SIGNIFICANCE OF MARINE ALGAL CALCIFICATION INHIBITORS IN THE GLOBAL CARBON CYCLE

ALLAN PENTECOST(1)

Keywords: marine algae, calcification, inhibitors, carbon dioxide

ABSTRACT

While the processes leading to the precipitation of calcium carbonate in algae have been well studied over the past few decades, little attention has been given to the possible role of nucleation inhibitors preventing calcification in algae. Since the majority of algae have never been observed to calcify, it is possible that nucleation inhibitors are widespread and have an important role in algal ecophysiology. In this paper, a method of detecting calcite nucleation inhibitors is described and preliminary evidence presented for inhibitors in members of the Chlorophyta, Phaeophyta and Rhodophyta. It is emphasised that more work is needed in this field, to identify the substance(s) responsible and assess the implications of calcification inhibitors in the marine carbon cycle.

INTRODUCTION

Over forty years ago, Joyce Lewin remarked on the fact that surprisingly few of the several thousand species of marine algae are calcified (Lewin, 1962). Many algologists have subsequently pondered this observation but we are no closer to understanding why the number of uncalcified species is so low. Seawater contains about 2.5 mmol L⁻¹ dissolved carbon dioxide, most of which is present as bicarbonate. The calcium concentration varies little from 10.5 mmol L⁻¹ and the ion activity product of Ca²⁺ and the CO₃²⁻ ions in shallow seawater indicates that it is supersaturated with respect to calcite and aragonite (e.g. Whitfield & Watson, 1983). Little energy would appear to be needed to overcome the activation energy barrier for the nucleation of calcite or aragonite to bring about calcification on the surface of marine organisms. During the carbon fixation process of photosynthesis, carbon dioxide is removed from the surrounding seawater, increasing the CO₃²⁻ ion activity, and further increasing the {Ca²⁺}{CO₃²⁻} activity product. Marine algae living in shallow seas, it would seem, would be hard pressed to prevent calcification on their cell wall surfaces.

Several styles of calcification have been observed in the marine algae, ranging from intracellular precipitation followed by exocytosis, to simple
extracellular calcification (Lowenstam, 1986). In the attached forms, most of the phyla of algae have calcified representatives, the bulk of which occur in the Chlorophyta and Rhodophyta, well-known examples being *Halimeda* and *Corallina* respectively. The relative scarcity of calcification in the algae is not understood, and within the calcified species, the pattern, and probably, the mechanism of calcification differs widely. For example, in *Halimeda* (Chlorophyta, Caulerpales), aragonite is deposited within intercellular spaces of the cortex and there is much evidence to demonstrate a direct relationship between cortical photosynthesis and aragonite precipitation (Borowitzka, 1989; De Beer & Larkum, 2001). In *Corallina*, calcification occurs within the narrow cell walls, consists of calcite, and enlarged intercellular spaces are lacking (Pentecost, 1985; 1990). Benthic calcified algae are often abundant in shallow tropical seas and make a significant contribution to both modern and ancient reef carbonates. The Haptophyta, which include the coccolithophorids with their calcified scales, are a major group of marine phytoplankters (Pentecost, 1991). Taken as a whole, the marine calcifying algae have played, and continue to play a major part in the cycling of carbon in the oceans.

In this article, an experimental system is described allowing rates of calcite precipitation in the presence of algal extracts to be compared. Extracts are added to a system which will spontaneously precipitate calcite from a supersaturated \( Ca^{2+} \cdot HCO_3^- \) solution. Supersaturation is achieved by aerating the solution leading to a progressive loss of excess carbon dioxide to the atmosphere, followed by carbonate deposition that is monitored by the electrical conductivity change. Evidence is sought for the presence of substances inhibiting calcite precipitation in marine algae.
MATERIALS AND METHODS

Calcium bicarbonate solutions are prepared by bubbling CO₂ gas into a suspension of calcite in distilled water. With dilution, a range of solutions become available for subsequent degassing of the excess CO₂ leading to calcite supersaturation. A calcium bicarbonate water was procured with a calcium concentration of 4 mmol L⁻¹, a total dissolved inorganic carbon concentration of c.13.5 mmol L⁻¹, and pH 6.5 in equilibrium with a partial pressure of CO₂ of 5%. The water was initially slightly undersaturated with respect to calcite. By removing carbon dioxide from the water by bubbling with air, the pH rises and the solution first becomes saturated then increasingly supersaturated with respect to this mineral. If suitable seed crystals of calcite are present, calcium is removed from solution as the carbonate is precipitated on the seeds. This process is identical to that operating in caves where stalactites (CaCO₃) are formed by the slow degassing of CO₂-rich ground waters. The precipitation of calcite in the experimental solution can be followed by measuring the specific conductivity over time. As calcite is precipitated, the conductivity falls approximately in proportion to the calcium lost from solution. Changes in conductivity also occur in response to the redistribution of CO₂ as pH rises but these changes are concurrent with calcite precipitation. This simple experimental system can be used to detect nucleation inhibitors of calcite by following changes in conductivity in the presence of test solutions and comparing them with a control solution where no inhibitors are present.

In the experiments, 1.5 L of the calcium bicarbonate water was placed in a clean 2 L glass beaker fitted with a small sintered glass aerator attached to a continuous flow air pump. Air was pumped through the water at a rate of 0.5 L min⁻¹ and 300 mg analytical grade calcium carbonate (BDH, UK) consisting of 1-5 µm calcite crystals added to the water. The top of the beaker was covered in metal foil to prevent splashing. The water was then aerated for 4 h and the conductivity monitored at a constant temperature of 10°C. Calcium concentration changes were monitored using a standard complexometric titration employing Eriochrome Blue-black R and EDTA (Greenberg et al., 1985). The initial undersaturation of the test solutions did not lead to calcite dissolution and the period of undersaturation was typically less than 2 min and not accompanied by conductivity change.

Three non-calcified marine algae were examined; Ulva lactuca L. (Chlorophyta); Fucus vesiculosus L. (Phaeophyta) and Chondrus crispus Lyngb. (Rhodophyta). They were collected from the intertidal zone of a Southern England shore. On return to the laboratory, the algae were washed twice in tap water, then once in distilled water, drained and air dried. The algae were coarsely ground to aid weighing, and 5 g immersed in 200 ml distilled water at 50°C for 2 h. The resulting supernatant was cooled, filtered and allowed to stand for 24 h at 5°C. The supernatants were tested for inhibitor activity by adding 50 ml extract to 1450 ml of the Ca-bicarbonate water described above. The water was then aerated and conductivity measured every 30 min. For controls, 50 ml distilled water was substituted for 50 ml supernatant. Small adjustments were necessary using NaCl to maintain the initial conductivity between 850 and 950 µS cm⁻¹. All initial conductivities were then normalized to 1000 µS cm⁻¹ to facilitate comparisons.

RESULTS AND DISCUSSION

Fig 1 shows the change in specific conductivity of the three algal extracts and the control. Calcium carbonate deposition on the seed crystals resulted in an almost linear decline in conductivity over the first 180 min, and began to slow at 240 min. In solutions spiked with the algal extracts, there was an initial decline in conductivity over the first 30-60 min, after which the conductivity remained more or less constant until 240 min. In more prolonged studies, this level of conductivity was found to be maintained. Calcium analyses of the solutions show that in these extracts, negligible Ca is lost from solution and the initial decline in conductivity is assumed to result from readjustment in the CO₂-HCO₃-CO₃ system as degassing of CO₂ began. In the control, Ca was lost from solution to the extent of about 0.4 mmol L⁻¹ over the 4 h period.

This experiment provides evidence for the inhibition of calcium carbonate precipitation by marine algal extracts. In samples with extracts, the waters became progressively supersaturated with
calcite as CO₂ degassing proceeded and the water was maintained in a metastable state. The presence of these inhibitors may be important in preventing these algae from calcifying but their nature is not known. In a study of the calcified freshwater cyanobacterium *Rivularia haematitites*, an extracted polysaccharide was found to inhibit CaCO₃ deposition at a concentration of 25 µg ml⁻¹ using the same technique (Pentecost, 1975). It may seem surprising that inhibitors also occur in calcified species, but calcification is not found throughout the cell walls of these algae, and inhibitors may have evolved in particular regions of the cell or cell wall to prevent or control mineralization. In a study of marine stromatolites, Kawaguchi & Decho (2001) also found evidence for an inhibitory role of extracellular polysaccharides produced by cyanobacteria. The coccolithophorid *Emiliana huxleyi*, a calcified phytoplankter possesses a polysaccharide inhibitor which may serve the same function (Borman et al., 1982). Wada et al., (1993) also found evidence of inhibitory effects of polysaccharides in experiments involving aragonite precipitation. Since many cell wall polysaccharides are soluble in hot water, it is possible that the inhibitory substances present in the experiment reported here are also polysaccharides. There has been a considerable amount of interest in the organic macromolecules associated with biological calcification (Mann, 1988). For example, closely matching organic-inorganic (CaCO₃) interfaces occurred in aspartic acid-rich macromolecules taken from molluscs, leading to calcification (Addadi & Wiener, 1985). Likewise, the inhibition of nucleation has been demonstrated using monolayers of organic molecules lacking complementarity between the crystal lattice parameters and the spacing of carboxylate groups (Mann, 1988). Thus, the three-dimensional structure and periodicity of molecular groups are important both for nucleators and inhibitors of calcification.

When calcium carbonate is precipitated, carbon dioxide is liberated (Ca²⁺ + 2HCO₃⁻ → CaCO₃ + H₂O + CO₂; Stumm & Morgan, 1981). The subsequent fate of the carbon dioxide is likely to depend on the environment into which it is liberated. In an 'open' system where calcification occurs on a surface in contact with the ocean seawater, the CO₂ may diffuse into the surrounding water and into the atmosphere. It is important to note that the above reaction does not require the intervention of photosynthesis since the surface seawater is already supersaturated with the mineral phase. If the energy barrier to nucleation is lowered further by an organic nucleation agent, calcification can occur. In this case, the existence of a nucleation inhibitor is not necessary to prevent nucleation at a surface since a low level of supersaturation will not lead to calcification, at least within a period of a few years due to kinetic effects. If the effects of photosynthesis are included, then a higher level of supersaturation is to be expected at the surface. The pH of seawater is close to 8 where most of the dissolved carbon dioxide is present as HCO₃⁻ and this ion is utilized by marine algae in photosynthesis (Shiraiwa et al., 1993). During photosynthesis, the bicarbonate is converted into CO₂ with the liberation of OH⁻, making the external medium more alkaline. Therefore the surface should experience an increase in pH, CO₂⁺ and calcite/aragonite supersaturation. Although no calculations appear to have been made, it is clear that the greater supersaturation will increase the likelihood of calcification, and the presence of a surface inhibitor may be necessary if calcification is to be prevented. While a number of marine algae show surface calcification (e.g. *Acetabularia, Dasycladus, Penicillus*) many calcify internally, either within their cell walls or within the semi-enclosed spaces between flask-shaped structures termed utricles. In these algae, the diffusion of molecules and ions into the surrounding seawater is retarded by the intervening cells and the effects of photosynthesis on the alkalization of the enclosed medium is more pronounced (Borowitzka, 1989). Calcification is often intense, and to prevent it, an inhibitor would need to be present. For instance, *Codium* and *Halimeda* (Chlorophyta) both possess a cortical layer of utricles, yet calcification only occurs between the utricles of *Halimeda*. Although there are minor structural differences between these genera, the calcification of *Halimeda* is believed to be largely dependent upon the anatomy of the cortex, so the absence of calcification in *Codium* is not easily explained without the existence of inhibitors.

In algal calcification, the liberated CO₂ may provide a further source of carbon for photosynthesis (McConnaughey & Falk, 1991). If however, some of the CO₂ escapes by diffusion, calcification becomes a potential source of CO₂ output to the atmosphere.
Marine algae are major primary producers on a global scale and any change in the ratio of calcifying to non-calcifying algae in the oceans is therefore of interest in the global carbon balance (Kitano, 1983). Further search and identification of nucleation inhibitors in marine algae is clearly of importance and may have practical applications. For example, in freshwaters, algal biofilms on boat hulls often calcify, reducing fuel efficiency, and a range of artificial inhibitors have been found effective in reducing the effect (Heath et al., 1995). It is suggested that much fruitful research can be undertaken in this field to increase our understanding of algal metabolism, ecophysiology and biomineralization in the oceans.

REFERENCES


(Received: March, 20, 2003. Accepted: June, 10, 2003)