ACTIVATION OF CARBONIC ANHYDRASE IN BRANCHIAL CAVITY TISSUES OF LOBSTER HOMARUS GAMMARUS CONDITIONED BY DILUTE SEAWATER

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Abstract
The activity of carbonic anhydrase (CA) and its distribution in the branchial cavity tissues has been studied in the lobster *Homarus gammarus* from ambient seawater (SW, salinity 38) and dilute seawater (DSW, salinity 20). In DSW-acclimated lobsters, almost 70% of total CA in tissues of the branchial cavity was found in epipodites and the rest was equally distributed between branchiostegites and gills. In cytosol fractions of tissues from DSW acclimated lobsters, the main proportion of 76% in epipodites, 61% in branchiostegite of total CA activity. Partially purified membranes contained 7% in epipodites and 16% in branchiostegite. These results indicate the importance of epipodites and branchiostegite in CA induction when lobsters are acclimated to DSW.

Keywords: carbonic anhydrase; dilute seawater; branchial cavity tissues; lobster Homarus gammarus

Introduction

Lobsters *Homarus gammarus* are mainly described as a non-regulating stenohaline species, able to tolerate only a narrow range of seawater salinity (1). However, recently it was found that lobsters can also temporary migrate to estuarine habitats where salinity fluctuates (2). In the lobsters acclimated to DSW, the specific activity of Na^+,K^+-ATPase was increased, particularly in the epipodites and the gills (3, 4). In addition to Na^+,K^+-ATPase, CA represents a key enzyme involved in the ion osmoregulation in hyperosmoregulating crabs, where it supplies ions for maintaining osmoconcentration gradients between the haemolymph and ambient seawater (5). CA is a multifunctional enzyme, with an additional role in respiration and acid-base regulation in the cells. CA supplies cells with H^+ and HCO_3^-, which then serve as counterions for the active uptake of important osmolytes Na^+ and Cl^- (6). In this work we studied CA of branchial cavity tissues during acclimation to DSW of the osmoregulating commercially important lobster *Homarus gammarus.*

Materials and methods

European lobsters *Homarus gammarus* (Linnaeus, 1758), weighing 312±110 g, were collected on the west Istrian coast of Adriatic Sea near Rovinj, Croatia. The activity of CA was measured using a modified method by comparison of uncatalyzed and catalyzed reaction times in tissue homogenates (with addition of Triton-100) (7). Differential centrifugation of tissues homogenates was used for measurements of the CA distribution in subcellular fractions, and to define the relation and distribution of cytosolic and membrane-bound CA (8).

Results and discussion

In lobsters acclimated to DSW, the adjustment of the blood hyperosmolarity is correlated with increased activity of Na^+,K^+-ATPase in epipodites and branchiostegite (3, 4). In the lobsters acclimated to DSW for two weeks, CA activity in the branchiostegite and epipodite was 8 and 6 times higher than the CA activity in lobsters held in SW (Fig. 1). These results show that epipodite and branchiostegite have an important osmoregulatory role when lobsters are acclimated to DSW.

Differential centrifugation of tissues showed that the cytosolic fraction of CA was inducible by exposure of lobsters to lower SW osmoconcentration (Fig. 2). In epipodites and branchiostegite the cytosolic fraction of CA in DSW acclimated lobsters amounted to 76% and 61%, respectively, of the total CA activity. The respective proportion of CA bound to membrane fraction from epipodites and branchiostegite of DSW-acclimated lobsters amounted to 7% and 16% of the total CA (Fig. 2). A slight activation of CA in homogenates and in partially purified membranes of gills was not confirmed as a statistically significant difference between the SW and DSW groups. Our results on the dominant proportion of the cytosolic CA are in accordance with results of Henry (5). We suggest that CA is mainly involved in the transport of osmolyte ions and respiration. The adaptive ion regulating mechanism in lobsters maintain homeostasis when lobsters migrate to brackish water habitats with a fluctuating salinity. Moreover, results provide knowledge to protect lobster populations in marine habitats.

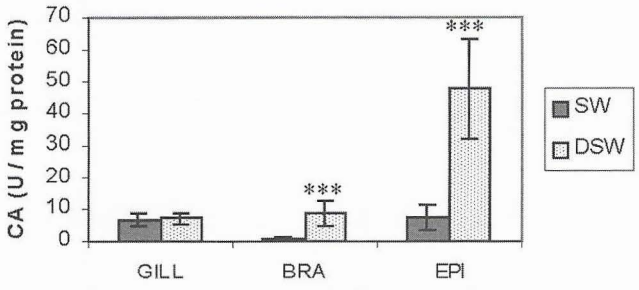

Fig. 1. Specific activities of carbonic anhydrase (CA) in epipodite, branchiostegite and gills of lobsters *Homarus gammarus* acclimated to seawater (SW) and to dilute seawater (DSW).
Mean values for 6 individual samples are given, error bars indicated SE. Asterisks denote significant differences from the SW value (Student's t-test p>0.001).

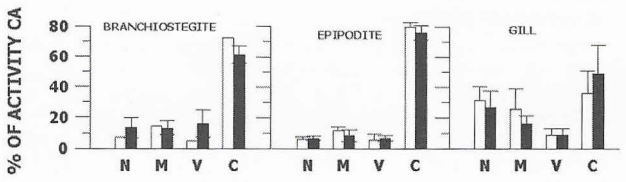

Fig. 2. Distribution of CA activity in subcellular fraction (N = nuclear; M = mitochondrial; V = membrane and C = cytosol) of epipodite, branchiostegite and gills from lobsters acclimated to SW (open bars) and DSW (dark bars).

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