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Perturbation of monovalent cation composition in *Ulva lactuca* by cadmium, copper and zinc

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Discs of thallus cut from the macroalga *Ulva lactuca* were incubated in filtered seawater containing cadmium, zinc, copper or cobalt (30 μM). The metal uptake rates differed for each metal in the order Cu > Zn > Cd > Co. Exposure of the macroalga to metals resulted in a disruption of intracellular monovalent cation composition. Intracellular potassium was irreversibly lost and sodium was accumulated by cadmium- or copper-treated *U. lactuca*, which was assumed to indicate irreversible disruption of the plasmalemma. Exposure to zinc caused an increase in sodium concentrations, whereas potassium concentrations were not significantly different from the controls, suggesting that the integrity of the plasmalemma had been maintained at the zinc concentration used. Intracellular magnesium was also lost from copper-treated algae, which again indicated a loss of integrity of the cell membrane.

Keywords: cadmium, cobalt, copper, potassium, sodium, *Ulva lactuca*, zinc

Introduction

Toxic metals can disrupt algal physiology and may affect photosynthesis, growth (Markham *et al.* 1980), enzyme activity (Van Assche & Clijsters 1990) and respiration (Webster & Gadd 1992, 1995). The physiological responses of organisms to toxic metals have been used as indicators of toxicity (Trevors *et al.* 1986). Axelsson & Axelsson (1987) used ion leakage to quantify the toxic effects of metals on *Laminaria*, while White & Gadd (1987) advocated the use of irreversible potassium release to monitor the toxic effects of metals on yeast. Reed & Moffat (1983) have described the loss of potassium from *Enteromorpha compressa* exposed to copper. Therefore, we have investigated the effect of toxic metals on the monovalent cation composition of *Ulva lactuca* as a potential organism for monitoring metal pollution.

Metals can be accumulated by algae by adsorption onto cell walls, which is independent of metabolism but which depends on other factors such as pH and metal concentration (Gadd & Griffiths 1978, Garnham *et al.* 1991, 1992). In addition to adsorption, ions can be taken up into the cell. To achieve this, ions must cross the plasmalemma, and the driving force for this is a complex function of the relative concentrations of the ion on either side of the membrane, the permeability of the membrane for the ion and the electrical transmembrane potential. The latter function is governed partly by the concentrations in the cytoplasm of a few ions, usually potassium, chloride and protons (Raven 1976).

Potassium is necessary for the efficient functioning of enzymes as an activator or as a component needed to maintain the precise conformation of the enzyme, thereby preventing the dissociation of subunits (Hughes 1981). Potassium is involved in osmotic regulation (Lobban *et al.* 1985) and is also a component of pyruvate kinase (Hughes 1981, Lobban *et al.* 1985). Sodium is tolerated in marine algae rather than required and the major regulatory problem for marine algae will be in excluding excess sodium from the cells (Higinbotham & Lüttge 1979). Potassium and sodium ions in algal cells are in a state of flux, with potassium being transported actively into the cell, but leaving the cell passively. For sodium the situation is reversed, i.e. influx is passive whereas efflux is active (West & Pitman 1967, Raven 1984, Ritchie 1988). Sodium and potassium in the cytoplasm are in the form of free ions, i.e. Na^+ and K^+; therefore disruption of the intracellular equilibrium between sodium and potassium may be a toxic response to exposure to metals. Magnesium is mostly bound to cellular constituents (Raven 1976) as a constituent of chlorophyll (Higinbotham & Lüttge 1979), participates in carbon fixation

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(Lobban *et al.* 1985) and is implicated in the transfer and hydrolysis of phosphate groups (Hughes 1981).

This work is part of an investigation of the uptake and accumulation of toxic metals by marine algae, which are the primary producers of the marine environment. Coastal waters, where most macroalgae are found, are the areas most likely to be affected by metal pollution (Rai *et al.* 1981) and any adverse effects resulting from exposure to toxic concentrations of metals could affect primary production. Macroalgae have also been used to monitor metal pollution in the marine environment (Scanlon & Wilkinson, 1987). Since potassium can be lost from macroalgae and other organisms in response to environmental stress (Dickson *et al.* 1980, Reed & Moffat 1983, White & Gadd 1987), analysis of changes in potassium (and sodium) concentrations in *U. lactuca* on exposure to toxic metals may provide an indicator and may reveal mechanisms of toxicity in this organism.

Materials and methods

Experimental organism, media and growth conditions

U. lactuca (Link) was collected from East Rocks adjacent to St Andrews harbour, Fife, Scotland. Thalli of a similar size and colouration were chosen, and discs (25 mm diameter) were cut from non-reproductive areas of the thallus and stored at 10°C in filtered seawater under constant illumination (35 μE m^{-2} s^{-1}) for up to 1 week. At 24 h before the start of the uptake experiments, 200 *U. lactuca* discs were transferred to 21 Azlon beakers containing filtered seawater and were maintained under the appropriate experimental conditions, in order to minimize any disruption of potassium concentrations caused by handling of the algal thalli at the start of the experiments. Seawater was collected from the same site, filtered through Whatman no. 1 filters and stored at 10°C.

Freeze-dried alga

U. lactuca was collected as detailed above, rinsed briefly (<15 s) in distilled, deionized water, then frozen (−10°C), freeze-dried and ground to pass a 600 μm mesh.

Determination of ionic composition of live U. lactuca

After exposure to cadmium, zinc, copper or cobalt (30 μM in filtered seawater), *U. lactuca* discs were removed from the medium, rinsed briefly (3 s) in 1 M mannitol and blotted between layers of tissue paper. Discs (six replicates of three discs per digest) were digested in 1 ml concentrated HNO_3 for 3 h at 90°C and then diluted with distilled deionized water for analysis using a Pye Unicam SP9 atomic absorption spectrophotometer, with reference to the appropriate standards. Standards were prepared by serial dilution from a stock solution using deionized, distilled water and stored in acid washed, plastic bottles at 10°C. Atomic absorption spectroscopy analysis was carried out on each digest using Pye Unicam lamps at the following wavelengths and bandpass settings (BP): Cd, 228.8 nm, 5 mA, BP 0.5 nm; Cu, 324.8 nm, 4 mA, BP 0.5 nm; Zn, 213.9 nm, 8 mA, BP 0.5 nm; Co, 240.9 nm, 9 mA, BP 0.2 nm; Na, 589.0 nm, 6 mA, BP 0.5 nm; K, 766.5 nm, 6 mA, BP; 0.5 nm; Mg, 285.2 nm, 3 mA, BP 0.5 nm. The experiment was repeated twice and the results shown are from one representative experiment.

Acid washing

Plastic vessels and disposable plastic test tubes were used throughout the experiments, and all were acid washed in 1 M HCl and rinsed in deionized, distilled water prior to use.

Chemicals

The chemicals used were $3Cd_2SO_4 \cdot 8H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$ and $CoCl_2 \cdot 6H_2O$ for the metal incubations

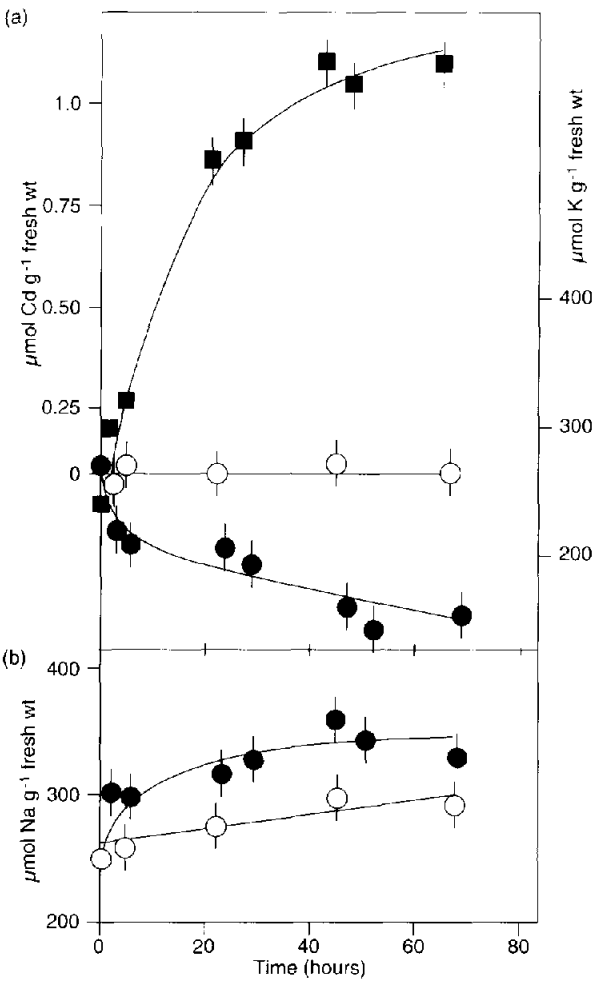

Figure 1. (**a**) Cadmium accumulation (■) and potassium concentrations for *U. lactuca* exposed to cadmium (●) and untreated (○) at 25°C. (**b**) Sodium concentrations for *U. lactuca* exposed to cadmium (●) and untreated (○). The final cadmium concentration (in filtered seawater) was 30 μM. Each point represents the mean of six replicates (±95% confidence limits).

and metal standards, and KCl, NaCl and $MgSO_4 \cdot 7H_2O$ for standards. All chemicals used were of the highest purity available.

Results

Potassium efflux

Live *U. lactuca* discs exposed to cadmium (30 μM) showed an immediate loss of potassium during the first 3 h (Figure 1a), after which time potassium continued to be lost, but at a slower rate. After 72 h potassium concentrations in the thalli were 62% of the control value (262 μmol K g^{-1} fr.wt). When potassium loss was plotted against amounts of cadmium accumulated (calculated from Figure 1a), there was a linear relationship between the two processes over the first 6 h ($r^2 = 0.9687$; $y = 264 \times 10^{-6} - 216x$) and the ratio of potassium lost to cadmium gained was 200:1. The rate of cadmium accumulation was greatest during the first 20 h, after which time uptake continued, but again at a reduced rate (1.1 μmol Cd g^{-1} fr.wt after 72 h). Exposure of live *U. lactuca* discs to copper (30 μM) (Figure 2a) also resulted in the efflux of potassium. The period of greatest potassium loss (the first 6 h) corresponded to the period of greatest copper accumulation. After 24 h potassium concentrations remained constant at 40% of the control value (250 μmol K g^{-1} fr.wt). Potassium loss was plotted against copper uptake and, as with cadmium, the relationship was found to be linear ($r^2 = 0.9915$; $y = 272 \times 10^{-6} - 56x$) with the ratio of lost potassium to copper uptake being 250:1 (calculated from Figure 2a). Zinc-treated discs and cobalt-treated discs (30 μM) showed no subsequent change in potassium levels (results not shown).

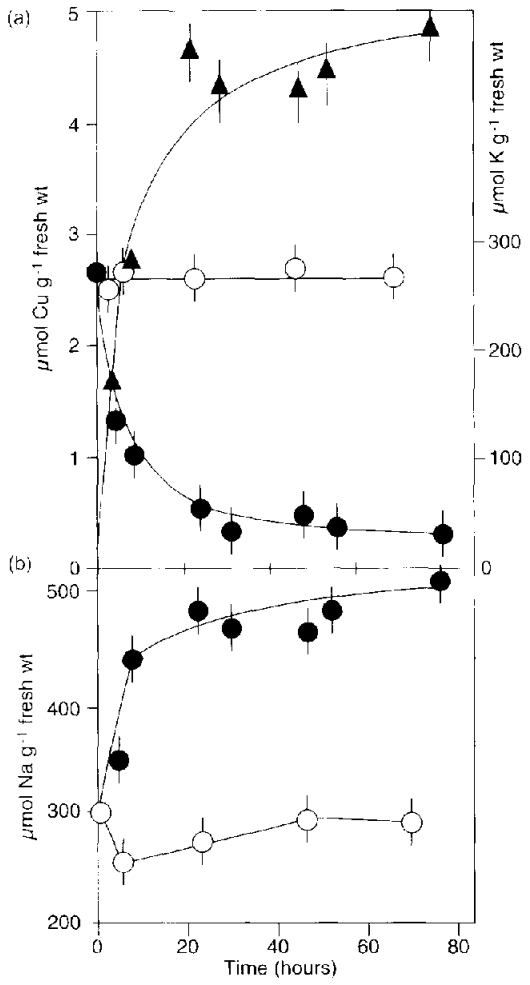

Figure 2. **(a)** Copper accumulation (▲) and potassium concentrations for *U. lactuca* exposed to copper (●) and untreated (○) at 25 °C. **(b)** Sodium concentrations in *U. lactuca* exposed to copper (●) and untreated (○). The final copper concentration (in filtered seawater) was 30 μM. Each point represents the mean of six replicates (±95% confidence limits).

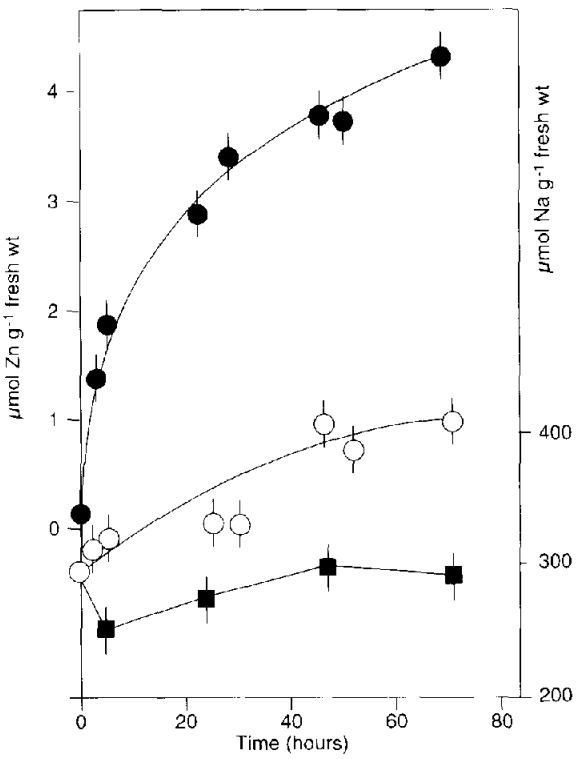

Figure 3. Zinc accumulation (●) and sodium concentrations in *U. lactuca* exposed to zinc (■) and untreated (○) at 25 °C. The final zinc concentration (in filtered seawater) was 30 μM. Each point represents the mean of six replicates (±95% confidence limits).

Sodium accumulation

When incubated in cadmium (30 μM; Figure 1b), sodium accumulation was greatest during the first 3 h and after 24 h remained at approximately 25% above the control value (275 μmol Na g^{-1} fr.wt). The rate of cadmium uptake continued to increase until 24 h, after which time the cadmium uptake rate slowed. Exposure to copper (30 μM; Figure 2b) resulted in a significant increase in sodium

concentration during the first 6 h and after 24 h sodium concentrations remained at 50% above the control (280 μmol Na g^{-1} ft.wt). When potassium efflux was plotted against sodium influx, there was a direct correlation between the two processes for both the cadmium- and the copper-treated discs ($r^2 = 0.9247$, $y = 467 \times 10^{-6} - 0.771x$; $r^2 = 0.8603$, $y = 491 \times 10^{-6} - 44x$, respectively) and the ratio of potassium lost to sodium gained was 1.4:1 for the cadmium treatment and 1.6:1 for the copper treatment. Zinc-treated discs (30 μM) showed no change in potassium concentrations but an increase in sodium (Figure 3) of 30% over the control value after 24 h. Sodium influx was plotted against zinc accumulation ($r^2 = 0.9136$, $y = 282 \times 10^{-6} - 44x$) and the ratio of sodium gained to zinc accumulated was 13:1. Cobalt (30 μM) had no significant effect on sodium or potassium concentrations.

Magnesium loss

Magnesium concentrations were also monitored. In discs of *U. lactuca* exposed to copper (30 μM), magnesium concentrations fell after 6 h to 20% below the initial value (120 μmol Mg g^{-1} fr.wt) (Figure 4).

Potassium, sodium and magnesium concentrations in freeze-dried U. lactuca

The concentrations of potassium, sodium and magnesium in freeze-dried *U. lactuca* were monitored over 80 h and were found not to fluctuate during this time (results not shown), suggesting that all of the changes in potassium, sodium and magnesium concentrations observed during the course of the experiments could be attributed to intracellular fluctuations.

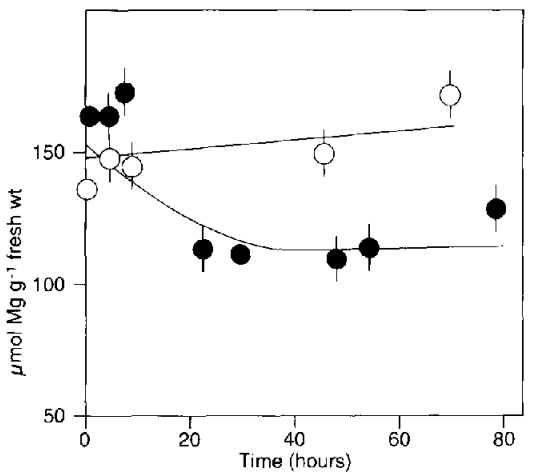

Figure 4. Magnesium concentrations in copper-treated (●) and untreated (○) discs of *U. lactuca* at 25°C. The final copper concentration (in filtered seawater) was 30 μM. Each point represents the mean of six replicates (±95% confidence limits).

Discussion

The cell membrane can act as a barrier to prevent the entry of potentially harmful cations or to confine their entry to specific transport systems, although such routes may not be absolutely specific and non-essential ions may be able to enter cells by utilizing the transport systems for essential ions such as potassium (Mehlhorn 1986).

Metals can also bind to metabolically inactive regions of algae such as the cell wall. This process is assumed to be independent of metabolism, but depends on such factors as the metal concentration in the bathing medium, the pH and the presence of competing ions (Rai *et al.* 1981, Reed & Gadd 1990). Metabolic activity by the cells may, however, influence the physico-chemical microenvironment around cells and thus indirectly affect 'metabolism-independent' binding. Potassium, sodium and magnesium concentrations in freeze-dried *U. lactuca* were found not to fluctuate during the time course of these experiments. This confirms that changes in concentrations of these metals in live *U. lactuca* were intracellular in origin, assuming that basic chemical parameters of binding were similar in whole thalli and freeze-dried preparations.

Early work on ion transport in *U. lactuca* (West & Pitman 1967) and further developed by Ritchie (1988) showed that potassium and sodium transport were two separate processes. Ritchie (1988) used $^{42}K^+$ and $^{86}Rb^+$ as tracers and microelectrodes to study membrane potential and ion fluxes in *U. lactuca*, and fitted his data to the Nernst equation to confirm that potassium concentrations within the cell were maintained via the interaction between two processes, i.e. active transport against a concentration gradient into the cell and passive efflux, a process termed 'pump and leak' by Raven (1984). Conversely sodium flux into the cell was passive down a concentration gradient, with intracellular sodium concentrations being regulated via an active efflux system (Ritchie 1988).

Metal uptake and potassium and sodium concentrations

If toxic metals enter the cell via potassium uptake system(s) (Mehlhorn 1986), then the cellular concentration of potassium should fall as a response. In the present study, copper uptake caused a decrease in potassium content with the rate of potassium loss correlating directly with copper uptake with a ratio of $1\,Cu^{2+}_{in}$:$250\,K^+_{out}$. This ratio is of the same order of magnitude as that observed for copper uptake and potassium release in *E. compressa* (Reed & Moffat 1983). The situation for cadmium and potassium was similar with a ratio of cadmium uptake to potassium release of $1\,Cd^{2+}_{in}$:$200\,K^+_{out}$. Thus, although potassium is lost in response to exposure to the toxic metal, the relationship between copper (or cadmium) uptake and potassium loss cannot be described as stoichiometric because the potassium loss is at least two orders of magnitude greater than metal accumulation (Reed & Moffat 1983). Sodium levels in *U. lactuca* rose in response to exposure to copper or cadmium, and in each case the ratio between potassium loss

and sodium uptake was close to 1:1, which did indicate a stoichiometric relationship between potassium loss and sodium uptake. The commonly observed 'antagonism' between potassium and sodium for monovalent cation transport systems, with affinities of K^+ being much greater than those for Na^+, suggests that sodium enters the cell passively in response to the loss of potassium. Potassium efflux will result in an electrochemical imbalance across the cell membrane and the passive influx of sodium will help to redress that balance. Uptake of zinc caused only a moderate accumulation of sodium, which suggests that the effect of zinc on *U. lactuca* is different from that of copper or cadmium, or that zinc is less toxic at the concentration used for these experiments.

White & Gadd (1987) reported that certain concentrations of copper and cadmium caused an irreversible loss of potassium from yeast cells, whereas after exposure to cobalt or zinc there was a temporary loss of potassium which was subsequently re-accumulated until control values were restored. These authors attributed these differences as being due to differing effects on membrane permeability, with cadmium and copper causing the integrity of the membrane to break down. In the present study, exposure to zinc did not inhibit the ability of the plasmalemma to exclude ions against a concentration gradient and the equilibrium for potassium was unchanged, although sodium concentrations remained above controls. The work of Dickson *et al.* (1980) indicated that *U. lactuca* could tolerate elevated cellular concentrations of sodium (in response to hyperosmotic stress) in the short term.

Toxic concentrations of metals are generally assumed to increase membrane permeability (Trevors *et al.* 1986). Reed & Moffat (1983) have reported a loss of potassium in copper-exposed *E. compressa*, although there was no loss of potassium from *E. compressa* which had previously been grown in a copper-enriched environment. Reed & Moffat (1983) attributed this to some form of membrane protection in the copper-tolerant ecotype. Thus, irreversible loss of potassium is indicative of membrane damage; the resulting inability of the cell to maintain chemical gradients across the membrane will result in the disruption of cellular processes and ultimately death (White & Gadd 1987, Karamushka & Gadd 1994).

From the data presented, it is unlikely that the metals studied are accumulated by entry as substitutes for potassium. It is theoretically possible that the metals could cause intracellular potassium concentrations to fall by inhibiting the active uptake of potassium (or the active efflux of sodium), either directly or indirectly such as by inhibiting ATP availability. There is some evidence for the latter phenomenon. De Fillipis *et al.* (1981) have reported a decrease in ATP concentrations when *Euglena* sp. was exposed to zinc or cadmium, although total adenylate concentrations remained unchanged. Van Assche & Clijsters (1990) observed an increase in induction of several enzymes involved in the tricarboxylic acid cycle in plants in response to exposure to toxic metals, which could ultimately increase the availability of ATP. The most likely explanation for the results obtained in the present study is that the metals used increased the permeability of the cell membrane leading to non-specific loss of potassium and accumulation of sodium. Further evidence for this can be seen from the data for the effects of copper exposure on cellular magnesium. The fall in magnesium concentration is relevant because magnesium is bound mostly to cellular components and does not form a significant pool of free ions in the cytoplasm (Raven 1984). Magnesium is an important component of chlorophyll, and is required for the efficient functioning of ATP and many enzymes. A decrease in magnesium concentrations suggests a disintegration of subcellular components which must be followed by a significant loss of cellular function. It is also possible that some free Mg^{2+} is released by disruption of the vacuolar membrane, vacuoles being an important storage organelle for Mg^{2+} (Raven 1984).

Potassium loss has been suggested as providing an index of toxicity by a number of authors (as detailed earlier), with the permanent loss of potassium signifying irreversible damage to the plasmalemma and the restoration of potassium concentrations suggesting the continued viability of the organism (White & Gadd 1987). The monitoring of magnesium concentrations could offer an additional index of toxicity, although further research would be necessary to investigate the applicability of this for toxicity screening and whether other algae respond in a similar manner.

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