Intracellular aragonite crystals in the fresh-water alga, *Spirogyra* sp.

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Abstract

Intracellular crystals of aragonite have been identified by selected area electron diffraction (SAED) in the freshwater filamentous alga *Spirogyra* sp. which grows sporadically as carpets in the Thames river, Ontario, Canada. The crystals are 2 to 24 μm in length, and characterized by a unique cross-shaped morphology, in which needle-like, or prismatic outgrowths develop from a common axis. Crystals may be dispersed through filaments, but tend to cluster as aggregates towards the centre.

KEYWORDS: intracellular, aragonite crystals, *Spirogyra*, algae, Canada

Introduction

CALCIUM CARBONATE deposition in algae can be initiated intracellularly, within intercellular spaces or within the thickened cell wall or sheath (Borowitzka, 1982; Okazaki and Furuya, 1985; Leadbeater and Riding, 1986). The major mineral phases deposited are calcite and aragonite. Although calcite is the thermodynamically favoured product, the majority of marine algae precipitate aragonite probably due to the high ionic strength and high Mg concentrations in these environments (Borowitzka, 1984; Cabioch and Giraud, 1986; Denizot, 1968). In contrast, no evidence has been presented, to date, implicating the biogenic deposition of aragonite in freshwater algae. This paper reports the characterization of intracellular aragonite in the freshwater alga *Spirogyra* sp. using transmission and scanning electron microscopy, electron diffraction and energy dispersive X-ray analysis (EDAX). The crystals develop from a common axis as needle-like or prismatic outgrowths such that the final mineral product has a unique cross-shaped morphology.

An unusually prolific bloom of bright green filamentous algae was observed on the west bank of the Thames river, close to the playing fields of the University of Western Ontario, London, Canada. Samples were collected and examined by light microscopy. Intracellular crystals were extracted and prepared for SEM and TEM by gently macerating a small quantity of the algae in a pestle and mortar followed by washings with deionized water. The mixture was then centrifuged, fractured algal cells were decanted, and the crystals recovered from the bottom of the centrifuge tube. The crystals were dispersed onto a glass slide and then transferred by hand, using a fine hair brush, onto copper electron microscopy grids for SEM and TEM analysis. SEM investigations were undertaken at 30 kV using a ISI-DS 130 SEM. Crystals were gold plated for effective SEM imaging. The EDAX facility was a PGT System. TEM investigations were undertaken using a JEOL 100CX electron microscope operating at an accelerating voltage of 100 kV. Electron diffraction was performed in the selective area aperture mode using a camera length constant calibrated from standard samples.

The collected algae had a slippery mucilaginous texture and was easily separated by hand into long hairlike threads. Light microscopy studies identified the genus as the green alga *Spirogyra* sp. Photomicrographs (Fig. 1) showed the presence of filaments of individual diameter approximately 70 μm. At higher magnifications (Fig. 1C,D) intracellular crystals with characteristic cross-shaped morphologies were readily observed. Although the crystals were dispersed throughout the filaments they were often localised towards the central region.

SEM micrographs clearly showed the apparent

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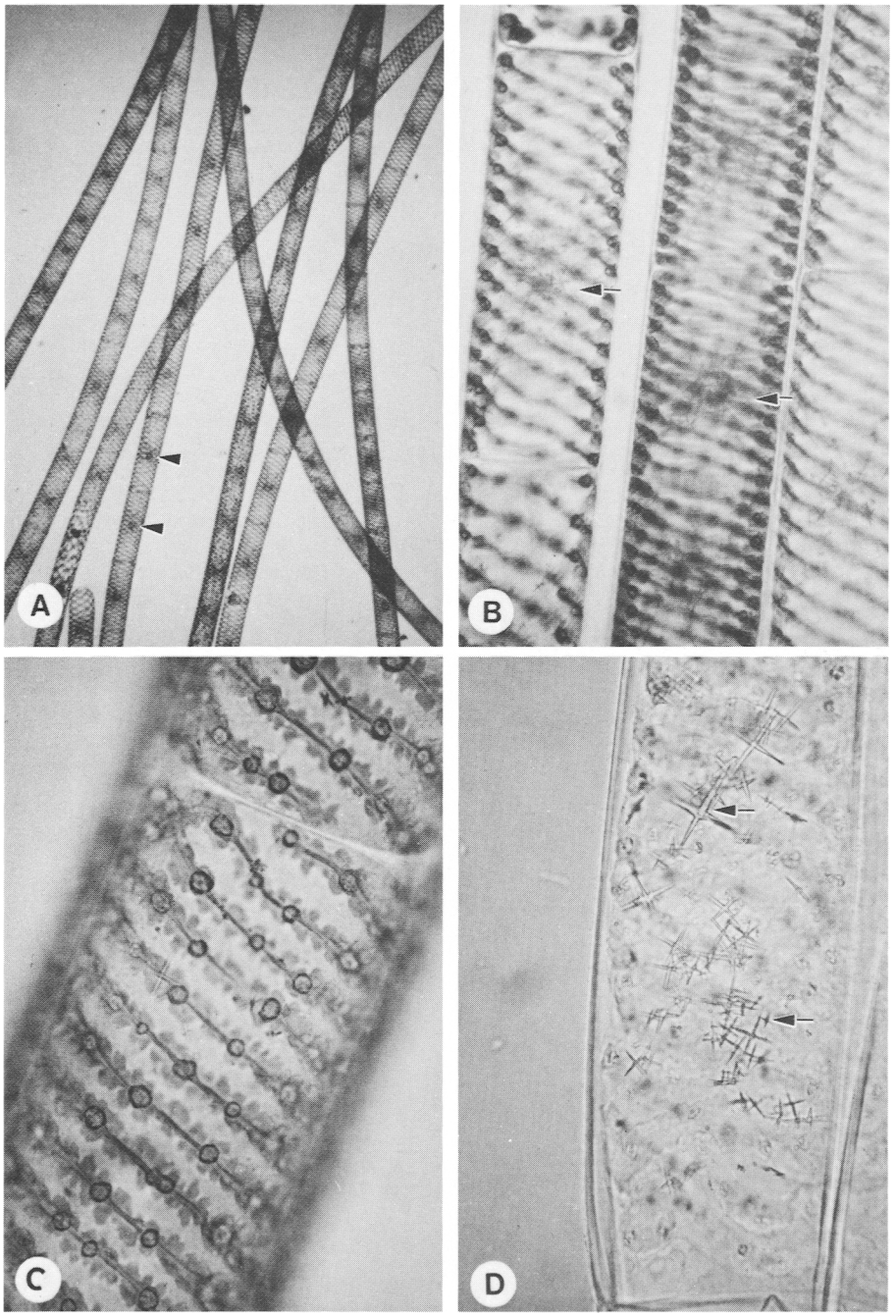
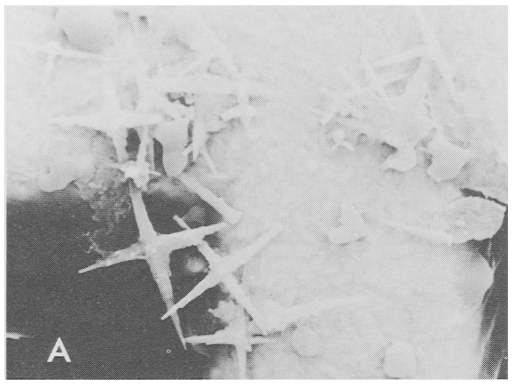


FIG. 1. Photomicrographs of *Spirogyra* sp. Diameter of all filaments 60–80 μm. (A) View at low magnification. Crystals are located towards the central region, arrows. (B) Detail of filaments illustrated in (A). (C) *Spirogyra* sp. filament containing aggregate of intracellular aragonite crystals. Length of crystals 10 to 24 μm. Note pronounced helical structure of chlorophyl. (D) Aggregate of intracellular aragonite crystals now well seen after treatment with commercial bleach. Filament diameter approximately 70 μm. Resistance of crystals to bleach rules out the possibility that they are crystalline Ca-oxalates.

four-fold symmetry and multidomain nature of the intracellular deposits (Fig. 2). Crystals were



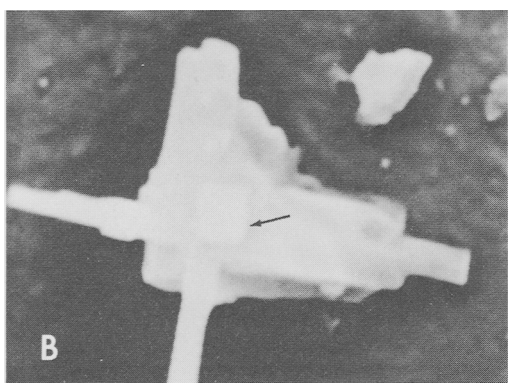


FIG. 2. (A) SEM micrographs of intracellular aragonite crystals isolated from *Spirogyra* sp. Bar 5.18 μm. (B) High magnification, SEM micrograph showing the disposition of crystal outgrowths along a fourfold axis. The outgrowths develop from cubic or rectangular nuclei sited along the rotational axis indicated by arrow. Bar 1.18 μm.

2–24 μm in length. The cross-like morphology was generated by the outgrowth of needle-like crystallites from the principal four-fold axis. However, close inspection showed that the outgrowths originated at different positions along the principal axis resulting in a staggered configuration of the arms. Several crystals showed secondary nuclei of cubic or rectangular geometry sited along the principal axis (arrow in Fig. 2B). The development of these nuclei into needle-like extensions with well-defined crystal ends and edges indicates a growth mechanism which results in discrete crystallographic faces being selectively enhanced.

Elemental analysis of individual crystals detected only Ca within these structures. Although these data confirm the absence of phosphate or sulphate minerals they provide no compositional evidence for either carbonates or oxalate phases because carbon and oxygen are not detected by this technique.

TEM micrographs showed that in some crystals there was considerable intergrowing of the original outgrowths such that the original cross-shape was no longer clearly distinguished (Fig. 3A). Selected area electron diffraction patterns were recorded from the crystal outgrowths and revealed that each outgrowth was a single crystal of aragonite (Fig. 3B). The biogenic mineral was exceedingly electron-beam-sensitive such that single crystal patterns changed to powder rings after prolonged exposure. However, these patterns confirmed the presence of aragonite as well as a decomposition product identified as CaO (Table 1).

Table 1. *Powder X-ray diffraction data (Å) for Spirogyra crystals and crystallographic assignment*

Spirogyra crystals	Aragonite	Calcium oxide (CaO)
3.39	3.39 (111)*	
2.73	2.73 (121)	2.77 (111)
	2.70 (012)	
2.34 (arc)	2.37 (112)	2.4 (200)
	2.34 (130)	
2.04	1.97 (221)	
1.68 (arc)	1.69 (222)	1.7 (220)
1.42	1.41 (312)	1.45 (311)
1.37	1.36 (242)	1.39 (222)
1.17	1.17 (162)	
1.04		1.07 (420)
0.97		0.98 (422)

* = Miller indices (hkl). The arc patterns are characteristic of (200) and (220) CaO decomposition products from $CaCO_3$ (Towe, 1978).

The data presented in this paper show, for the first time, to our knowledge, the occurrence of aragonite in freshwater algae. The crystals are formed intracellularly, although detailed investigations of cellular ultra-structure need to be undertaken to determine the exact sites of mineralization. Very few algae are known to date to synthesize calcium carbonate phases intracellularly. The coccolithophorids (Class: Prymnesiophyceae) are unique in forming intracellular membrane-bounded sculpted plates of calcite

which are subsequently translocated to the exterior of the cell where they are assembled to form exoskeletons (Westbroek *et al.*, 1984). The further evidence for intracellular $CaCO_3$ deposition is the formation of needle-shaped aragonite crystals in the cytoplasm of a marine green algae *Penicillus dumetosus* (Perkins and McKenzie, 1972).

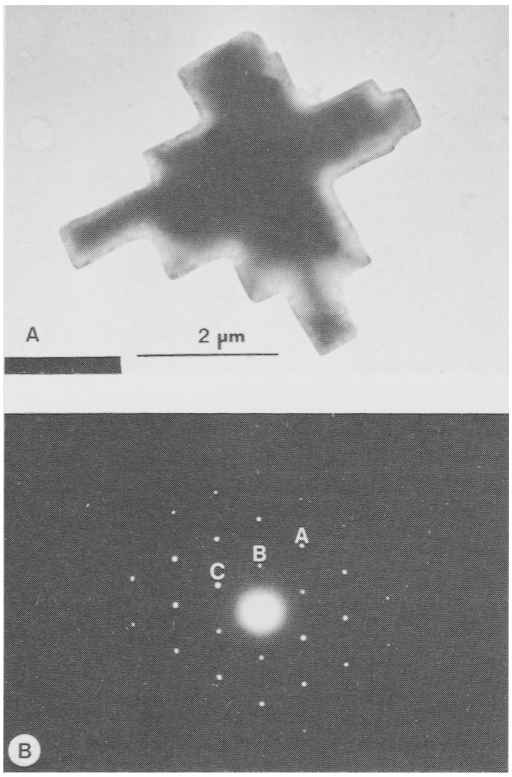


FIG. 3. (A) TEM image of an aragonite crystal from *Spirogyra* sp. Bar 2 μm. (B) Selected area electron diffraction pattern of a crystal outgrowth from the mineral product shown in (A). Tilt angle = 12°. The pattern corresponds to the [001] zone of aragonite. Reflection A = (200) (2.5Å); Reflection B = (110) (4.2Å); Reflection C = (020) (3.9Å). Angles: 100 ∧ 010 = 90°; 100 ∧ 110 = 32°. (Crystallographic data: space group *Pmcn*; for [001] zone, i.e. *hk*0 reciprocal lattice net, reflections $h + k = 2n + 1$ are forbidden; $a = 4.959$, $b = 7.968$, $c = 5.741$Å).

Lowenstam and Margulis (1980) have tabulated the distribution of biomineralization products among the five kingdoms. *Spirogyra* is the largest genus, in terms of the number of species, of the phylum Zygnematophyta. The genus is known to deposit biominerals such as intracellular calcium oxalate (Arnott and Pautard, 1970; Kamiya, 1961) and baryte ($BaSO_4$) within vacuoles in the desmids (Kreger and Boere, 1969; Brook, 1980; Brook and Williamson, 1985). These observations in combination with the results reported here confirming the presence of aragonite in the phyllum Zygnematophyta, suggests that *Spirogyra* can initiate the mineralization of a range of biominerals at various cellular locations under suitable environmental conditions.

The occurrence of at least three different biominerals in *Spirogyra* suggest a common response to demands placed on the organism from its immediate environment. In deeply cold springs and pools *Spirogyra* sp. flourishes abundantly and vegetatively, forming green patches several centimetres in diameter in favourable habitats. In shallow warm water *Spirogyra* sp. will often develop near the sediment surface in areas protected from swift currents (Darley, 1982), as is the case in the Thames river, Ontario, where algal 'carpets' ranged from 1–5 m^2 in area. The role of the local chemical environment appears to be critical. For example, the same *Spirogyra* sp. with cross-shaped crystal inclusions were observed at the the above specific location during previous years (1984–85) but at other observation spots, the same species of algae was present without the intracellular crystals.

The formation of intracellular aragonite in *Spirogyra* is possibly linked to the dissolved CO_2 levels in the surrounding environment. For example, studies of the precipitation of inorganic $CaCO_3$ in calcareous springs around Urach (Germany) (Carr and Whitton, 1973) has shown that the restoration of the carbonate equilibrium with atmospheric CO_2 produces chemical gradients along the water flow. The amounts of dissolved CO_2 and dissolved calcium carbonate decreased whereas O_2 and pH increased as a function of the distance from the source. A pronounced initial decrease in dissolved CO_2 was followed by intensive $CaCO_3$ precipitation. Also, an increase in temperature diminished the capacity of the water to dissolve CO_2 and accounted for part of the precipitation. The rate of chemical change along these gradients was highest at the source. Further down the river the rate decreased as evidenced by measurements of alkalinity and conductivity. Such changes in gradient were correlated with a corresponding zonation of organisms. Within the first 20 m of flow the pH changed from 7.5 to 9. *Rivularia* sp. and *Pleurocapsa* sp. were found closest to the spring, followed by mosses and later by *Zygnema* sp. and *Spirogyra* sp. (Carr and Whitton, 1973). Note that the carbonate equilibrium can also be shifted in a similar direction biogenically such that

carbonate can be precipitated by the process of photosynthesis which removes CO_2.

Acknowledgements

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