Biocalcification of Corals and their Response to Global Climate Change

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***Abstract*—The response of calcifying marine organisms, especially from corals – arguably among the most biologically diverse and ecologically important ecosystems on the planet – could have a potential mitigating role in buffering atmospheric CO_2. Here we report that the organic substances that participate in biocalcification in coral skeletons contain a carbonic anhydrase (CA) enzyme which is a biological catalyst responsible for the interconversion of CO_2 and bicarbonate. Also, it appears that the internal physiological condition of the body of corals has precisely evolved to respond to external environmental conditions. We find that the CA in the organic matrix acts as "keys" to control those internal conditions to enable a response to external environmental change. This study of biocalcification process can be used as a tool for understanding coral mineralization in nature and global climate change, and also has implications for CO_2 capture from the atmosphere.**

Keywords- biocalcification; coral mineralization; carbonic anhydrase ; climate change; organic matrix

I. INTRODUCTION

Calcifying marine organisms make extensive use of calcium carbonate (CaCO3), one of the most abundant minerals in nature, as a structural or protective material. Biologically formed calcium carbonates are mainly calcite, aragonite and vaterite as well as high Mg-calcites. During the work on biocalcification, it is seen that morphology, mineralogy and chemistry of biologically formed calcium carbonate are largely dependent on both biological species and physical-chemical environmental conditions. Proteins and enzymes may act as "keys" to control internal conditions and respond to external environmental change.

Identification and elucidation of proteins and their enzyme activity involved in calcium carbonate skeleton or spicules formation in corals are very important to understand the calcification process, biodiversity of their ecosystems and carbon cycling, along with global environmental change. Atmospheric CO_2 is expected to reach double the preindustrial levels by the year 2065. It is believed that the increase in CO_2 concentration is responsible for global warming. Biological sequestration of carbon dioxide (CO_2) in geological formations is one of the proposed methods to reduce the carbon dioxide released into the atmosphere. Mineralization of CO_2 can be achieved by direct contact of gaseous CO_2 with mineral sources of calcium or magnesium or by dissolving CO_2 in water and then bringing the solution into contact with the minerals. Either way will produce calcium or magnesium carbonate, which are solids and will precipitate [1, 2]. In the present work, the feasibility of using CA enzyme as a catalyst for hydration of CO_2, as well as its precipitation in the form of calcium carbonate, was studied. The effects of enzyme concentration and temperature on the hydration of CO_2 and formation of calcium carbonate were investigated. Here we applied CA enzyme extracted from the soft coral sclerites, *Sinularia polydactyla* as a model. The information gained from this study would be applicable in understanding the biomineralization process of corals and their response to global climate change.

II. MATERIALS AND METHODS

A. Sample preparation

Sclerites were separated from the coral colony (*Sinularia polydactyla*) according to the mechanical and chemical treatments followed by Rahman et al., 2006 [3]. Briefly, the collected sclerites were stirred vigorously in 1M NaOH for 2 hours and subsequently in 1% NaClO solution for 2 hours to remove the fleshy tissues and debris. Treated samples were washed under tap water until the sclerites were completely cleaned. Finally, samples were washed with distilled water (five times) to remove unwanted substances.

B. CA assay

To examine the CA activity, the chemically treated sclerites were decalcified in 0.5 M EDTA (pH 7.8) overnight, followed by dialysis against H_2O for 48 hours. Proteins were separated from the soluble organic matrix of sclerites using SDS-polyacrylamide gel electrophoresis according to the method of Laemmli [4]. After electrophoresis, the bands of interest were excised from the SDS-PAGE and the pure proteins were extracted by electro-elution treatment, using

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the Electro-eluter (Model 422, Bio-Rad) according to the procedure of Rahman et al., [5]. The Micron centrifugal Filter Devices (Millipore) were used for further purification of the sample.

The amount of protein concentration in each fraction was measured by the method of Lowry et al. [6] using chicken ovalbumin (Kanto chemical) as a standard protein followed by a spectrophotometric assay of protein. We measured CA activity using the CO_2-Veronal indicator method [7] as follows. Six drops of phenol red, 3 ml of 20mM Veronal buffer (Sodium 5, 5-Diethylbarbiturate, pH 8.3), and 0 .5 ml of a sample were mixed and placed in ice water; then the reaction was started by adding 2 ml of ice-cold water saturated with CO_2 followed by observation of the time until the color changed from red to yellow for a pH drop to 7.3. The EU was calculated according to the following equation.

Activity unit (EU) = (To – T)/ T

[where T and To are the reaction times required for the pH change from 8.3 to 7.3 at 0°C with and without a catalyst, respectively].

C. Enzymatic Hydration of CO_2. Carbonic anhydrase catalyzes the hydration reaction of CO_2, and consequently, hydrogen ions are transferred between the active site of the enzyme. This results in a change in pH. Therefore, measuring pH via the delta pH method is a viable method to monitor the progress of this enzymatic reaction [8]

D. Enzymatic Precipitation of Calcium Carbonate.

The influence of CA enzyme on the precipitation of CO_2 in the form of calcium carbonate ($CaCO_3$) was studied according to Mirjafari et al [9] and Rahman et al. [10]

III. RESULTS AND DISCUSSION

A. Biocalcification

For understanding biocalcification process in corals and their response to climate change, we purified proteins and enzyme from a soft coral sclerites, *S. polydactyla*. The SDS-PAGE analysis of the preparation showed seven proteins with the apparent molecular masses of 109, 83, 70, 63, 41, 30 and 22 kDa (Fig. 1, lane 2) that we named SP-1 *(Sinularia polydactyla-1)*, SP-2, SP-3, SP-4, SP-5, SP-6 and SP-7 respectively. In the present work, we purified a protein with a molecular mass of about 83-kDa (SP-2) (Fig. 1, lane 2), which has high CA activity (Fig. 2). We excised the protein bands from the SDS-PAGE and extracted the purified proteins by electro-elution treatment. We confirmed the CA protein (SP-2) followed by the technique as indicated above (Fig. 2).

In order to identify CA activity associated with the purified proteins, the samples were assayed independently. Fig. 3 shows that the SP-2 possessed specific CA activity; where other has lower activity. The CA activity of both the matrix protein (SP-2) and bovine erythrocyte enzyme (BECA) was inactivated by heat treatment at 100°C for 10 min, which showed no activity. Since SP-2 showed the highest and most significant activity among the seven proteins, we can consider only SP-2 to be a CA domain protein in S. *Polydactyla.*

Internal and external reactions of coral body including hard endoskeleton of sclerites are occurred with biocalcification process. In the mineralization of CO_2, calcium carbonate is produced through a reaction between calcium ions and aqueous CO_2. The following reactions take place in this process:

(1) First, gaseous CO_2 dissolves in water to form aqueous CO_2.

$$CO_{2(g)} \leftrightarrow CO_2 \qquad (1)$$

(2) Then, aqueous CO_2 reacts with water to form carbonic acid:

$$CO_{2(aq)} + H_20 \leftrightarrow H_2CO_3 \qquad (2)$$

(3) In the next step, carbonic acid dissociates to bicarbonate and carbonate ions in the presence of CA enzyme:

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad (3)$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \qquad (4)$$

Reaction 3 is very rapid and is virtually diffusion controlled.

(4) At the end, in the presence of calcium cations, calcium carbonate forms and precipitates:

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\downarrow \qquad (5)$$

Among reactions 1−5, reaction 2 is the slowest, and it is the rate-limiting step. It is proposed that a biological catalyst be used to increase the rate of this reaction.[11-13]. The biological catalyst for this reaction is enzyme carbonic anhydrase.

B. Enzymatic Precipitation of Calcium Carbonate

The amount of $CaCO_3$ decreased as the temperature increased. The weight of $CaCO_3$ at 0, 30, and 50 °C was 0.2097, 0.1282, and 0.095 g, respectively. This is because the solubility of CO_2 in water decreases with increasing temperature [14]. This result did not depend on the temperature or concentration of the enzyme. When the buffer is absent from the reaction mixture, addition of CO_2 to the mixture drives the pH down to low values (near 4). The chemistry of CO_2 hydration and bicarbonate dissociation shows that, in low pH, there is not enough carbonate ion present [15]. As a result, the solution does not become saturated with $CaCO_3$. This is the reason precipitation was not observed in this condition.

In the absence of the enzyme, precipitation was observed after 2 h. The amount of $CaCO_3$ was almost the same as its amount in the presence of the enzyme (Table 1). However, Figure 3 indicates that precipitation of calcium carbonate was much faster in the presence of CA enzyme. This figure

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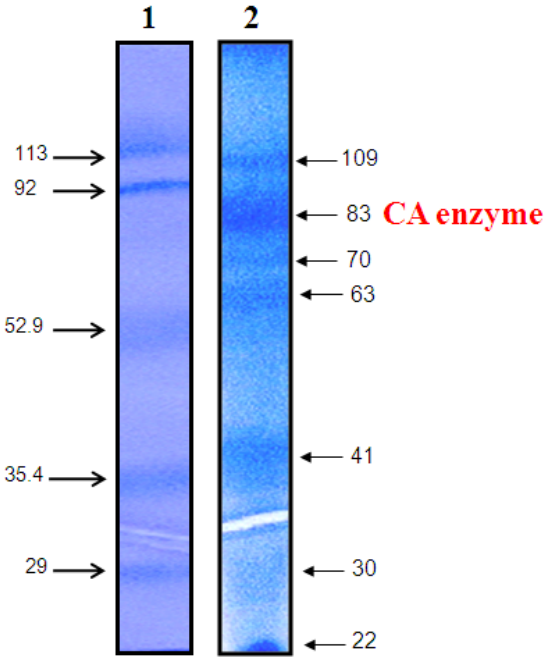

Figure 1: Identification of proteins. Lane 1, Protein marker, Lane 2, SDS-PAGE separation of the total assemblage of soluble matrix proteins.with CBB staining. *Arrows* indicate significant protein bands.

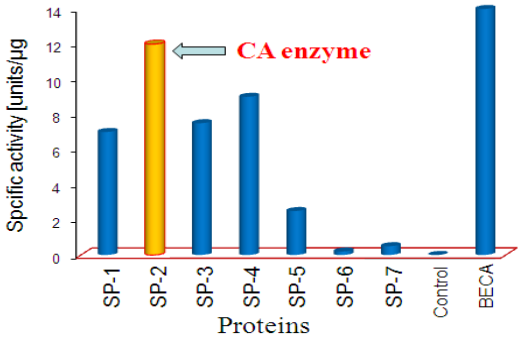

Figure 2: Determination of CA activity with purified matrix proteins. Specific activity was measured in the presence of matrix proteins at concentration of 1.5 ml (contain 30µg protein) for each protein; SP-2 showed the highest activity. H_2O (control) and bovine erythrocyte CA (BECA) was used as a standard in comparing the efficacy of CA proteins. Ten experiments were conducted for each protein. The novel matrix protein of SP-2 showed significant CA activity.

presents a comparison of the precipitation in the presence and absence of the enzyme. It is clearly seen that, in the presence of the enzyme, $CaCO_3$ reached its maximum value in less than 10 min; however, when no enzyme was added to the reaction mixture, the formation of calcium carbonate took place very slowly.

Carbonic anhydrases are very well-known enzymes that are ubiquitous in nature. They can be found in animals and plants and even in the human erythrocyte. They exist in different forms, with different structures and molecular weights, and their activities vary from one to another. They are among the fastest enzymes known. For instance, each molecule of isozyme C from the human body can catalyze 1.4×10^6 molecules of CO_2 in 1 s [16].

In the presence of an anhydrase enzyme, the mechanism of hydration of CO_2 changes completely. The evidence suggests that the catalysis of CO_2 hydration is initiated by the nucleophilic attack on the carbon atom of CO_2, by zinc-bound OH^-, to produce bicarbonate, which is then displaced from zinc by a water molecule [17]. In this study, the Mineralization of carbon dioxide is investigated as a method of converting CO_2 to mineral carbonates. The slow rate of hydration of CO_2 has been a limiting factor to make this

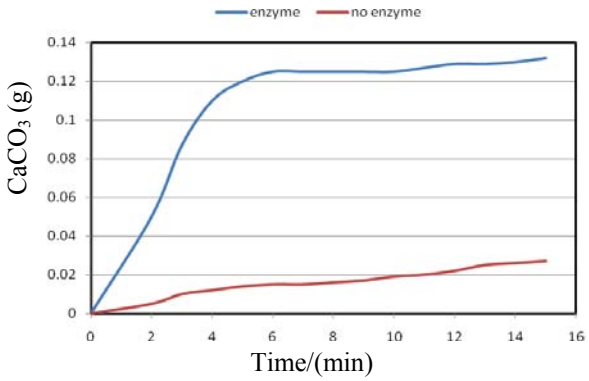

Figure 3: Comparison of precipitation with and without enzyme.

Table 1: Summary of $CaCO_3$ Precipitations

Set	Temp (°C)	Enzyme con. (µM)	Wt. of precipitate (g)	No. of units	Error (%)
1	0	3	0.2097	2	0.1
2	0	6	0.2105	4	4.2
3	0	6	0	3	N/A
4	0	No enzyme	0.18	2	1
5	30	3	0.1279	2	o.16
6	30	6	0.1282	4	1.8
7	50	6	0.095	3	1.7
8	50	No enzyme	0.0940	2	1.5

method widely accepted. Carbonic anhydrase enzyme isolated from soft corals sclerites has been shown to be credible as biological catalysts to overcome this shortcoming. The results showed that this enzyme was a very effective catalyst for coral mineralization. Overall, it promoted the hydration of CO_2 and, consequently, the precipitation of $CaCO_3$.

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