SHORT COMMUNICATION

Aluminium Uptake by *Anabaena cylindrica*

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Atomic absorption spectrophotometry was used to characterize aluminium uptake by the cyanobacterium *Anabaena cylindrica*. An EDTA-washing procedure was used to distinguish between adsorbed and intracellular aluminium. The intracellular aluminium content increased with increasing external concentration and time. The phosphorus concentration in the growth medium did not affect the rate of aluminium uptake nor did dark treatment or addition of CCCP, an uncoupler of phosphorylation. We therefore conclude that aluminium toxicity is due to intracellular aluminium rather than to interactions with nutrients in the growth medium and that aluminium uptake is independent of phosphorus uptake. The accumulation of aluminium in polyphosphate granules and cell walls of phosphorus-rich cells noted earlier is rather due to an increased binding capacity in these cellular compartments. Also, the rapid uptake of aluminium by *A. cylindrica* mainly occurs via passive diffusion.

INTRODUCTION

The toxic effects of aluminium on higher plants are well known (Foy *et al.*, 1978) although the mechanism of its uptake, its distribution in individual cells and its metabolic effects are not fully understood (for review see Haug, 1984). Few reports have described the effects of aluminium on micro-organisms.

N₂-fixing cyanobacteria are important primary producers in both soils and waters of areas now affected by acidification and thereby with elevated concentrations of solubilized aluminium. We previously reported the inhibitory effects of aluminium on the growth, photosynthesis and N₂ fixation of *Anabaena cylindrica* (Pettersson *et al.*, 1985*a*). The intracellular localization of aluminium was investigated by X-ray microanalysis; aluminium was rapidly taken up and accumulated in polyphosphate granules and cell walls of *A. cylindrica* (Pettersson *et al.*, 1985*b*). The concentration of phosphorus in the growth medium affected the accumulation pattern; more aluminium was found in both the polyphosphate granules and the cell walls during incubation in phosphorus-rich medium.

Here we describe the kinetics of aluminium uptake in *A. cylindrica* as investigated by atomic absorption spectrophotometry.

METHODS

Organism and culture conditions. *Anabaena cylindrica* strain 1403/2a (Culture Centre of Algae and Protozoa, Cambridge, UK) was grown axenically in 0.5 l growth medium (Pettersson *et al.*, 1985*a*). The culture was harvested after 7–8 d of growth (exponential growth phase), washed with sterile water and resuspended to 130–150 mg dry wt l⁻¹ in autoclaved half-strength growth medium, buffered to pH 6.0 with 10 mM-MES. Phosphorus, supplied as K₂HPO₄·3H₂O, was added to the desired final concentration, up to 90 µM. The cultures were preincubated for 30 min in the dark or in continuous light (70 µmol photons m⁻² s⁻¹) at 25 °C and aerated before aluminium was supplied as AlCl₃·6H₂O from freshly prepared stock solutions to appropriate concentrations.

Abbreviation: CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone.

CCCP (10 μ M) was added 10 min before aluminium was supplied. The treatment lasted 1–24 h. All experiments were run in triplicate and repeated at least twice.

Atomic absorption spectrophotometry. The cyanobacteria were harvested by centrifugation and the pellet was resuspended in either 75 ml redistilled H₂O or 1 mM-EDTA. EDTA was used to distinguish aluminium adsorbed to the surface of the cyanobacterial cells and aluminium taken up by the cells according to Bates *et al.* (1982). After incubation with gentle shaking for 10 min in darkness, the sample was again centrifuged (1500 g, 10 min), the supernatant carefully removed and the pellet resuspended in 5 ml redistilled H₂O or EDTA. The cells were collected by filtration on preweighed cellulose papers, 50 $\times$ 50 mm (Munktell no. S3-90-500, acid-washed). The filters were oven-dried for 24 h at 100 °C and the dry weights determined. To the filters was then added 3 ml redistilled H₂O and 15 ml of a mixture of 70% HClO₄ and 65% HNO₃ (1:2, v/v) 30 min before combustion started. Samples were heated at 250 °C for 45 min and then the temperature was slowly increased to 300 °C for about 15 min until a clear solution remained. After combustion samples were diluted to 50 ml with redistilled H₂O and NaCl and LaCl₃ (each 0.1%) were added to reduce ionization interference. The aluminium concentration was determined by flame atomic absorption spectrophotometry (Perkin–Elmer 2380).

RESULTS AND DISCUSSION

Effect of washing aluminium-treated cells with EDTA

The EDTA-washing procedure has been successfully used in several studies to distinguish adsorbed from cellular metal (Bates *et al.*, 1982; Les & Walker, 1984). In our experiments incubation of cells for 10 min with 1 mM-EDTA proved optimal for obtaining a stable cellular bound aluminium concentration. Washing with redistilled H₂O only showed that all aluminium added was adsorbed to the cells while cells treated with EDTA retained intracellularly 60% of the aluminium added (Table 1). This result is in accordance with our estimations of aluminium remaining in the growth medium. After incubation for 10 min, only trace amounts of added aluminium remained (data not shown). In all experiments control flasks without added aluminium were run. In these cells the aluminium content was calculated to be 0.27 ± 0.05 mg (g dry wt)⁻¹. This background value was subtracted from the aluminium levels given in the Tables.

Effect of external aluminium concentration and time on aluminium accumulation

Accumulation of aluminium by *A. cylindrica* after 24 h incubation was dependent on the applied concentration (Table 1), in accordance with previous results (Pettersson *et al.*, 1985a). Also, the aluminium content of cells increased with incubation time from 2.78 ± 0.04 (SD) mg (g dry wt)⁻¹ (42% of the added aluminium at a concentration of 36 μ M) at 1 h to 3.25 ± 0.04 (49%) at 5 h to 3.84 ± 0.05 (61%) at 24 h. Within the first hour after the addition, however, as much as 72% of the aluminium accumulated after 24 h was already adsorbed. These results indicate that aluminium toxicity is due to aluminium accumulated in the cells rather than to interactions with nutrients in the growth medium.

Effect of phosphorus concentration on aluminium accumulation

The uptake of aluminium in medium with or without phosphorus, and in low or high phosphorus medium, was not significantly different, irrespective of treatment (Table 2). Previously X-ray microanalysis showed that high concentrations of aluminium accumulated in polyphosphate granules and cell walls and that the accumulation in these structures was increased in phosphorus-rich medium (Pettersson *et al.*, 1985b). These results suggested that aluminium and phosphorus uptake could in some way be coupled. However, this is not the case, as the present results show that aluminium is rapidly taken up even in medium with no phosphorus supplied. The increased aluminium accumulation in polyphosphate granules and cell walls of phosphorus-rich cells is therefore probably due to an increased selective binding capacity by these cellular compartments.

Dependency of aluminium uptake on light and ATP production

Intracellular aluminium accumulation did not show any dependency on light (Table 2) as dark-grown cells accumulated aluminium at the same rate as light-grown cells. Addition of carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), an uncoupler of phosphorylation, increased rather than decreased the aluminium content of the cells (Table 2), although the

Table 1. *Accumulation of aluminium by Anabaena cylindrica*

Cells were incubated for 24 h in growth medium pH 6.0 containing $90 \mu\text{M-K}_2\text{HPO}_4$ and various concentrations of aluminium, harvested and washed in redistilled H_2O or 1 mM-EDTA before the aluminium concentrations in the cells were determined. The results are means $\pm$ SD of triplicate samples.

Treatment		Aluminium in cells	
Aluminium concn (μM)	Resuspended in	mg (g dry wt) $^{-1}$	Percentage of added aluminium
36	H_2O	9.21 ± 0.50	101
18	EDTA	0.40 ± 0.05	12
36	EDTA	3.84 ± 0.05	60
180	EDTA	32.00 ± 0.50	95

Table 2. *Effect of phosphorus, light and ATP production on accumulation of aluminium by Anabaena cylindrica*

Cells were incubated for 1 or 2 h in the light or dark in growth medium pH 6.0 containing $36 \mu\text{M}$ -aluminium and different phosphorus concentrations, harvested and resuspended in 1 mM-EDTA before the aluminium concentrations in the cells were determined. CCCP ($10 \mu\text{M}$) was added 10 min before the aluminium. The results are means $\pm$ SD of triplicate samples.

Treatment	Aluminium in cells	
	mg (g dry wt) $^{-1}$	Percentage of added aluminium
No K_2HPO_4 , 1 h, light	4.56 ± 0.98	59
$90 \mu\text{M-K}_2\text{HPO}_4$, 1 h, light	4.42 ± 0.46	57
$18 \mu\text{M-K}_2\text{HPO}_4$, 2 h, light	4.74 ± 0.28	52
$90 \mu\text{M-K}_2\text{HPO}_4$, 2 h, light	5.11 ± 0.68	56
$90 \mu\text{M-K}_2\text{HPO}_4$, 1 h, dark	4.14 ± 0.54	54
$90 \mu\text{M-K}_2\text{HPO}_4$, 1 h, light + CCCP	4.82 ± 0.10	63

concentration of the inhibitor ($10 \mu\text{M}$) was sufficient to prevent growth of *A. cylindrica*. Aniol (1983) studied the effect of two inhibitors, dinitrophenol and sodium azide, on the accumulation of aluminium in roots of two winter wheat varieties. Both inhibitors lowered the accumulation of aluminium but increased the aluminium toxicity. He concluded that the action of the inhibitors was not on the energy-mediated transport but rather on the increased sensitivity of the membrane structures responsible for aluminium toxification. Our study shows that aluminium uptake in the cyanobacterium *A. cylindrica* mainly occurs as a passive diffusion, although the aluminium species taken up is still unknown.

We therefore conclude that aluminium toxicity in cyanobacteria is due to intracellular accumulation of aluminium, which occurs via passive diffusion. The uptake of aluminium is independent of the phosphorus availability although the intracellular localization of aluminium is dependent on the phosphorus status of the cells. The intracellular events caused by aluminium are currently being investigated.

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