THE UNIVERSITY OF MIAMI

ATOMIC ABSORPTION AND ENZYME
ANALYSES OF CALCIFICATION IN THE ALGA,  
HALIMEDA TUNA

By

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A DISSERTATION

Submitted to the Faculty of the University of Miami in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Coral Gables, Florida

January 1973
THE UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of
the requirements for the degree of
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Subject

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INTRODUCTION

Living tissue appears to exert a catalytic and selective influence on the calcification process. This role in its narrowest (greatest degree of biological control) sense, according to Isenberg and Levine (1965):

... involves the transport of cationic and anionic ions in solution from the environment into a cell where a matrix has been or is being formed through the efforts of the cell. The ions are deposited selectively upon the organic matrix as crystals in a manner not as yet understood.

This definition implies the active accumulation of charged species above environmental levels. Thus, in its simplest terms, active mineralization means active uptake and selective deposition upon a preformed organic matrix.

To attempt to attach a particular definition of calcification on to all calcifying organisms, regardless of their level of organization is a gross oversimplification. The wide spectrum of biological control must be superimposed upon the evolutionary continuum of calcifying systems. The mechanism of mineralization observed in bacteria may be functionally similar in many respects to those which lead to crystal deposition in bone, but there is obviously a wide difference in the degree of control. However, the fundamental factors which cause calcium carbonate precipitation to occur are: (1) physico-chemical, (2) supersaturation, and (3) factors of structure and
metabolism. The generally accepted reaction sequence for the carbon dioxide-carbonate system is as follows:

1. \( \text{CO}_2 \rightleftharpoons \text{CO}_2 \text{ (solution)} \)
2. \( \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \)
3. \( \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \)
4. \( \text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-} \)
5. \( \text{Ca}^{++} + \text{CO}_3^{2-} \rightleftharpoons \text{CaCO}_3 \)

\( \text{CO}_2 \) in solution reacts with \( \text{H}_2\text{O} \) to form carbonic acid (2) which then dissociates in two steps (3, 4). The dissolution steps (1, 2) are relatively slow, but the ionization steps are very rapid. Reactions 2 and 3 are usually combined to give: \( \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \). The thermodynamic constants for these equilibria increase with increasing temperature but are inversely related to ionic strength.

In addition, the whole \( \text{CO}_2 - \text{HCO}_3^- - \text{CO}_3^{2-} \) system is strongly influenced by pH.

When these equilibria occur within a living system, the precipitation of calcium carbonate is strongly influenced by substances or structures made by the individual. The general metabolism of the organism affects the pH and the concentrations of ions and energy sources which contribute to calcification. In addition, enzyme systems may regulate diffusion of ions and the formation of the organic matrix upon which deposition initially occurs.

In order to understand the mechanism of biogenic calcification, several questions must be answered. They are:
1. Do carbonates precipitated under defined conditions differ from those produced when the unknown variables of organic complexing and biochemical fractionation are present?

2. Is there a specific substrate or matrix, synthesized by living tissue present at the site of calcification?

3. If so, what are its characteristics?

4. To what degree is the microenvironment adjacent to the site of calcification isolated from the external environment?

5. What are the mechanisms which maintain an ionic equilibrium favorable to calcium carbonate deposition?

6. How is a pH conducive to precipitation maintained?

7. What is the adaptive value of calcification to the particular system under consideration?

In dealing with these components of the calcification problem, several corollary questions, of interest to both the biologist and the geochemist must also be confronted. The first is: what determines which particular mineralogical form of the carbonate precipitate is deposited in living systems? Secondly, what controls the incorporation or co-precipitation of trace elements in these carbonate minerals?

The present study was designed to answer several of these questions by determining the degree of control which
the alga *Halimeda tuna* has over the deposition of its calcium carbonate polymorph, aragonite. However, in order to understand the functional mechanism of calcification in a particular calcareous taxon, it is necessary to examine the morphology, physiology, and molecular systems at its depositional site. The rationale for a close examination *Halimeda* is that it represents an organism which may be considered relatively simple when related to the degree of organizational complexity represented by the various invertebrate groups, especially the Molluscs. *Halimeda*'s depositional site is not completely exposed to the environment, as in the case of the cell walls of the calcifying bacteria, nor is it completely isolated, as in the Molluscs. Moreover, there is literature available describing its morphology, ultrastructure, physiology and filament wall chemistry, and its calcareous skeleton is of considerable importance geologically and geochemically.

In addition, the carbonate skeleton in *Halimeda* is most suitable for mineralogic analysis since the aragonite crystal incorporates strontium into its lattice. Small amounts of this element can be measured very accurately using atomic absorption spectrophotometry and its concentration relative to calcium in the skeleton can be used as an indicator to help explain various aspects of calcification. This approach of studying the trace and minor element chemistry of skeletal carbonate materials
has been used quite extensively by both geologists and biologists and this literature has been extensively reviewed by Dodd (1967).