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Comparison of hemocytic carbonic anhydrase activity of bivalves

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ABSTRACT

Carbonic anhydrase (CA), which is involved in shell formation processes in bivalves, is one of the major biocatalysts for carbon capture and storage. In this study we investigated CA activity in the total hemocytic proteins of five bivalves. The highest CA activity was observed in *Scapharca broughtonii*, which had more than twice the activity found in *Crassostrea gigas*. No CA activity was observed among the total hemocytic proteins of *Pinctada fucata* and *Saxidomus purpuratus*. The results suggest that marine invertebrates may provide a better source of CA, as an alternative to mammalian sources.

Key word: Carbonic anhydrase (CA), Hemocyte, Bivalve, Carbon capture and Storage (CCS)

Introduction

In the last 45 years, the atmospheric carbon dioxide concentration has risen by 19%, causing the global atmospheric phenomenon termed global warming. Global warming has become a worldwide concern, and the Kyoto Protocol has set binding targets for 37 industrialized countries and the European community to reduce greenhouse gas (GHG) emissions. Among the most important GHGs including CO_2, CH_4, N_2O, HFCs, PFCs, SF_6, the carbon dioxide (CO_2) comprises the major GHG and is the most controllable. Therefore, CO_2 has become the principal focus as a point of control for the global warming concerns (Lacis *et al*., 2010).

Carbon capture and storage (CCS) is considered to be technically feasible at a commercial scale. This is based on a broad range of technologies including those that enable CO_2 emissions from fossil fuel use at large point sources to be transported to safe geological storage, rather than being emitted to the atmosphere (Gibbins and Chalmers, 2008). Among CCS technologies, there is growing interest and substantial investment in biomimetic CO_2 storage. Carbonic anhydrase (CA), which is involved in bivalve shell formation process, is one of the most biocatalyst options for CCS, and become a key focus of government and private enterprise in developed countries including the USA and Canada (Lee *et al*., 2010).

In present study, we evaluated the CA activity in five different marine bivalves, with the potential aim of developing this enzyme as an efficient catalyst for biomimetic carbon-capturing technologies.

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Materials and Methods

Five species of marine bivalve purchased from regional market including Oyster (*Crassostrea gigas*), Arkshell (*Scapharca broughtonii*), Scallop (*Chlamys farreri*), Pearl oyster (*Pinctada fucata*), and Pruple Washington clam (*Saxidomus purpuratus*). Hemocyte was withdrawn from pericardial cavity or adductor

Table 1. Comparison of CA activity of total hemocytic proteins from various shellfish

Species	Protein (mg/mL)	T (Sec)	Activity (Unit/mg)	Specific activity
C. gigas	3.06 ± 0.03	15	0.109	1.00
S. broughtonii	1.47 ± 0.01	14.5	0.282	2.59
C. farreri	1.01 ± 0.01	16.5	0.120	1.10
P. fucata	0.24 ± 0.00	18.5	-	-
S. purpuratus	2.23 ± 0.00	19.5	-	-
Blank	-	17.5	-	

muscle sinus using ice-cooled 1 mL syringe and plated on ice-cooled Petri dish. After incubation of cell adhesion for 30 min at room temperature, cell was washed two times with PBS saline and then harvested by cell scrapper. Harvested cell was lysed for total protein by commercial kit (Invent Biotechnologies Inc., USA). Total protein was quantified by Bradford assay (sigma B6916). CA activity was measured by electrometric method of Wilber and Anderson (1948) in which the time required (in seconds) for a saturated CO_2 solution to lower the pH of 0.012 M Tris · HCl buffer from 8.3 to 6.3 at 0°C is determined. A unity of activity was calculated as: = 2 x (T_0-T) / T, which T_0 and T are the time of pH shift at blank and lysate.

Result and Discussion

CA catalyzes the conversion of CO_2 and water to bicarbonate and protons, and contributes to CO_2 metabolism (Henry, 1996) and ion regulation (Henry and Saintsing, 1983). It is also known as an catalyst for the formation of the exoskeleton of bivalves, which is mainly composed by $CaCO_3$ (Miyamoto *et al*., 1996; Lee *et al*., 2010). Since first reported from erythrocytes in 1933 (Meldrum and Roughton, 1933), CA has been reported in at least six gene families (α, β, γ, δ, ζ and η) that have no significant amino acid sequence similarity.

CA activity in the tested bivalves ranged from 0 to 0.282 unit/mg protein. The highest CA activity was found in *S. broughtonii* and was significantly higher than that found in *C. gigas*. However, in *P. fucata* and *S. purpuratus*, the CA activity was almost zero. The CA activity in mammalian source is much higher than those in the present study. The low CA activities found in the study may be attributable to the low level of purification of the total proteins tested. CA activity can be increased by a factor of more than 200 when several purification steps are included (unpublished data).

Nacrein, which includes the carbonic anhydrase domain, has been identified from invertebrates (Miyamoto *et al*., 1996; Yu *et al*., 2006), where it functions as a catalyst of bicarbonate (HCO_3^-) formation, and participates in $CaCO_3$ crystal formation in the nacreous layer (Miyamoto *et al*., 1996; Norizuki and Samata, 2008; Miyashita *et al*., 2012). No CA activity was observed in *P. fucata*, which may have been a result of the low efficiency of protein extraction used, but the reason for the absence of CA activity in *S. purpuratus* is not clear. Mammalian CA has been widely used for CCS technologies. We found that magnitude differences in CA among bivalves, which suggests that marine invertebrates may offer a better source of CA, as an alternative to mammalian source.

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