production under normal submerged conditions (Widdows et al., 1979). Using these strategies the blue mussel can survive in oxygen depleted water for up to 35 days (Theede et al., 1969) and under aerial exposure for up to 20 days (Babarro and de Zwaan, 2008). Natural or anthropogenic stressors increase the metabolic rates of mussels and concomitantly the energy demand, thus, reducing aerial survival time compared to unstressed mussels (de Zwaan and de Kock, 1988).

The aim of this experimental study was to obtain deeper insights into the influence of long-term aerial exposure on the lysosomal membrane stability of the digestive gland cells of *M. edulis* depending on sampling site and habitat conditions (intertidal/submerged). In addition, the participation of autophagic processes as underlying mechanisms of the acclimatisation to tidal related aerial exposure was studied.

2. Materials and methods

Mussels were sampled at four locations along the coast of the German Bight (Fig. 1). Sampling was conducted between the 20th of April and the 5th of May 2007. Three areas were wild mussel

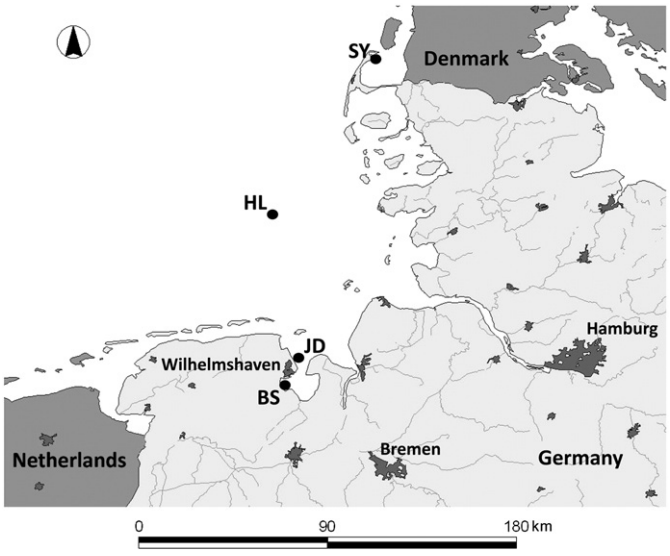

Fig. 1. Map of the German Bight showing the sampling sites. Three intertidal sampling sites at Bordumer Sand (BS), Lyster Strand at the island of Sylt (SY), and the dune of the island of Heligoland (HL) and from one suspended hanging culture at the *Niedersachsenbrücke* (nearshore) near Wilhelmshaven in the Jade (JD) Bay were sampled in April/May 2008.