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Chemical Control of Cadmium Uptake by *Peltigera*

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Abstract

Earlier studies of cadmium uptake by *Peltigera* using a sequential elution technique have distinguished between intercellular, extracellular, and intracellular fractions. This paper deals with interactions between cadmium and other cations during uptake by extracellular exchange sites and trans-membrane movement into the cell. The possible value of a pretreatment with potassium, to remove pre-existing cations from extracellular sites, is discussed. Intracellular uptake of cadmium is shown to display complex kinetics. Possible explanations are discussed, emphasising the potential toxicity of the supplied cadmium, and potassium-induced alterations to cell physiology and cation binding to exchange sites.

Keywords: cadmium uptake kinetics, extracellular and intracellular sites, cation interactions, potassium pretreatment, *Peltigera horizontalis*, toxicity

1. Introduction

Using a sequential elution technique it is possible to recover elements from three main cellular locations in lichens: intercellular, extracellular, and intracellular (Brown and Beckett, 1984; Brown and Wells, 1988). The intercellular fraction comprises elements in solution between the cells and will reflect the chemistry of recently supplied solutions (e.g. rainfall or experimental solutions)

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and any elements displaced from the other fractions. The extracellular fraction represents material temporarily bound in an exchangeable form to charged groups on the polysaccharide and protein components of the cell wall and outer surface of the plasma membrane. For elements in cationic form, extracellular binding is to anionic groups from which they can be displaced by the addition of a further cation with greater affinity for the site or supplied at a higher concentration. The intracellular fraction consists of material that has been transported across the plasma membrane, probably by a carrier system, and may be soluble or insoluble within the cell.

Laboratory studies on heavy metal uptake by lichens have shown that, while extracellular uptake reflects predictable physico-chemical reactions and is quantitatively the dominant process, intracellular uptake of cadmium appears to be a carrier-mediated process displaying conventional Michelis-Menten kinetics (Beckett and Brown, 1984a,b). Comparable processes occur in fungi (Gadd and White, 1989), algae (Trevors et al., 1986) and higher plants (Woolhouse, 1983; Clarkson and Lüttge, 1989) but with differing distributions of elements between the two locations. Supplied heavy metals have been shown to move from extracellular to intracellular locations, emphasising that extracellular binding is a reversible equilibrium process (Brown and Beckett, 1985). Divalent cations, particularly calcium, have often been reported to ameliorate the toxic effects of heavy metals and to reduce their uptake (Woolhouse, 1983; Clarkson and Lüttge, 1989). Beckett and Brown (1984a) reported competition experiments which showed that calcium was a more efficient competitor of extracellular cadmium uptake than is magnesium, whereas the reverse applied to its intracellular uptake. In addition to showing that samples of *Peltigera* from a disused zinc mine had reduced rates of intracellular cadmium uptake, compared with samples from uncontaminated sites, Beckett and Brown (1984b) demonstrated that magnesium was a competitive inhibitor of intracellular cadmium uptake in unpolluted material, whereas samples from the zinc mine appeared to show non-competitive kinetics.

Uptake of heavy metals by mosses, such as *Rhytidiadelphus squarrosus*, show many features in common with lichens (Brown and Beckett, 1985; Wells and Brown, 1987). However, recent studies have shown that the proportion of cell wall to cytoplasm (Wells and Brown, 1987) and the nature of chemicals on the cell wall (Wells, 1988; Wells and Brown, 1990) significantly modify the intracellular uptake rate for cadmium. Incubating a moss, or lichen, in a cation-containing solution will cause the release of elements previously located on the extracellular exchange sites, and these elements may then interact with the intracellular uptake of the supplied cation. Pre-treatment of moss samples

with potassium (Wells and Brown, 1990) substantially reduced the concentration of cations released during cadmium uptake experiments and eliminated differences in intracellular uptake rates between two populations from sites with contrasting heavy metal contamination (Wells, 1988).

Wells and Brown (1990) showed that intracellular cadmium uptake was competitively inhibited by calcium, and non-competitively inhibited by magnesium, whereas Beckett and Brown (1984b) showed that magnesium was a competitive inhibitor in *Peltigera*. We now hypothesise that the results that were obtained with *Peltigera* might have been complicated by the substantially greater concentrations of calcium, compared to magnesium, displaced from the lichen cell walls, producing the equivalent of a partly inhibited cadmium uptake rate when this element was supplied alone. Addition of calcium in inhibitor tests could have been masked by this "background" calcium already present. Tests have now been made to establish if potassium pre-treatment modifies intracellular cadmium uptake in lichens as well as in mosses.

2. Materials and Methods

Plant material

Peltigera horizontalis (Huds.) Baumg. was collected from woodland at Goblin Combe, Avon and used either immediately or stored, moist, for no more than 2 weeks, under continuous fluorescent illumination (95 μmol $m^{-2}s^{-1}$).

Experimental procedures

Discs (5 mm diam., with rhizinae removed) were cut from cleaned thalli washed in deionised distilled water. Discs were bubbled in 1 litre of the appropriate solutions, generally for 60 min in the light (110 μmol $m^{-2}s^{-1}$), rinsed with 1 litre of deionised distilled water and subjected to the sequential elution procedure, involving shaking in 10 ml volumes of water (twice) = intercellular elements, 20 mM nickel chloride (twice) and water (once) = exchangeable elements, and 1 M HNO_3 (once) = intracellular elements (Beckett and Brown, 1984a,b; Brown and Wells, 1988). Elements were determined by atomic absorption spectrophotometry using an air/acetylene flame with the addition of caesium and lanthanum. Plant metal concentrations are expressed on a pre-acid dry weight basis.

Photosynthesis and respiration were measured by infra-red gas analysis using the procedure of Snelgar et al. (1980) involving following the changes in CO_2 concentration in closed containers in the light and dark respectively. Photosynthesis was corrected for dark respiratory gas exchanges.

3. Results and Discussion

When increasing concentrations of cadmium were supplied to discs of *Peltigera horizontalis*, uptake to the cell wall showed saturation kinetics. Whereas similar saturation kinetic were observed for cadmium in the presence of additional magnesium (Fig. 1) or calcium (Fig. 2), the quantities of

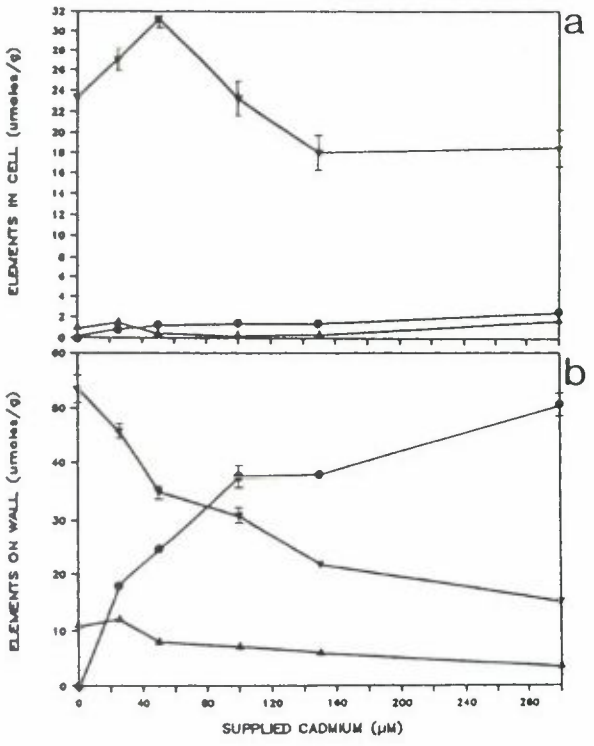

Figure 1. Changes induced by incubation with varying cadmium concentrations and 300 μM magnesium on the calcium (▲), magnesium (▼) and cadmium (•) concentrations of (a) intra- and (b) extracellular sites in *Peltigera horizontalis*. Bars = standard error, if larger than the symbol, n = 3.

the pre-existing and additional magnesium or calcium recovered from the wall decreased with increasing cadmium concentration. Apart form the uptake of cadmium, few obvious changes to pre-existing intracellular divalent elements were observed.

Intracellular cadmium uptake, over a wide range of concentrations, was reduced in the presence of calcium (Fig. 3a). Pre-treating samples with 80 mM potassium sulphate, followed by observing cadmium uptake from a range of

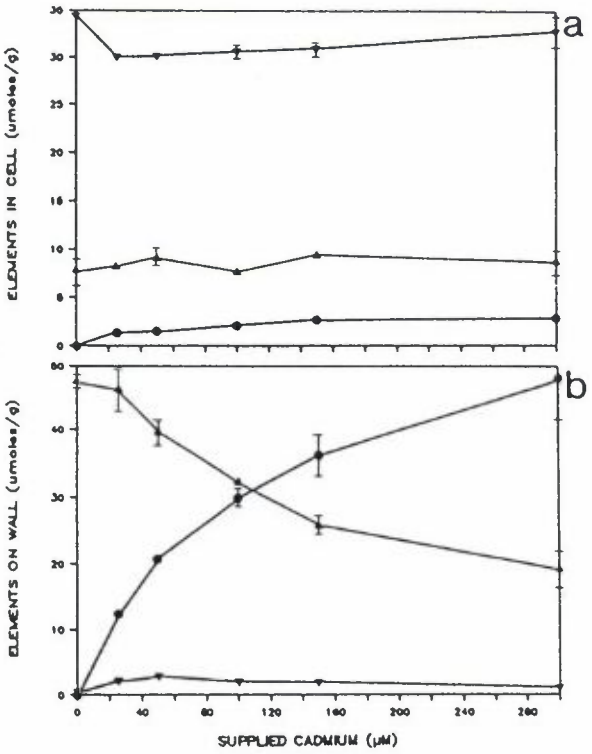

Figure 2. Changes induced by incubation with varying cadmium concentrations and 300 μM calcium on the calcium (▲), magnesium (▼) and cadmium (●) concentrations of (a) intra- and (b) extracellular sites in *Peltigera horizontalis*. Bars = standard error, if larger than the symbol, n = 3.

concentrations in the presence of calcium, resulted in an intermediate intracellular cadmium uptake rate. The same relationship also held for extracellular cadmium uptake, although here the difference between the two treatments with added cadmium was less distinct (Fig. 3b). Lineweaver and Burke double reciprocal plots of the same data show straight-line relationships for extracellular cadmium uptake, with the intercept of the three lines being coincident on the y-axis (Fig. 4b). This is indicative of a competitive interaction between calcium and cadmium uptake. However, unlike Wells and Brown (1990) using a moss, no such clear-cut relationship was seen for the effect of calcium on intracellular cadmium uptake (Fig. 4a). The evidence is ambiguous (Fig. 4a) as to whether or not the inhibition is competitive, because simple linear expressions cannot be provided for the experimental results, particularly in the presence of calcium.

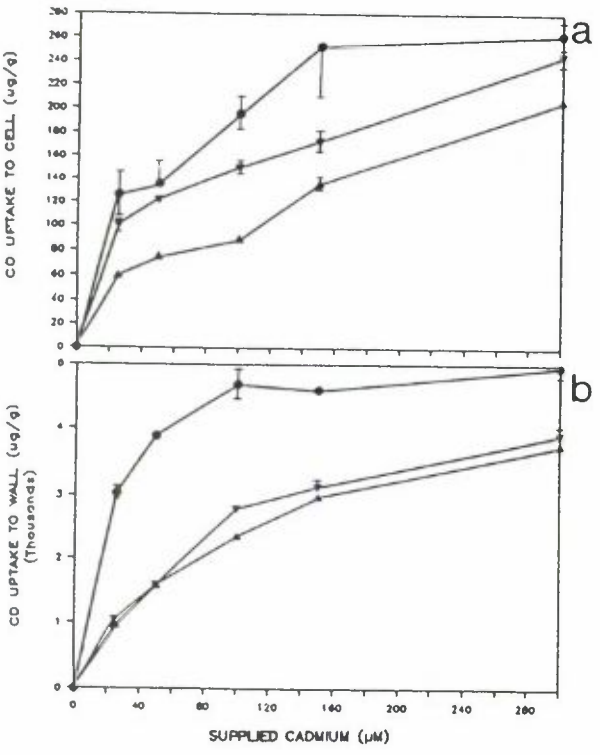

Figure 3. Cadmium uptake to (a) intra- and (b) extracellular sites of *Peltigera horizontalis* with cadmium alone (•), in the presence of 300 μM calcium (▲), or after pretreatment with 80 mM potassium and with added cadmium and calcium (▼). Bars = standard error, if larger than the symbol, n = 3.

Consideration needs to be given to a number of features of the experimental design used here, in order to explain the apparent disparity between moss and lichen species in the kinetics of cadmium uptake.

In order to study cadmium uptake kinetics an adequate concentration range must be employed. Frequently this includes observation at near-saturating concentrations. During this study it became apparent that such high concentrations caused alterations to cell metabolism. Thus both photosynthesis and respiration were appreciably depressed after supplying 300 μm cadmium for 60 min (Table 1). Hence the lichen is not physiologically unresponsive to the added cadmium concentrations and measurements made at different cadmium concentrations will, therefore, not reflect the behaviour of physiologically identical material. It should be noted that in these experiments the lowest cadmium concentration tested was below that used by Beckett and Brown (1984a,b). As

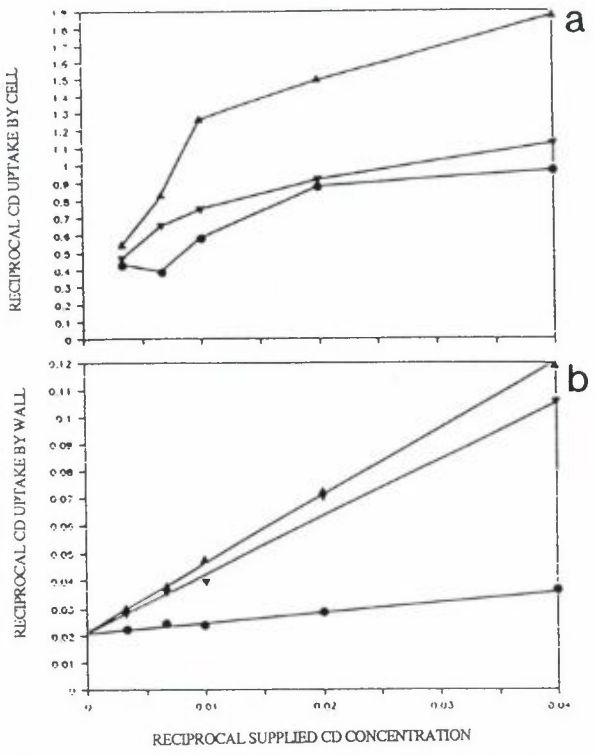

Figure 4. Lineweaver and Burke plot of data presented in Fig. 3.

Table 1. The effect of pre-treatment with 80 mM potassium sulphate or 300 μM cadmium sulphate on respiration and photosynthesis in *Peltigera horizontalis*

Condition	Respiration (ppm CO_2 $min^{-1}g^{-1}$)	Photosynthesis (ppm CO_2 $min^{-1}g^{-1}$)
After 1 hr		
Water	505±150	960±205
Potassium	570±10	1060±200
Cadmium	450±150	235±200
After 24 hr		
Water	430±90	670±140
Potassium	610±340	690±55
Cadmium	235±190	105±100

Photosynthesis corrected for respiratory gas exchange, n = 3.

this concentration showed a higher rate of uptake than might have been predicted from the other concentrations employed (Fig. 4a), this may be further evidence of underestimation of carrier-mediated intracellular cadmium uptake at elevated levels of this potentially toxic element. Recently it has been suggested that competition experiments should be conducted at lower and more physiologically realistic concentrations, when many of the previously claimed interactions do not occur (Ross and Parkin, 1989).

Observation of intracellular cadmium uptake with time showed that at 100 μM cadmium the rate was substantially linear, whereas at 300 μM cadmium the rate may alter with time, finally resulting in a uptake possibly in excess of the initial rate (Fig. 5a). This is interpreted as indicating an altered transfer of cadmium across the cell membrane, probably finally involving diffusive, rather than exclusively carrier-mediated, transfer. Wells and Richardson (1985) reported similar problems when investigating uptake of potentially toxic anions by the moss *Hylocomium splendens*, from which they hypothesized that earlier kinetic studies on lichens of some of the same anions (Nieboer et al., 1984) required re-assessment.

Because potassium is mainly soluble within lichen cells, loss from intracellular sites has been taken to be a measure of membrane damage (Buck and Brown, 1979). There is some evidence to suggest that the 80 mM potassium sulphate pretreatment, rather than causing an increased intracellular potassium concentration, may result in some loss of intracellular potassium (Table 2). Although experiments considered individually may not be statistically significant, the same pattern was always apparent. Brown et al. (1987) reported a substantial loss of intracellular potassium when the lichen *Evernia prunastri* was treated for up to 16 hr with 75 mM sodium phosphate buffer or, to a lesser extent, 40 mM urea. Although the study with *E. prunastri* showed substantially greater loss of potassium, a minor version of a comparable phenomenon may be occurring with potassium sulphate treatment of *Peltigera*. Potassium pre-treated samples cannot therefore be considered to be functionally identical to the original field material.

The physiological basis of the potassium sulphate-induced reduction in intracellular potassium is not known. However, it should be noted that the subsequent addition of a divalent cation generally resulted in a greater concentration of intracellular potassium being detected (Table 2). As calcium is frequently cited as being required for fully functional membranes, it is possible that high potassium concentrations displaced some calcium from functionally important sites in the membrane, thereby permitting the loss of soluble intracellular substances, for example potassium. Calcium, or to a lesser extent

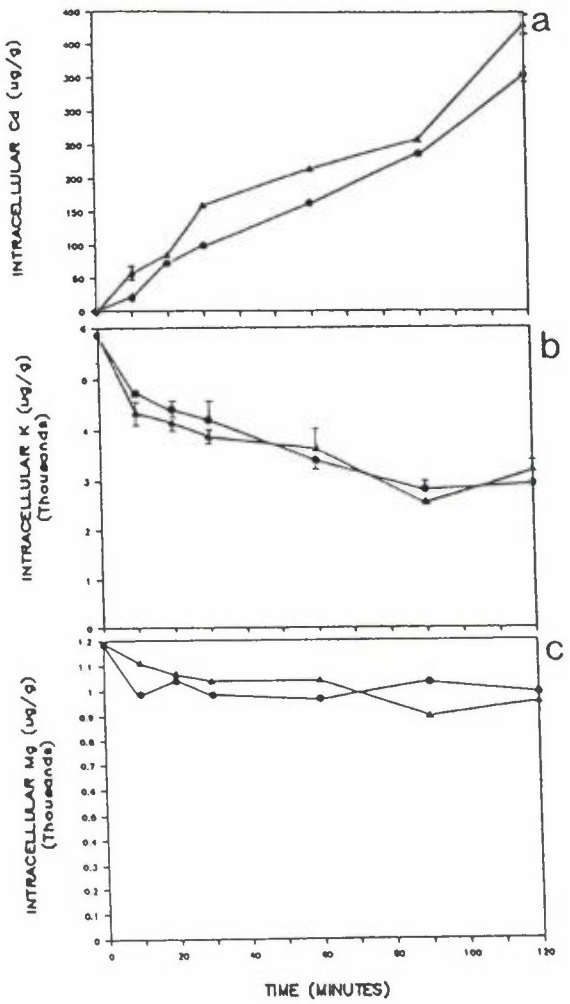

Figure 5. Effect of incubation time in cadmium at 100 μM (•) or 300 μM (▲) on the intracellular concentrations of cadmium (a), potassium (b), and magnesium (c) in *Peltigera horizontalis*. Bars = standard error, if larger than the symbol, n = 3.

other divalent cations, added after potassium treatment may functionally restore the membrane, so that less potassium was released during subsequent washings in different solutions. This discussion implies that potassium loss may be due to enhanced membrane permeability. Although magnesium also appears to be, in part, a soluble intracellular cation, its behaviour did not apparently parallel that of potassium, following the above treatments. Table 1

Table 2. Changes to intracellular potassium and magnesium levels in *Peltigera horizontalis* induced by 80 mM potassium sulphate pre-treatment and addition of divalent cations

Condition		Potassium (μmoles g^{-1})		Magnesium (μmoles g^{-1})	
		-K*	+K	-K	+K
-	Calcium	109	78	36	31
+	Calcium	134	100	35	31
-	Magnesium	158	138	25	25
+	Magnesium	135	148	23	34
-	Cadmium	145	95	48	48
+	Cadmium	108	117	45	44

* With or without 80 mM potassium sulphate pre-treatment. Calcium and magnesium added at 300 μM, cadmium at 100 μM, n = 3.

shows that the relatively small loss of potassium observed did not result in a significant change in either respiration or photosynthesis.

Buck and Brown (1979) observed a decline in intracellular potassium and magnesium in *Peltigera horizontalis* following desiccation stress, which is assumed to result from temporary non-specific damage to cell membrane integrity. Intracellular potassium concentrations also appear to be influenced by the addition of cadmium. During time-course experiments, intracellular potassium concentrations declined (Fig. 5b) while magnesium levels remained constant (Fig. 5c). Increasing concentrations of cadmium also caused a reduction in intracellular potassium, but not magnesium levels (data not shown). The data shown in Fig. 5b suggest that loss of potassium is closely comparable during exposure to 100 and 300 μm cadmium. This, coupled with the behaviour of magnesium, suggests that potassium loss may be a specific response to this toxic element rather than a general effect on membrane integrity.

When attempting to measure the effect of additional calcium on intracellular cadmium uptake it must be appreciated that some will become bound to the extracellular exchange sites. This results in a lower residual concentration remaining in solution to interact with the carrier systems. In these experiments calcium was used at 300 μM because this had previously been shown to be saturating. With a constant calcium concentration and increasing cadmium, less calcium was found bound to the cell wall and hence more, potentially interactive, calcium remained in solution (Fig. 6). Potassium pre-treatment also alters the ability of the cell wall to remove cations from solution, by removing

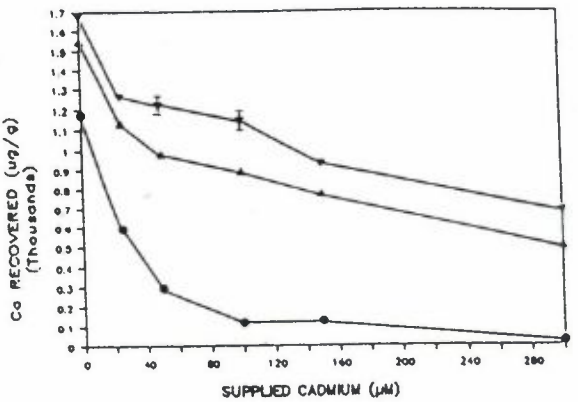

Figure 6. Calcium uptake to extracellular sites of *Peltigera horizontalis* supplied with cadmium alone (•), in the presence of 300 μM calcium (▲), or after pre-treatment with 80 mM potassium and with added cadmium and calcium (▼). Bars = standard error, if larger than the symbol, n = 3.

many of the potentially competitive elements previously bound to the cell wall anionic sites. When calcium is used at saturating concentrations, this results in more becoming bound to the cell wall and lower concentrations remaining in solution. At non-saturating concentrations less calcium is recovered from the cell wall after potassium treatment, because of the removal of pre-existing calcium (unpublished results).

The above comments show that it is extremely difficult to achieve stable physiological conditions during tests of competition between cations. While potentially toxic elements have been shown to alter physiological processes, even ostensibly non-toxic elements such as potassium may cause some changes. Equilibria between cell wall exchange sites and the solution bathing the outer surface of the plasma membrane, and hence the carrier systems, are complex and depend on both the added cations and those already on the walls. The present work has identified some of the problems involved and suggests how they may create a situation where it is still impossible to state clearly if intracellular cadmium uptake is competitively inhibited by either calcium or magnesium. Unlike bryophytes, lichens have the additional complication of two dissimilar organisms living together with potentially different carrier systems.

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