Ultrastructural Changes in Spirogyra submargaritata Growing on Iron-ore Tailing

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High concentrations of heavy metals have been reported from different environmental sources and these heavy metals might exert detrimental effects on the associated vegetations (Turner 1969, Wong et al. 1978). Algae, which are one of the most common groups of plants found in various environments, therefore suffer from the toxic actions of heavy metals (Whitton 1970a, 1970b, Malanchuk and Gruendling 1973, Bartlett et al. 1974, Klass et al. 1974, Burnison et al. 1975, Overnell 1975a, 1975b, Silverberg 1976). On the other hand, heavy metal-tolerant populations of various plant species have been reported (Turner 1969, Antonovics et al. 1971). In algae, it has been found that certain populations of Stigeoclonium tenue (McLean 1974, Harding and Whitton 1977), Ectocarpus siliculosus (Russell and Morris 1970), Ankistrodesmus falcatus, Scenedesmus quadricauda and Chlorella pyrenoidosa (Silverberg 1976), and Hormidium rivulare (McLean 1975, Say et al. 1977) were highly tolerant to certain heavy metals.

During a recent survey on the algal flora growing on the iron-ore tailing area of Ma On Shan, Hong Kong, it was found that the filamentous green alga Spirogyra submargaritata was one of the few algal species which could grow well in this environment (unpublished data). The iron-ore tailing area of Ma On Shan has been known to contain high levels of heavy metals especially Pb, Cd, Fe, Mn, Zn, Cr and Cu (Wong et al. 1978). Apparently this population of S. submargaritata tolerate the high concentrations of heavy metals. A subsequent attempt was made to determine whether ultrastructurally discernible changes in cells of S. submargaritata could be conspicuously correlated with their heavy metal tolerance. The present paper reports the results of this study.

Materials and methods

Spirogyra submargaritata was collected in a very shallow stream (2 inches in depth) which run through the iron-ore tailing area in Ma On Shan, Hong Kong. The filaments of S. submargaritata were found growing attached to the sediment of the stream and the sediment was found to consist solely of tailing materials. In order to determine the metal contents in both the sediment and in the cells, samples of the sediment were collected, air-dried at room temperature (25°C), and were extracted with 1 N ammonium acetate (pH 7.0) in deionized water through a Whatman No. 42 filter paper; ten grams (fresh weight) of the algal filaments were rinsed with deionized water twice and then dried at 105°C for 24 hr. These sediment
and dried algal samples were ashed with the mixed acid digestion method (Allen
et al. 1974). The metal contents (Zn, Pb, Mn, Fe, Cu, Cr and Cd) were detected
with atomic absorption spectrophotometry (Perkin-Elmer model-360).

For electron microscopy, the algal filaments were cut into 3–5 mm long seg-
ments and were fixed in 6% glutaraldehyde in 0.1 M sodium cacodylate buffer
(pH 7.0) at 22°C for 6 hr. After 3 rinses (30 min each) with buffer, postfixation
was in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer at 4°C for 1 hr.
The segments were dehydrated through a graded ethanol series up to 70% ethanol
and were then transferred to 2% uranyl acetate solution in 70% ethanol for 1 hr.
After transfer through 80%, 95% and 100% ethanol, the segments were embedded
in 30-min steps with Spurr’s low-viscosity embedding medium (Spurr 1966) in in-
creasing concentrations in 100% ethanol and finally to 100% embedding medium
for 12 hr. Polymerization was at 60°C for 24 hr. Sections were cut with glass
knives on a Reichert Om-U2 ultramicrotome and were mounted on uncoated—300
mesh grid, stained for 10 min with lead citrate (Reynolds 1963) and viewed with
a Zeiss EM 9S-2 electron microscope. For comparison, normal filaments of
S. submargaritata growing in an unpolluted stream on the campus of The Chinese
University of Hong Kong were also collected and processed for electron microscopy.

Results and discussion

Table 1 shows that high levels of various heavy metals were found in the sedi-
ment of the stream where the algal samples were collected. Table 1 also indicates
that higher concentrations of heavy metals were found in the cells of S. submar-
garitata growing on the sediment. Various organisms are known to accumulate
metals at levels greatly in excess of those of their surrounding environment (Wong
et al. 1978). Green algae such as Chaetomorpha brychagona and Entermorpha crinita
were found to concentrate heavy metals up to many folds of those existed in their
environment (Wong et al. 1979).

Ultrathin sections of the normal filaments of S. submargaritata revealed that
the cells contained ribbon-like chloroplasts with pyrenoids and the associated starch
grains, specialized ‘karyoids’, mitochondria, Golgi bodies, a centrally located nu-
cleus with nucleolus and several large central vacuoles similar to those reported in
cells of other Spirogyra species (Dawes 1965).

In cells growing on iron-ore tailing, the metals were observed as electron-dense
precipitates adhered to the outer surface of the cell wall (Figs. 1, 2, 3, 5 and 7).
Apparently, gentle rising with water would not remove the firmly attached metal
precipitates from the cell wall surface. Clusters of metal ions were also found
distributing unevenly in the cell wall (Fig. 2). Fig. 1 shows a terminal cell of a
young filament in which the ribbon-like chloroplasts and starch grains were re-
mained intact. In Spirogyra cells, a periplasmalemma space which locates bet-

Fig. 1. Showing the ribbon-like chloroplast (Ch) and the associated starch grains (S), several
large central vacuoles (CV) and a number of smaller vacuoles (V) in a terminal cell on a young
filament. Also note the metal precipitates (MP) on the outer surface of the cell wall (W), inside
the periplasmalemma space (PS) and in the plasmalemma ingrowths (IG) which are proliferations
of the plasmalemma (PI). ×5000.
Figs. 2–3. 2, showing the clusters of metal precipitates (MP) trapped in the cell wall (W) and in the periplasmalemma space (PS). Also note the ingrowths (double arrow) forming vacuoles (V) and massive release of vesicles (Ve) from the plasmalemma (P1). ×12000. 3, showing the metal precipitates (MP) trapped inside the vacuoles (V) and inside the vesicles (Ve). ×12000.
Fig. 4. Showing the fewer numbers of ingrowths (IG), vacuoles (V) and vesicles (Ve) from the plasmalemma (Pl) along a newly-formed cross wall (CW). Metal precipitates (MP) are found in the periplasmalemma space (PS) and inside the vacuoles (V). The starch grains (S) and pyrenoid (P) are seen inside the chloroplast (Ch). ×14000.
ween the cell wall and the plasmalemma has been reported (Jordan 1970). The periplasmalemma space was rather prominent in *S. submargaritata* and the finely granular electron-dense metal precipitates were found to accumulate in this space as well as adhered on the plasmalemma surface even in young cells exposed to iron-ore tailing materials (Fig. 1). The plasmalemma showed an undulating appearance and produced proliferations in the form of finger-like ingrowths into the central vacuoles (Fig. 1). The metal precipitates were found filling in these ingrowths (Fig. 1). Later the complete separation of these ingrowths from the plasmalemma might result in the formation of metal-containing vacuoles along the inner surface of the plasmalemma (Fig. 3). In addition, massive release of vacuoles and small vesicles from the plasmalemma into the periplasmalemma space was observed (Fig. 2). This outwardly proliferation of the plasmalemma probably could account for the existence of large numbers of vesicles and vacuoles in the periplasmalemma space (Fig. 2). It has been reported that plasmalemma proliferation was of relatively frequent occurrence in plant cells (Marchant and Robards 1968) and these structures have been reported in several algae including *Chara* (Barton 1965, Crawley 1965), *Fucus* (McCully 1968), *Eudesme virescens* (Cole 1969), and in *Sphacelaria tribuloides* (Galatis et al. 1977). However, in *Spirogyra submargaritata*, plasmalemma proliferations are probably an unusual phenomenon which occurs only in response to certain unfavorable environment since plasmalemma proliferations are not observed in normal cells. Although vacuoles and vesicles have been reported to exist in the space between the cell wall and the plasmalemma in normal cells of several *Spirogyra* species (Jordan 1970), the number of these vacuoles and vesicles was much less than that observed in the present study. Since large amounts of metal precipitates were found mainly to accumulate inside the vacuoles and vesicles along both sides of the plasmalemma (Fig. 3), the role of these vacuolar structures formed by plasmalemma proliferations for absorbing the metal ions in order to reduce metal toxicity by rendering the non-availability of the metal ions for contact with the cellular organelles is evident. McCully (1968) and Cole (1969) also suggested a role in absorption for this membrane system in other algae.

In contrast to the active plasmalemma proliferations along the side walls, only few or no outgrowth of the plasmalemma into the periplasmalemma space on both sides of the newly formed cross wall between two adjacent cells was observed, although ingrowths forming vacuoles in the cytoplasm were detected (Fig. 4). The fact that the plasmalemma along a newly-formed cross wall was a young membranous structure might account for the fewer numbers of plasmalemma outgrowths and plasmalemma-originated vesicles in the periplasmalemma space adjacent to the cross wall.

During the continuous deposition of metal precipitates into the cytoplasm,

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Figs. 5-6. Showing the metal precipitates (MP) trapped inside the vacuoles (V) and in the highly compartmented central vacuole (CV). Only few thylakoids (T) remain in the distorted chloroplast (Ch) with large numbers of starch grains (S). ×8500. 6, showing the inflated thylakoids (T), pyrenoid (P) and starch grains (S) inside the chloroplast (Ch). Metal precipitates (MP) are seen on the outer surface of the chloroplast envelope (ChE) and on the surface of the intact mitochondria (M). ×12000.
the several large central vacuoles (Fig. 1) became compartmented into numerous smaller vacuoles (Fig. 7) until a highly vacuolated situation was established (Fig. 5). Many of the vacuoles thus formed tended to trap and store the metal precipitates (Fig. 5). The ribbon-like chloroplasts eventually became distorted, presumably due to the active formation of new membranes during the compartmentation process of the large central vacuoles (Fig. 5). The number of the chloroplast thylakoids in a stack were found to be greatly reduced and became inflated (Fig. 6). Lead induced chloroplast abnormality has also been reported in *Stigeoclonium tenue* (Silverberg 1975). In *Spirogyra submargaritata*, metal precipitates were not observed in the chloroplast proper however (Fig. 6). Ultrastructural changes of mitochondria resulted as cadmium toxicity have been reported in *Ankistrodesmus falcatus*, *Chlorella pyrenoidosa* and *Scenedesmus quadricauda* (Silverberg 1976). Also formation of intranuclear complexes due to lead poisoning has been observed in some organisms (Goyer 1973). However, observations on *Spirogyra submargaritata* revealed that the structures of the mitochondria (Fig. 6) and the nucleus (Fig. 8) were not affected even though cadmium and lead were found present in high concentrations in both the sediments and inside the cells (Table 1). In addi-

<table>
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<th>Heavy metal</th>
<th>Heavy metal contents of sediment (ppm)*</th>
<th>Heavy metal contents in cells (ppm)*</th>
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<tr>
<td>Cd</td>
<td>0.16</td>
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<tr>
<td>Cr</td>
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<tr>
<td>Zn</td>
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* Figures represent the means of three replicates.

The toxicity of heavy metals on various physiological processes of freshwater algae (Steemann and Kamp-Nielsen 1970, Whitton 1970b, Gachter *et al.* 1973, Bartlett *et al.* 1974, Overnell 1975a, Monahan 1976) and marine algae (Mandelli 1969, Hessler 1974, Zingmark and Miller 1975, Overnell 1976) has been well documented. However, very few studies on the ultrastructural changes induced by long term exposure to heavy metal environments in algal cells have been pursued (Silverber 1975, 1976). In light of the fact that certain algal species can develop heavy metal-tolerant populations (Jensen *et al.* 1974, Silverberg 1975, Bentley-Mowat and Reid 1977, Harding and Whitton 1977, Say *et al.* 1977, Wong *et al.* 1979), it is anticipated that the presence of special structural mechanisms exist in the algal cells, which might account for the removal or prevention of excess metal ions so as not to affect the organism's normal physiological processes.
to affect the general metabolism or damage the sensitive structures of the cells. It has been reported that the cell wall and the pinocytotic vacuoles were such structural mechanisms in *Stigeoclonium tenue*, so that only a relatively low cytoplasmic concentrations of lead was maintained and thereby reducing the toxic effect of the lead ions (Silverberg 1975). The metal tolerance exhibited by *Spirogyra submargaritata* probaly results from the partial exclusion of the metal ions by binding the metal ions within the cell wall. Brown and Bates (1972) found that in several plants, lead was taken up passively and became ionically bound to the aniotic groups in the polyuronic acids of the cell wall. Since large amounts of metal precipitates were observed in the periplasmalemma space in *Spirogyra submargaritata*, it is suggested that this space serves as a temporary reservoir for the metal ions and delays their entrance to the cytoplasm. The plasmalemma proliferations in forms of vacuoles and vesicles are considered as an additional line of defense of the algal cells for the sequestration of excess metal ions entering the cell. These vacuolar structures might contribute to the translocation of intracellular metal precipitates from the cytoplasm to the enlarged periplasmalemma space. When excessive amounts of metal ions enter the central portion of the cell, the formation of numerous vacuoles by compartmentation of the large central vacuoles in order to trap the metal ions serves to strengthen the mechanisms for reducing the metal toxicity on the cell organelles. In addition, the chloroplast envelop, mitochondrial membrane and the nuclear membrane seem to be extremely effective barriers in resisting the entrance of the metal ions into these organells. Since the iron-ore tailing contains a mixture of a number of metals (Table 1), the ultrastructural changes observed in the cells should be considered as the combined effects of these metals rather than any particular metal alone. From the results obtained in the present study, it is also suggested that the structural mechanisms of the cells for limiting the availability of the metal ions for binding with the sensitive organelles are indifferent for various metals detected.

Reference

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