CARBONIC ANHYDRASE IN MUSSEL *MYTILUS GALLOPROVINCIALIS*: A PRELIMINARY STUDY

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Abstract

We investigated the esterase activity of carbonic anhydrase (CA) on total esterase activity level in mussels sampled from 19 locations along the Croatian coast of Adriatic Sea. The results of total esterase activities in winter were lower than in summer at almost all investigated locations. CA activities determined in gills of mussel sampled in winter ranged from 1.75% to 24.65% of total esterase activity. CA activities in summer samples were practically not detectable. Despite *in vitro* research showed potential application of CA activity in bioassay and biomarker studies for application of CA as a biomarkers of environmental pollution further research are needed.

Keywords: Adriatic Sea, Ecotoxicology, Enzymes, Mollusca, Pollution

INTRODUCTION

Carbonic anhydrase (CA), a ubiquitous enzyme in the bacteria, plant, and animal kingdoms catalyses the reversible hydration of CO_2 to produce H^+ and HCO_3^- using zinc as a cofactor. To date 15 CAs or CA-like proteins have been identified in mammals. In humans, for CAII isozyme, turnover number for CO_2 hydration is the highest known for any enzyme, while for the other isozymes activities are lower in the order CAII > CAIV > CAI > CAIII. There are several CAs which have shown esteraze activity, enzymes known to hydrolyse endogenous substrates, and the majority hydrolyse lipid ester substrates. In *in vitro* conditions, the esterase activity of CA was determined, both in physiological and pathological conditions. Additionally, besides Na,K-ATPase, CA represents a key enzyme involved in the adaptation of marine organisms to environmental conditions [1]. Experimental studies performed on Crustacea gills have shown two main isozymes of CA located in membrane-bound and in cytosolic fractions, functionally similar to mammalian CAIV and CAII [2]. So far no report has dealt with the esterase activity of CA on total esterase activity level in mussel.

MATERIAL AND METHODS

In this study the esterase activity of CA on total esterase activity level was investigated in mussels sampled from 19 locations along the Croatian coast. The gills were the target tissue because the respiratory, ionic transport and pH regulatory enzyme function of CA and it's potential usage as a biomarker of environmental pollution was the main topic. Previously it was suggested the possible application of CA activity inhibition as an *in vitro* bioassay for the detection of heavy metals in pollution monitoring using the mussel *Mytilus galloprovincialis* Lamarck, 1819 [3,4]. Total esterase activity was measured in the cytosolic fraction of gills homogenates by colorimetric end point reaction using p-nitrophenyl acetate as enzyme substrate, and CA activity was estimated by the same enzymatic reaction using acetazolamide as a specific CA inhibitor.

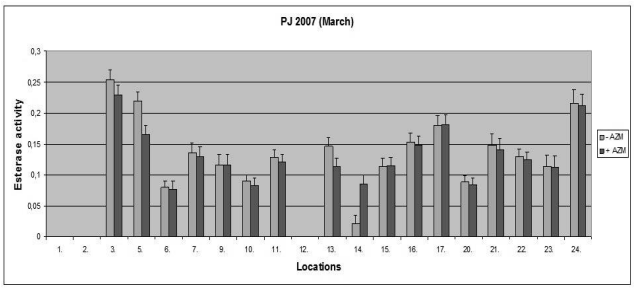

Fig. 1. Total esterase activity in cytosolic fraction of mussel gills: (-AZM) without specific CA inhibitor and (+AZM) with acetazoamide inhibition.

RESULTS

The results of total esterase activities in winter (March; average value 0.137±0.057) were lower then determined for the summer season (August; 0.153±0.036) at almost all investigated locations (Fig. 1). CA activities determined in gills of mussels sampled in winter ranged from 1.75% to 24.65% of total esterase activity. CA activities in summer samples were practically not detectable. This is preliminary research; it is difficult to assume a direct relationship between pollution at investigated sites with determined CA activities, especially in the summer period.

INSTEAD OF CONCLUSIONS

Inspite *in vitro* studies showing clear inhibition of CA activity with heavy metals, for the application of CA as a biomarker of pollution further detailed research on the effects of environmental and physiological factors and different CA isozymes activity using different inhibitors, protein characterization and sequencing are needed. With this paper we report a partial CAII sequence of *M. galloprovincialis* (Fig. 2) which is, according to the NCBI database the first normal one for Mollusca, Bivalvia, besides nacrein, nacrein-like proteins and the novel CA from *Tridacna gigas* with two CA domains.

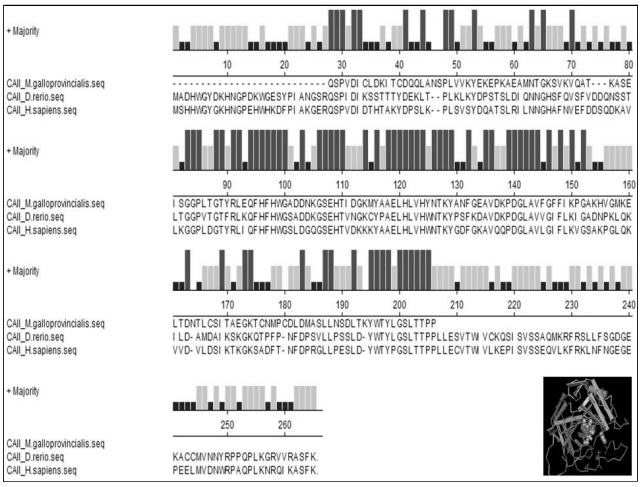

Fig. 2. Alignment of our partial *M. galloprovincialis* cDNA sequence with similar human and fish CAs after NCBI blast.

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