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The formation of calcium carbonate crystals in *Halimeda incrassata* with special reference to the role of the organic matrix

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This study deals with the formation of calcium carbonate crystals in the green alga *Halimeda incrassata* at the ultrastructural level. Crystal formation occurs within tube-like organic envelopes in the intercellular space. These envelopes are derived from the organic matrix adjacent to the cell wall of the coenocytic filament and exhibit a double walled structure. The envelopes act as sites of crystal initiation and also regulate the size and shape of the crystals.

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It has been reported that a feature common to all calcareous algae is the presence of gelatinous or mucilaginous (organic) substances associated with cell walls (LEWIN 1962). It was further suggested that this substance may be involved in the deposition of crystalline material (KOBAYASHI 1971). Recent studies (WILBUR *et al.* 1969; BOROWITZKA *et al.* 1977, 1974) dealing with the problem of calcification in *Halimeda*, a typical calcareous alga, have failed to confirm the presence of an organic matrix in the intercellular space, the site of crystal formation.

The discrepancy in regard to these observations prompted us to re-examine *Halimeda* at the electron microscope level in order to ascertain whether an organic matrix is present, and if so, what role it plays in crystal initiation and growth. The present report describes the results of this study.

Halimeda incrassata was collected in Harrington Sound, Bermuda, during the month of July. Immediately after removal from the water, the apical and second segment of the thallus was removed, cut into small pieces and fixed as follows: (1) in veronal buffered (pH 7.4) glutaraldehyde (5%) sol. for one hour, then washed in buffer and post fixed in cacodylate buffered OsO_4 1% for 45 min. (Fixation times were of relatively short duration to avoid dissolution of aragonite crystals.) The materials were routinely dehydrated and embedded in Araldite 502. Sections were cut with a glass and diamond knife and stained with lead citrate (2% in 1/10 N NaOH) or 2% solution of uranyl acetate followed by lead citrate. Thick sections were also prepared and stained with toluidine blue in 40% alcohol.

The basic structure of *Halimeda* is the coenocytic filament. The filaments of the outer surface of the thallus are in contact with one another and form a boundary

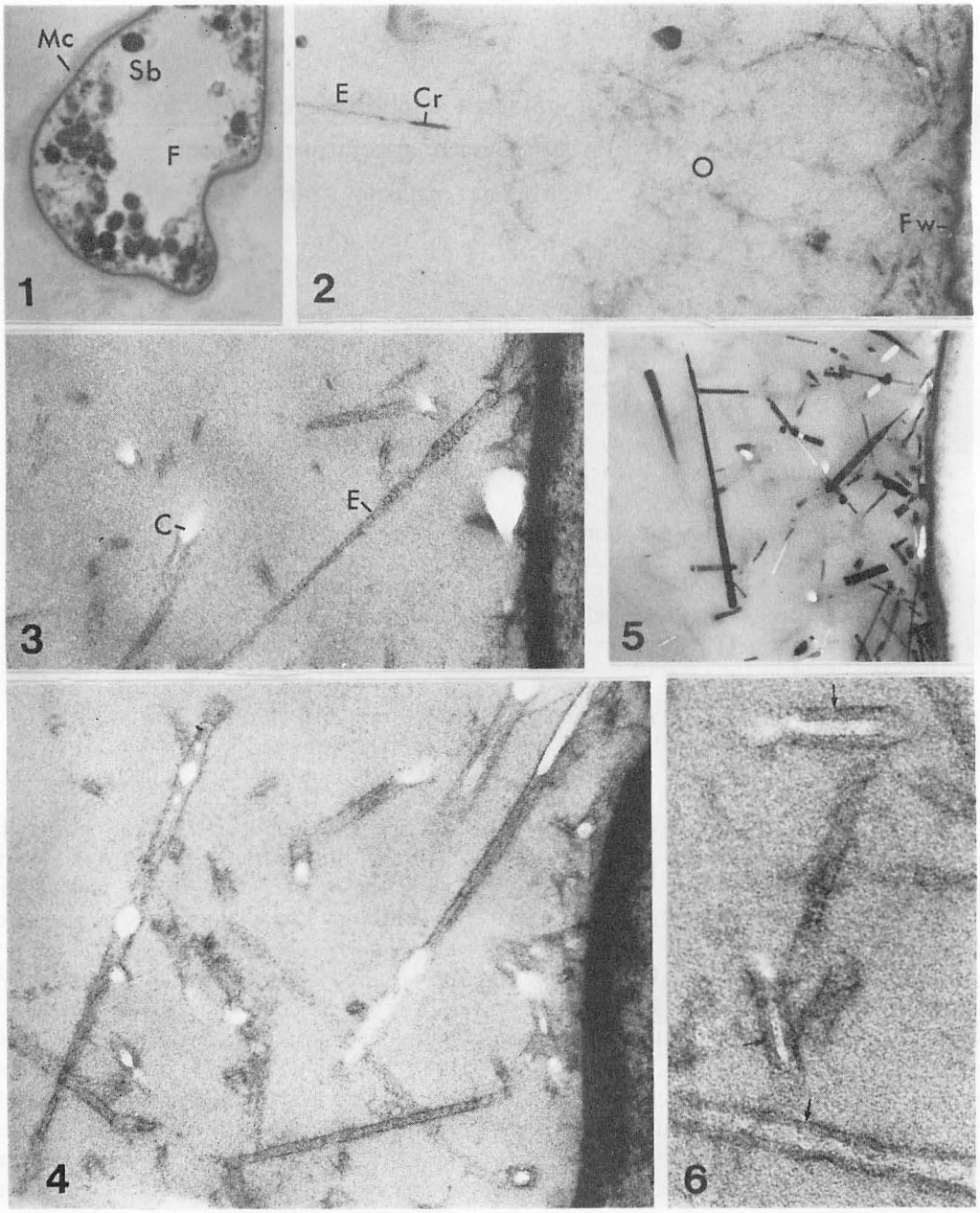

Fig. 1. Transverse section of filament, (F), showing numerous spherical bodies (Sb), and metachromatic coat (Mc), adjacent to filament wall. ×800 Figs. 2-6. Transmission electron micrographs of portions of the filament wall showing development of crystals. Fig. 2. Cr, crystal; E, associated organic envelope; Fw, filament wall; O, network of organic substance (matrix). Fixed in veronal buffered 1% OsO_4 for 30 min., unstained. ×43,000 Figs. 3-4. Sections illustrated in these figures were fixed in veronal buffered 1% OsO_4 for 30 min., stained with uranyl acetate and lead citrate and demineralized during the staining process. Fig. 3. Tube-like envelope (E), projecting from filament wall. Small crystals (C), present in some envelopes. ×60,000 Fig. 4. Tubes (envelopes) more highly developed showing crystals growing within tubes. ×60,000 Fig. 5. Thick section showing randomly arranged large mineralized crystals. ×20,000 Fig. 6. Selected area of intercellular space showing thin inner and thick outer wall of envelopes surrounding growing crystals (arrows). Uranyl acetate-lead citrate stain. ×140,000

against the sea water. Between the filaments there are extensive spaces in which aragonite crystals are deposited as the thallus matures. The filaments are bounded by a filament wall and contain in addition to several cytological components such as mitochondria and chloroplasts, large spherical bodies that stain metachromatically with toluidine blue (WILBUR *et al.* 1969). These bodies also occur in the intercellular space.

Adjacent to the filament wall is a zone of organic matrix that stains metachromatically (Fig. 1). This matrix also occurs as scattered patches throughout the intercellular space. Originally, the matrix appears somewhat fibrilar. Subsequently, this material undergoes a reorganization (polymerization ?) to form randomly arranged tube-like envelopes. After this occurs, crystals appear within the envelopes (Fig. 2). Initially, the envelopes are most numerous in the region adjacent to the filament wall. Continued development results in the differentiation of additional envelopes throughout the intercellular space. One, or more, crystals are initiated within the envelopes (Fig. 3, 4); they continue to grow until envelopes are completely filled, giving rise to straight needle-like crystals characteristic of the mature state (Fig. 5). At a relatively high magnification, the crystal envelope (Fig. 6) is shown to be double layered, consisting of a thin inner and thick outer wall.

We have demonstrated the presence of an organic substance in the intercellular space in *Halimeda* which is in agreement with the observations concerning other calcareous algae (LEWIN 1962). Our investigations disagree, however, with those in which an organic matrix was not observed (WILBUR *et al* 1969; BOROWITZKA *et al.* 1974, 1977).

Matrix have been defined (EASTOE 1968, KOBAYASHI 1971) as a substance that is (1) in existence before crystal initiation occurs, (2) is the medium in which crystals develop, and (3) subsequently encloses the crystals spacially. The intercellular organic material observed in *Halimeda* conforms to the above criteria and accordingly, justifies the use of the term matrix. Further, the high degree of organization is in agreement with the previous contention in regard to the character and role of the matrix in algae.

The presence of envelope-like structures has been described in association with crystals or crystal formation in higher plants (ARNOTT 1966), Earthworms (NAKAHARA and BEVELANDER 1969) and Molluscs (BEVELANDER and NAKAHARA 1969; ERBEN and WATABE 1974).

This study has demonstrated that the matrix is involved in the following in regard to crystal formation: (1) the matrix gives rise to envelopes, (2) the envelopes act as specific sites in which crystal initiation occurs, (3) they also regulate the growth (size) and shape of the crystals.

The matrix derived envelopes are highly organized randomly arranged structures. Since the crystals are enclosed within envelopes, the arrangement of the crystals coincides and is dependant on the arrangement of the envelopes.

It is apparent that an organic matrix is present in the intercellular space, and further, it is intimately associated and plays a decisive role in the initiation, growth, shape and arrangement of the aragonite crystals in *H. incrassata*.

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中原　晧*. Gerrit Bevelander: *Halimeda incrassata*（ミツデサボテングサ）における炭酸カルシウム結晶の形成，特に Organic matrix の役割**

超薄切片法—透過型電子顕微鏡によって *Halimeda incrassata*（緑藻）における炭酸カルシウム結晶の形成を観察した。結晶の生成，成長は細胞壁の外側に形成された管状の有機質 envelope 中で行われる（Fig. 2～4）。このような envelope は coenocytic filament の外側に接して存在する organic matrix に由来するものと考えられ，その壁は二重の構造を示す（Fig 6）。envelope は結晶の initiation の場となり，また結晶の成長にあたって，その大きさと形を調節する役割を有するものと考えられる。（*350-02 埼玉県坂戸市けやき台 1-1，城西歯科大学. **Bermuda Biological Station for Research, Bermuda）

斎藤　譲：ソゾ属の本邦新産種 II. Yuzuru SAITO: *Laurencia* species new to Japan II.

Laurencia nidifica J. AGARDH, Species, genera et ordines algarum, 2, p. 749, 1863. 和名：ミナミソゾ（新称）産地：高知県沖の島ニウドガタネの低潮線下 2～3 m の岩上（1964年 6 月28日，喜田和四郎採集；1977年 6 月 5 日，筆者採集）

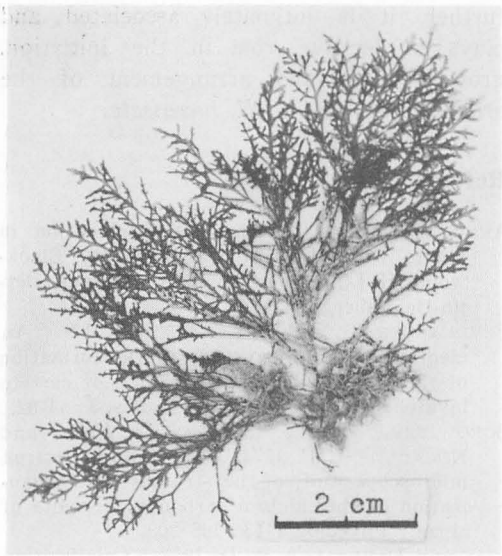

Fig. 1. *Laurencia nidifica* J. AGARDH (Nyûdogatane, Okinoshima Isl. near Shikoku, Japan, 5-VI-1977).

以前，基準産地のハワイに多産し，外形や色彩の変化に富むことについてものべた（SAITO Pac. Sci., 23, pp. 152-3, fig. 5, 1969）が，その際は喜田博士から贈られていた上記標本のことを失念しており，最近に到って別の目的で現地におもむき，自分で採集して検討したところ，本種にあてるべきものであることを知った。形態的には，ハワイのオアフ島の Kuloa 渓流河口にほど近い低潮線下約 1 m の岩上から採集したものに類似し（**Fig. 1**），約 10 cm まで高く，紅褐色である。表皮細胞相互間には，縦方向の原形質連絡の存在が明らかで，四分胞子嚢は平行型配列を示すので，Subgenus *Laurencia* マソゾ亜属に所属することになる。なお，髄細胞の膜に半月形肥厚もある種なので，南日本で採集され，従来いくぶんの疑問を残しながら *Laurencia okamurai* YAMADA ミツデソゾと同定されていた標本中に本種の含まれている可能性が考えられる。

ここに標本を提供された三重大学の喜田和四郎博士にお礼を申し上げる。

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