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Cadmium replaces calcium in the cell wall of *Ulva lactuca*

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Electron microscopy, in conjunction with X-ray microanalysis, was used to investigate the effects of exposure to cadmium on the elemental composition of the macroalga *Ulva lactuca*. The cell wall was the only region of the cell to show any marked change in chemical composition as a result of exposure to cadmium, with less calcium evident in cadmium-treated thallus compared with untreated thalli. The cell wall of *U. lactuca* is a complex structure made up of polysaccharides consisting of many-branched chains composed mostly of rhamnose and galactose subunits. Some of the hydroxyl groups on the subunits are substituted by sulphate groups. Borate is associated with the rhamnose subunits, which contain no sulphate groups, and calcium binds to borate, cross-linking the rhamnose groups. The borate–calcium complex adds rigidity to the cell wall; the replacement of calcium by cadmium will, therefore, influence the rigidity of the thallus. The ecological significance of this work is discussed with respect to the ability of the alga to withstand grazing or emersion.

Keywords: cadmium, calcium, cell wall, *Ulva lactuca*

Introduction

Algal cell walls can act as cation exchange systems (Ritchie & Larkum 1982, Kloareg *et al.* 1987, Mariani *et al.* 1990) and bind toxic metals as a result of covalent, electrostatic or redox reactions between the metal ion and sites on the cell wall (Greene & Darnall 1990). The cell wall in the macroalga *Ulva lactuca* is a substantial structure consisting of negatively charged polysaccharides (Haug 1976, Percival 1979) which present a number of potential metal binding sites, such as sulphate and carboxyl groups, each with different affinities for metals (Greene & Darnall 1990). The functional significance of negatively charged polysaccharides in the cell wall of marine macroalgae is that binding of cations allows the ionic environment of the alga to be regulated during the tidal cycle (Mariani *et al.* 1985, 1990, Kloareg 1991). The availability of metals for such accumulation will dependent on the speciation of the metal which is influenced by the physico-chemical characteristics (Reed & Gadd 1990, Hughes and Poole 1991) such as pH (Skowronski 1991).

The accumulation of metals may have a toxic effect on algal physiology (Webster & Gadd 1992, 1996). In addition, previous work has shown that microbial biomass (including microalgae) can act as efficient systems for accumulating metals, a phenomenon which is believed to have potential for the removal of toxic metals or radionuclides from contaminated effluents, thus allowing safe disposal into water (Greene & Darnall 1990, Reed & Gadd 1990, Volesky 1990). There is also a growing need to recover metals of economic importance in an effort to conserve existing resources (Volesky 1990). The aim of this investigation was to determine using X-ray microanalysis, in conjunction with electron microscopy, whether cadmium is preferentially accumulated in particular regions of the cell and whether there were any changes in the elemental composition of the cell resulting from exposure to cadmium.

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Materials and methods

Experimental organism and media

U. lactuca (Link) was collected from the Eash Rocks adjacent to St Andrews harbour, Fife, Scotland (OS grid reference: 517168). Seawater was collected from the same site and was filtered through Whatman no. 1 filter paper before use. Algae of similar size and colouration were chosen, cleaned using tissue paper and filtered seawater, and stored at 5°C under constant illumination (35 μE m^{-2} s^{-1}).

Electron microscopy

Discs were cut from non-reproductive areas of live *U. lactuca* thallus and incubated in filtered seawater at 20°C illuminated (35 μE m^{-2} s^{-1}) with constant stirring for 2 days. Algal discs were incubated in either filtered seawater or 10 μM Cd (as $3CdSO_4 \cdot 8H_2O$) in filtered seawater. The discs were then removed and rinsed briefly (3 s) in fresh filtered seawater, fixed in 5% (w/v) glutaraldehyde in 0.2 M PIPES (piperazine-*N*,*N*′-bis(2-ethanesulphonic acid buffer)) containing 0.5 M NaCl, pH 8, at 4°C for 16–18 h. The fixative was replaced by washing 2 × 1 h in 0.2 M PIPES buffer. Post-fixation was carried out using 0.2% (w/v) osmium tetroxide in 0.1 M cacodylate buffer for 16–18 h. The discs were then washed (2 × 30 min) in distilled deionized water and cut into pieces of approximately 2 × 10 mm before being embedded in 1% (w/v) agar. Blocks were cut from the agar containing the excised thallus and were transferred to 5% (w/v) uranyl acetate for 16–8 h. This was followed by two washes (30 min) in distilled deionized water before dehydration in an ascending ethanol series. Infiltration was carried out in 1:1 (v/v) absolute ethanol/L R White resin for 48 h on a turntable at 1–2 r.p.m., then a further 48 h in 100% L R White. The infiltration steps were carried out at 20°C. The samples were then transferred to gelatin capsules which were filled with fresh L R White resin and polymerized at 60°C for 24 h. The blocks were cut after a further 24 h at room temperature. Sections were cut on a Reichert OMU-3 ultramicrotome using glass knives before being mounted on pyroxylin-coated 200 mesh copper grids. Sections for electron micrographs were stained using 2% (w/v) Reynolds uranyl acetate (10 min) (Reynolds 1963), washed in distilled deionized water, then 20% (w/v) lead citrate in 0.01 M NaOH (10 min) and finally washed in distilled deionized water (Kierans *et al.* 1991). Sections were examined using a JEOL-1200 EX transmission electron microscope in conjunction with a LINK series II X-ray microanalysis system fitted with a LZ-5 detector. The sections required for X-ray microanalysis were unstained. The voltage used was 80 kV; counts were accumulated over 800 s on five sections of thallus (area 20 μm^2).

Results and discussion

The subcellular organization of *U. lactuca* has been described by other authors (West & Pitman 1967, Lobban & Wynn 1981) and no conspicuous differences in the appearance of subcellular organelles were observed between the two treatments (Figure 1).

X-ray microanalysis of the unstained sections of the thallus of *U. lactuca* (as in Figure 1) indicated that cadmium was present in all subcellular organelles within the cell, but was not concentrated in any one region. This contradicts the results of McLean & Williamson (1977) who used

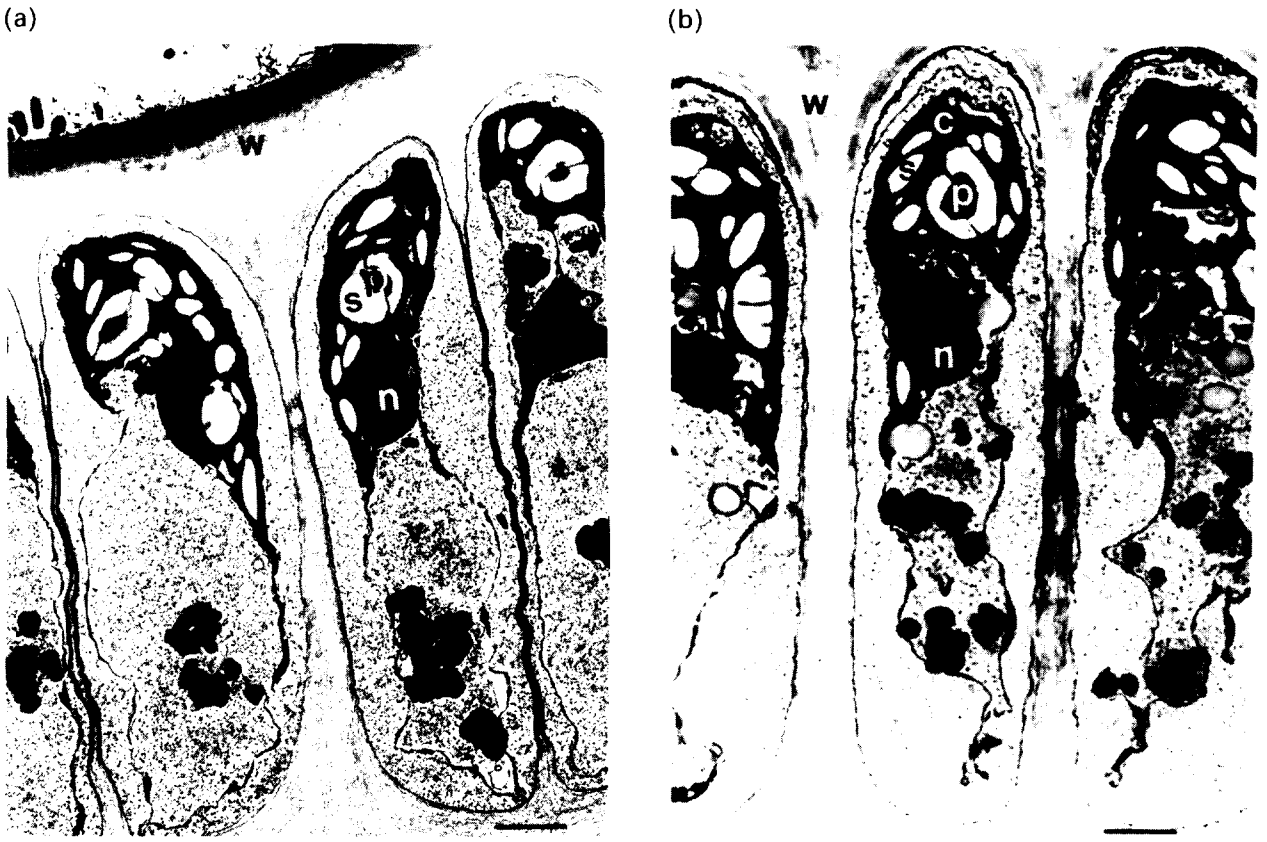

Figure 1. Electron micrograph (transverse section) of (**a**) cadmium-treated (10 μM in filtered seawater for 3 days) and (**b**) untreated *U. lactuca* thallus showing nucleus (n), pyrenoid (p), starch grains (s), cytoplasm (c), vacuole (v) and cell wall (w). The bar represents 2 μm.

autoradiographic techniques and reported that cadmium appeared to be located in or around the nucleus in *Porphyra umbilicalis*. This current study showed that cadmium was found in the nucleus of *U. lactuca*, but was also located in all other areas of the cell. In addition to indicating the presence (or absence) of cadmium in the thallus, X-ray microanalysis of sections of *U. lactuca* thallus also indicated changes in the chemical composition of the thallus in response to cadmium. The cell wall was the only part of the thallus to show any apparent change in chemical composition as a result of exposure to cadmium, with less calcium being present in the cadmium-treated thallus (Figure 2). The cell walls of algae have been described as cation

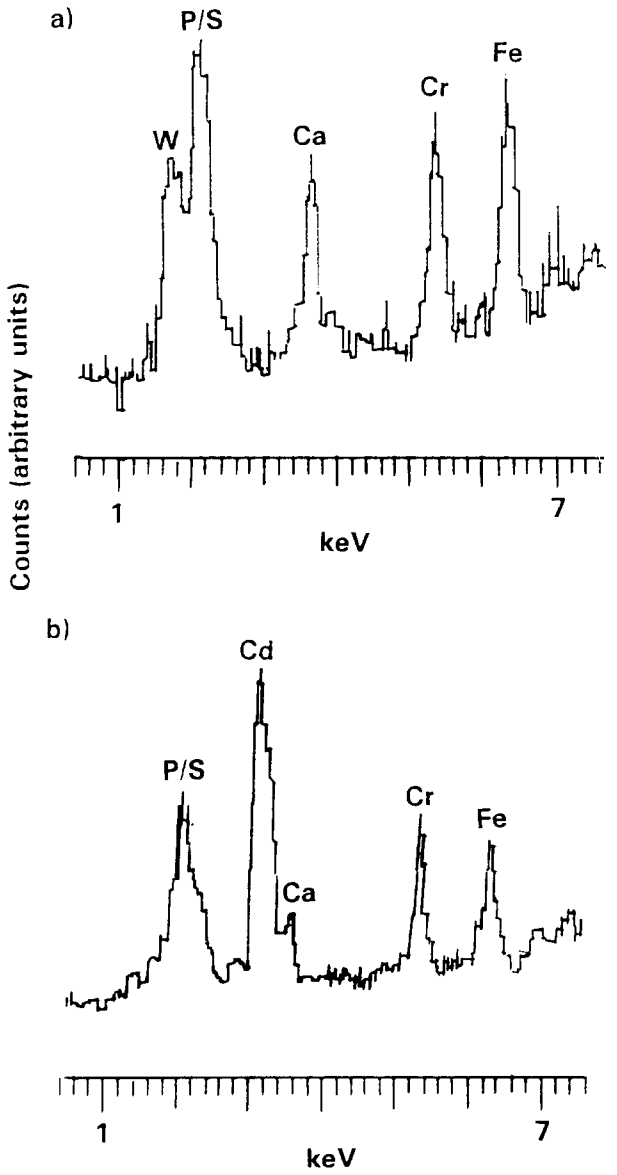

Figure 2. X-ray microanalysis spectra of (**a**) the cell wall of *U. lactuca* incubated in filtered seawater only and (**b**) the cell wall of *U. lactuca* incubated in filtered seawater plus cadmium.

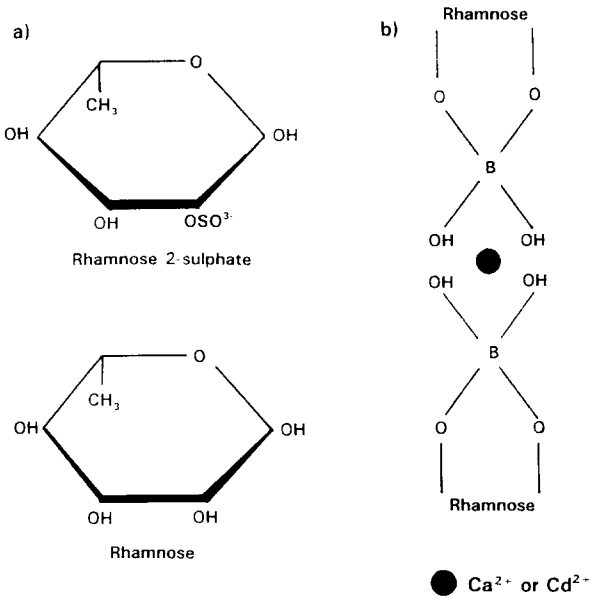

Figure 3. The proposed binding site for cadmium showing (**a**) the polysaccharide subunit of the cell wall of *U. lactuca* and (**b**) the cadmium binding site where cadmium may substitute for cadmium (adapted from Percival, 1979).

exchange systems by previous authors (Ritchie & Larkum 1982, Mariani *et al.* 1990, Crist *et al.* 1994) and some metals are integral components of the structure. Percival (1979) has described the cell wall of *U. lactuca* as consisting of highly branched chains of heteropolysaccharides where rhamnose and galactose are the principal saccharide subunits. Some hydroxyl groups on the saccharide subunits are replaced by sulphate groups (Figure 3a). The hydroxyl groups on the rhamnose subunits which lacked sulphate groups complexed with borate, and calcium was bound to the borate groups of two adjacent rhamnose subunits forming a calcium–borate complex. This configuration stabilized the borate complex, adding to the rigidity of the cell wall (Haug 1976, Percival 1979). From the results presented in this current study, it is possible that cadmium replaced calcium in the borate–rhamnose complex, in which case the rigidity of the thallus could be affected. One implication of this observation is that the consistency of the gel-like thallus may contribute towards the ability of the alga to withstand grazing (Hay *et al.* 1994, Schupp & Paul 1994) or to protect the alga from wave action or from desiccation during emersion (Percival 1979, Kloareg 1991). Other work on the characterization of metal-binding properties of the cell wall of *U. lactuca* has determined that there are at least two cadmium-binding sites available on the thallus, each having a different affinity for cadmium, the availability of which varies according to the cadmium concentration of the bathing medium (Webster & Gadd, submitted). In addition, NMR spectroscopy has revealed that the cadmium-binding functional group probably contained oxygen (Webster & Gadd, submitted), providing further evidence that the hydroxyl groups on the polysaccharide subunits (which contain oxygen) may

therefore act as cadmium-binding sites. The sulphate groups on the subunits also contain oxygen and while the results presented in this current study support the suggestion that cadmium binds to hydroxyl groups, they do not exclude the possibility that cadmium may also bind to sulphate groups on the saccharide subunits.

In conclusion, cadmium was taken up by *U. lactuca* but was not preferentially accumulated by any particular subcellular organelle. The cell wall accumulated cadmium and was the only part of the thallus to show any change in chemical composition as a result, with calcium apparently being displaced by cadmium. The ecological significance of this work has not been investigated, but the implications are that where cadmium replaces calcium in the cell wall, the rigidity of the thallus may be affected which may, in turn, influence the ability of the alga to withstand grazing, wave action or emersion.

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