ENZYMES INVOLVED IN OSMOLYTE TRANSPORT PROCESSES IN THE ADRIATIC LOBSTER HOMARUS GAMMARUS

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Abstract
Transport related enzymes Na,K-ATPase and carbonic anhydrase (CA) were studied in the branchiostegite and antennal gland of the commercially important Adriatic lobsters Homarus gammarus acclimated to seawater (SW; 38 ppt) and dilute seawater (DSW; 20ppt) . In homogenates of branchiostegite from DSW-acclimated lobsters, specific activities of Na,K-ATPase was about 2- fold higher of the native (3.2 mmol Pi h-1 per mg protein) and saponin treated homogenates (6.6 mmol Pi h-1 per mg protein) than those of the SW-acclimated lobsters. In DSW –acclimated Homarus, CA activity of branchiostegite homogenates was 5.6-fold of activities reported in SW (4.42 U per mg proteins). In DSW specific activity of the Na,K-ATPase in antennal gland native homogenates was 2- fold increased of the SW lobsters (3.2 mmol Pi h-1 per mg protein). However, the CA in antennal gland was not activated keeping Enhanced activity of branchiostegites CA and not these in the antennal gland when acclimated to DSW suggest different strategies of the enzyme fitting in these osmoregulatory tissues.

Key words. Lobster, Homarus gammarus, branchiostegite, antennal gland, Na,K-ATPase, Carbonic anhydrase

There is a lack of information on the biochemical aspects of adaptivness to various seawater osmoconcentration in commercially important Adriatic lobster Homarus gammarus. The osmoregulatory role of the branchiostegite, as a epidermal tissue underlying carapace in the region of the trichobranchiate chamber as well as these of antennal gland is unknown. We have studied effect of 38 ppt seawater (SW) and 20 ppt dilute seawater (DSW) on the branchiostegite and antennal gland activities of the Na,K-ATPase and carbonic anhydrase (CA), enzymes which are respectively involved in the primary-driven Na pump, and in supply of the carriers for secondary active transport of osmolytes.

Preparation of the homogenates of branchiostegite and antennal gland and measurements of the enzymes was carried out by modified procedures described in details (1, 2). The Na,K-ATPase was measured in native homogenates and saponin treated homogenates of the branchiostegite (1). CA was determined following the rate of hydration and dehydration of CO2 comparing the uncatalysed (slightly buffered saline) and catalysed (branchiostegite homogenate in slightly buffered saline) reaction times of the enzyme (3). The total carbonic anhydrase was measured in detergent Tryton-100 treated homogenates. In the lobsters two weeks acclimated to DSW, the Na,K-ATPase activity in the branchiostegite homogenates (6.2 mmol Pi h-1 per mg protein) and in saponin treated homogenates (12.8 mmol Pi h-1 per mg protein) was 1.96 and 1.94 times higher of the respective activities in the SW acclimated lobsters (Fig.1.). Moreover, under the identical experimental conditions CA activity in the branchistegite was 5.6 –fold over the seawater acclimated lobsters. When lobsters acclimated to DSW the increased Na,K-ATPase activity are consistent with the previously published results on the enzyme activation in trichobranchiate gills and epipodites of the osmoregulating lobster Homarus gammarus (2, 4) and no activation in stenohaline conforming Crustacea occur (5). Enzymes activation was followed by keeping osmoconcentration gradient between the haemolymph and hypoosmotic DSW. Increased activity of CA in DSW in gills (6) and branchiostegite (this study) supporting the conclusion that branchiostegite CA plays important role in ion regulation processes (Fig.1). However, when lobster acclimated to DSW, there was no activation of the CA in the antennal gland homogenates of the SW and DSW acclimated lobsters. Slightly increased Na,K-ATPase in the antennal gland homogenates in DSW-acclimated lobsters, suggests probably active role in divalent ions regulation and in elimination of nitrogenous waste products.

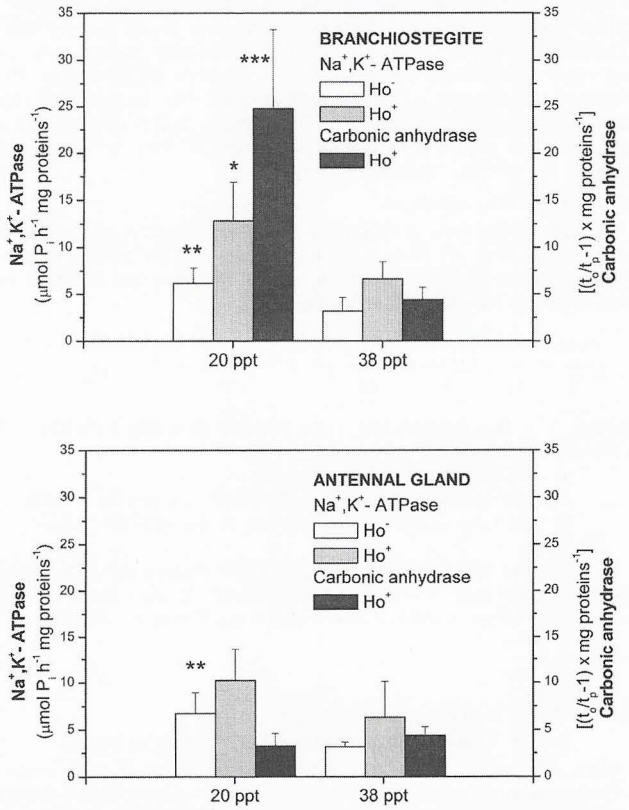

Fig.1. Na,K-ATPase and carbonic anhydrase activities in branchiostegite and antennal gland of the lobster *Homarus gammarus* acclimated to seawater (38ppt) and dilute seawater (20ppt).
H-0 = native homogenate; H+0 = saponin (Na,K-ATPase), and Tryton-100 (CA) treated homogenates. Mean values for 4-6 individual samples are given, error bars indicated SE. Asterix denote Student's t-test a value significantly different from the seawater value
(P<0.04; ** P<0.02; ***P<0.006).*

References

1- Lucu, C. and Flik,G. 1999. Na-K-ATPase and Na^+/Ca^{2+} exchange activities in gills of hyperregulating *Carcinus maenas*. *Am. J. Physiol.* 27,: R490-R499.
2- Lucu, C and Decescovi,M. 1999. Osmoregulation and branchial Na,K-ATPase in the lobster I acclimated to dilute seawater. *J. Exp. Mar. Biol. Ecol.*, 234: 291-304.
3- Böttcher K., Siebers D. and Becker W., 1990. Localization of carbonic anhydrase in the gills of *Carcinus maenas*. *Comp. Biochem. Physiol.*, 96B: 243-246.
4- Flik G. and Haond C., 2000. Na and Ca pumps in the gills, epipodites and branchiostegites of the European lobster *Homarus gammarus*: effects of dilute seawater. *J. Exp. Biol.*, 203: 213-220.
5 - Lucu C. Devescovi M. Skaramuca B. and Kozul V., 2000. Gill Na,K-ATase in the spiny lobster *Palinurus elephas* and other marine osmoconformers. Adaptivneness of enzyme from osmoconformity to hyperregulation. *J. Exp. Mar. Biol. Ecol.*, 246: 163-178.
6- Wheatly M.G. and Henry R., 1987. Branchial and antennal gland Na/K dependent ATPase and carbonic anhydrase activity during salinity acclimation of the euryhaline crayfish *Pacifastacus leniusculus*. *J. exp. Biol.*, 133, 73-86.