Caesium accumulation and interactions with other monovalent cations in the cyanobacterium *Synechocystis* PCC 6803

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Growth of *Synechocystis* PCC 6803 in BG-11 medium supplemented with 1 mM-CsCl resulted in intracellular accumulation of Cs⁺ to a final level of approximately 510 nmol (10⁹ cells)⁻¹ after incubation for 10 d. The doubling time was increased by 64% and the final cell yield was decreased by 70% during growth in the presence of Cs⁺ as compared to growth in control BG-11 medium. When the total monovalent cation concentration of the medium was doubled by adding either K⁺ or Na⁺, levels of accumulated Cs⁺ were decreased by approximately 50% to 220 and 270 nmol (10⁹ cells)⁻¹, respectively, after 28 d with little inhibition of growth being apparent. Short-term experiments revealed that extracellular K⁺ and Na⁺ inhibited Cs⁺ accumulation to a similar extent, with 90% inhibition of Cs⁺ accumulation occurring at the highest concentrations used (50 mM-K⁺ or Na⁺; 1 mM-Cs⁺). In all experiments, Cs⁺ accumulation resulted in a reduction in intracellular K⁺, except when cells were grown in K⁺-depleted medium, although a stoichiometric relationship was not apparent, the amount of Cs⁺ accumulated generally being greater than the amount of K⁺ released. Cs⁺ accumulation had no discernible effect on intracellular Na⁺. When K⁺, Na⁺, Rb⁺, Li⁺ or TI⁺ were supplied at equimolar (1 mM) concentrations to Cs⁺, only TI⁺ significantly reduced Cs⁺ accumulation. However, an approximately 50% inhibition of Cs⁺ accumulation resulted when concentrations of K⁺, Na⁺, Rb⁺ or Li⁺ were increased to 10 mM, which suggests that Cs⁺ may have a higher affinity for the monovalent cation transport system than K⁺. Rb⁺ and TI⁺ also caused a decrease in intracellular K⁺, whereas Na⁺ and Li⁺ stimulated K⁺ uptake. Cs⁺ accumulation was dependent on the external Cs⁺ concentration and showed a linear relationship to external Cs⁺ concentrations ≤ 2 mM over 12 h incubation. However, prolonged incubation in external Cs⁺ concentrations ≥ 0.8 mM resulted in Cs⁺ release from the cells and after 48 h, similar amounts of Cs⁺ and K⁺ were present in cells incubated at these higher concentrations. Cs⁺ accumulation was energy- and pH-dependent. Incubation in the light at 4 °C, or in the presence of 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), or at 22 °C in the dark resulted in decreased Cs⁺ accumulation and decreased K⁺ release from the cells. Increased amounts of Cs⁺ were accumulated as the pH of the external medium was increased, with maximal accumulation [approximately 1330 nmol Cs⁺ (10⁹ cells)⁻¹ after 24 h incubation] occurring at pH 10, the highest pH value used. It is suggested that an important mechanism of Cs⁺ toxicity in *Synechocystis* PCC 6803 arises through replacement of cellular K⁺ by Cs⁺. The possible role of primary producers such as cyanobacteria in the mobilization of this radionuclide in aquatic habitats is discussed.

Introduction

Caesium is a critical fission product in waste effluents from power reactors and in atomic fallout because of its long half-life of 30 years. Although some work involving microbial interactions with caesium was conducted in the 1950s and 1960s because of concern over nuclear weapons testing, interest in the environmental effects of this radionuclide has re-awakened recently following the Chernobyl accident in 1986. Recent reports of biological

caesium accumulation have focussed on bacteria and higher fungi from terrestrial environments (Haselwandter *et al.*, 1988; Elstner *et al.*, 1987; Dighton & Horrill, 1988; Bossemeyer *et al.*, 1989; Korky & Kowalski, 1989) and there is a marked lack of information relating to microbial interactions with this element in the aquatic environment. This is surprising since microalgae and cyanobacteria are important primary producers in aquatic ecosystems and any accumulated caesium may be transferred to organisms further up the food chain, as is the case for other heavy metal and radionuclide pollutants (Gadd, 1988, 1990; Hughes & Poole, 1989; Reed & Gadd, 1990).

Abbreviation: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

Caesium has no known essential biological role, although its chemical similarity to other monovalent cations, particularly potassium (ionic radii of K^+ and Cs^+ being 133 and 165 pm respectively; Greenwood & Earnshaw, 1984), suggests that a possible route of caesium uptake in cyanobacteria might be via potassium transport system(s), as in yeast (Borst-Pauwels, 1981) and bacteria (Jasper, 1978; Nakamura *et al.*, 1982; Bossemeyer *et al.*, 1989). It is well known that cyanobacteria concentrate potassium from their external environment (Reed *et al.*, 1981*a*; Walderhaug *et al.*, 1987) whilst an active Na^+/H^+ antiport maintains low intracellular levels of sodium (Paschinger, 1977; Raven, 1980). This potassium uptake is an energy-dependent process, the immediate driving force being ATP (Walderhaug *et al.*, 1987; Padan & Vitterbo, 1988). It has been suggested that passive uniport of K^+ may also occur in cyanobacteria under conditions where active uptake is blocked, although this system is thought to be relatively unimportant (Reed *et al.*, 1981*a*).

Cyanobacterial uptake of potassium has been compared to the specific high-affinity, *kdp*-coded K^+ -ATPase of *Escherichia coli* (Padan & Vitterbo, 1988), although Reed *et al.* (1981*b*) have demonstrated the existence of an alternative, low-affinity system in *Anabaena variabilis*. More recently, Bossemeyer *et al.* (1989) have shown that the Kup system of *E. coli* takes up caesium with a moderate rate and affinity, this particular system showing little discrimination between the alkali metal ions. The possibility that a similar system may accumulate Cs^+ in cyanobacteria is highly likely, particularly in view of work on the low-affinity system of *A. variabilis* which has been shown to be a route for significant Rb^+ uptake (Reed *et al.*, 1981*b*).

This study describes the effects of caesium on growth of *Synechocystis* PCC 6803 and the influence of external potassium and sodium on caesium accumulation by the cells. Experiments with non-growing cell suspensions further characterize the nature of caesium accumulation, including interactions with other monovalent cations and energy- and pH-dependence.

Methods

Organism, media, growth conditions and preparation of cell suspensions.

Axenic cultures of *Synechocystis* PCC 6803 were grown at 22 °C in 100 ml BG-11 medium (Stanier *et al.*, 1971). Cultures were incubated in 250 ml Erlenmeyer flasks with rotary aeration at 150 cycles min^{-1} , with a photon fluence irradiance, incident on the surface of the flasks, of $12 \mu E m^{-2} s^{-1}$, provided by white fluorescent tubes.

For growth experiments, cells were inoculated to an initial cell density of approximately 4×10^6 cells ml^{-1} ($OD_{680} \approx 0.1$) in 100 ml BG-11 medium containing desired amounts of Cs^+ , K^+ and Na^+ (added as chlorides). These were incubated as above. Cell numbers were determined using a modified Fuchs-Rosenthal haemocytometer after appropriate dilution with distilled water.

To prepare cell suspensions for short-term Cs^+ experiments, cells in the exponential growth phase were collected by centrifugation (1200 g, 10 min), washed thoroughly with distilled deionized water and then suspended to a cell density of approximately 5×10^7 cells ml^{-1} in 100 ml 10 mM-HEPES buffer, adjusted to pH 8 using solid tetramethylammonium hydroxide, unless stated otherwise. Cells were allowed to equilibrate for 1 h prior to addition of Cs^+ and/or other monovalent cations. To assess the effect of pH on Cs^+ accumulation, cells were treated as above and suspended in 100 ml of the following buffers (all 10 mM) at the specified pH values: PIPES, pH 6.5 and 7.0; HEPES, pH 7.0 and 8.0; TAPS, pH 8.0 and 9.0; CHES, pH 9.0 and 10.0. The final pH was again adjusted with solid tetramethylammonium hydroxide. All cell suspensions were incubated at 22 °C in the light ($12 \mu E m^{-2} s^{-1}$) with rotary shaking (150 cycles min^{-1}). All glassware was washed with 1 M-HCl and rinsed thoroughly with distilled deionized water prior to use.

Metal analyses. Cells were harvested by centrifugation (1200 g, 10 min) and washed twice with distilled deionized water. Cell pellets were digested for 1 h in 0.5 ml 6 M- HNO_3 at 100 °C and, after subsequent addition of 2.5 ml distilled deionized water and mixing, cell extracts were centrifuged (1200 g, 10 min) to remove debris. Caesium, potassium and sodium concentrations in the supernatants were determined using a PYE Unicam SP9 atomic absorption spectrophotometer, with reference to appropriate standards.

Results

Growth experiments

Addition of CsCl to a final concentration of 1 mM in BG-11 medium resulted in inhibition of growth of *Synechocystis* PCC 6803. Growth was exponential for the first 4 d and the doubling time in the absence and presence of 1 mM- $CsCl$ was approximately 16.8 h and 27.6 h respectively (Fig. 1*a*). The presence of 1 mM- $CsCl$ reduced cell yield; after incubation for 28 d, cell numbers were approximately 1×10^9 and 3×10^8 ml^{-1} in the absence and presence of $CsCl$, respectively. Accumulation of Cs^+ continued until the tenth day of growth, after which time the level remained approximately constant at 510 nmol Cs^+ (10^9 cells) $^{-1}$ (Fig. 1*a*). When the K^+ or Na^+ concentration of BG-11 medium (0.46 mM- K^+ , 18 mM- Na^+) was increased by adding either KCl or NaCl (to final concentrations of either 20.46 mM- K^+ , 18 mM- Na^+ or 0.46 mM- K^+ , 38 mM- Na^+) the growth-inhibitory effects of Cs^+ were modified, and doubling times and cell yields showed little difference in the absence or presence of Cs^+ (Fig. 1*b*, *c*). These observations were accompanied by changes in Cs^+ accumulation throughout growth. Cs^+ accumulation was reduced over the first 4 d of growth (particularly evident in 20.46 mM- K^+) and although the maximum amount of Cs^+ accumulated by the cells again occurred at the end of the exponential phase of growth, this was significantly reduced when compared with control BG-11 medium and Cs^+ was subsequently lost from the cells (Fig. 1*b*, *c*). The intracellular level of Cs^+ in cells grown for 28 d in BG-11

Fig. 1. Effect of caesium accumulation on growth of *Synechocystis* PCC 6803. Cells were grown in the absence or presence of 1 mM-CsCl in (a) normal BG-11 (0.46 mM-K⁺, 18 mM-Na⁺), (b) high-K⁺ BG-11 (20.46 mM-K⁺, 18 mM-Na⁺), (c) high-Na⁺ BG-11 (0.46 mM-K⁺, 38 mM-Na⁺) or (d) low-K⁺ BG-11 (46 μM-K⁺, 18 mM-Na⁺). The graph shows intracellular Cs⁺ (●), log₁₀ cell number in medium lacking Cs⁺ (□), and log₁₀ cell number in medium supplemented with Cs⁺ (■). Mean values from three replicate determinations are shown ± SEM where these exceed the dimensions of the symbols.

Table 1. Effect of caesium on intracellular potassium and sodium levels in growing cells of *Synechocystis* PCC 6803

Cells were harvested after 15 d growth in the media specified. Cs⁺_{int}, K⁺_{int} and Na⁺_{int} refer to intracellular Cs⁺, K⁺ and Na⁺ respectively. Mean values from three replicate determinations are shown ± SEM.

Medium	Cs ⁺ concn (mM)	Cs ⁺ _{int} [nmol (10 ⁹ cells) ⁻¹]	K ⁺ _{int} [nmol (10 ⁹ cells) ⁻¹]	Na ⁺ _{int} [nmol (10 ⁹ cells) ⁻¹]
BG-11	0	0	715 ± 21	78 ± 12
(0.46 mM-K ⁺ , 18 mM-Na ⁺)	1	499 ± 56	449 ± 32	85 ± 11
High-K ⁺ BG-11	0	0	1177 ± 10	81 ± 25
(20.46 mM-K ⁺ , 18 mM-Na ⁺)	1	291 ± 5	611 ± 10	92 ± 17
High-Na ⁺ BG-11	0	0	1268 ± 55	124 ± 5
(0.46 mM-K ⁺ , 38 mM-Na ⁺)	1	311 ± 8	540 ± 2	122 ± 2
Low-K ⁺ BG-11	0	0	266 ± 20	97 ± 32
(46 μM-K ⁺ , 18 mM-Na ⁺)	1	222 ± 8	274 ± 16	89 ± 8

medium supplemented with excess K⁺ or Na⁺ was approximately half that of cells grown in normal BG-11 (Fig. 1a, b, c). Fig. 1d shows growth of *Synechocystis* PCC 6803 under potassium-depleted conditions (46 μM-K⁺ as compared to 460 μM-K⁺ in normal BG-11). Rates of growth over the first 4 d were relatively unaffected under these conditions, although final cell yields were considerably reduced as compared with control cultures (Fig. 1d). The addition of CsCl to a final concentration of 1 mM had little effect on growth rate and cell yield and, after an initial increase, Cs⁺ accumulation remained approximately constant over the incubation period (Fig. 1d).

Intracellular levels of K⁺ and Na⁺ were also examined after 15 d growth in the absence or presence of CsCl

(Table 1). A significant reduction in intracellular K⁺ was evident in cells which had accumulated Cs⁺, except those incubated in K⁺-depleted conditions. Of the cells which were not incubated in K⁺-depleted conditions, the lowest level of cellular K⁺ occurred in control cells which had accumulated the most Cs⁺ and, as described above, where the greatest growth inhibition was apparent (Fig. 1a; Table 1). No significant reduction in intracellular Na⁺ was detected following Cs⁺ accumulation (Table 1).

Effect of other monovalent cations on Cs⁺ accumulation by *Synechocystis* PCC 6803

The effect of Cs⁺ on levels of intracellular K⁺ and Na⁺ was examined in short-term experiments. Cs⁺ accumu-

Fig. 2. Depression of caesium accumulation in *Synechocystis* PCC 6803 by potassium and sodium. Cells were suspended to $5 \times 10^7 \text{ ml}^{-1}$ in (a) unsupplemented 10 mM-HEPES buffer, pH 8, or supplemented with (b) 50 mM-KCl or (c) 50 mM-NaCl. Test cells were also incubated in the presence of 1 mM-CsCl. The graph shows intracellular values of K^+ ($\square$) and Na^+ ($\triangle$) in control cells, and intracellular values of Cs^+ ($\bullet$), K^+ ($\blacksquare$) and Na^+ ($\blacktriangle$) in cells incubated with CsCl. Mean values from three replicate determinations are shown $\pm$ SEM where these exceeded the dimensions of the symbols.

lation generally resulted in K^+ loss from the cells (Fig. 2a), although the amount of K^+ lost was not as large as the amount of Cs^+ accumulated. After 24 h incubation, the amount of Cs^+ accumulated was approximately $1000 \text{ nmol } (10^9 \text{ cells})^{-1}$ whereas the amount of K^+ lost was approximately $900 \text{ nmol } (10^9 \text{ cells})^{-1}$. However, control cells lost K^+ in the absence of added Cs^+ and, when this was taken into account, the net loss of K^+ by cells incubated with Cs^+ was about $500 \text{ nmol } (10^9 \text{ cells})^{-1}$ after 24 h (Fig. 2a). There was little increase in cell Cs^+ or further reduction in cell K^+ after 24 h (Fig. 2a). Increasing the external concentration of K^+ or Na^+ to 50 mM markedly reduced accumulation of Cs^+ (Fig. 2b, c). This reduced Cs^+ accumulation was correlated with a greater retention of intracellular K^+ in both cases and in the presence of high external K^+ , cellular K^+ showed an approximate 25% increase in control cells over 48 h (Fig. 2b). Cells incubated with 1 mM-CsCl showed an increase in cell K^+ , although this was reduced as compared to control cells, the extent of K^+ reduction being approximately matched by levels of Cs^+ accumulation (Fig. 2b). While more variable, cell K^+ in the presence of high external Na^+ was also slightly reduced in the presence of Cs^+ (Fig. 2c). Intracellular Na^+ was slightly increased in the presence of high external Na^+ (Fig. 2c) although there was little difference in cellular Na^+ in the absence or presence of Cs^+ in all the experimental treatments (Fig. 2). The inhibitory effects of increasing extracellular concentrations of K^+ and Na^+ on Cs^+ accumulation were approximately equivalent and a 75% reduction resulted at K^+ or Na^+ concentrations up to 12 mM; 90%

Fig. 3. Influence of increasing extracellular concentrations of potassium and sodium on caesium accumulation in *Synechocystis* PCC 6803. Cells were suspended to $5 \times 10^7 \text{ ml}^{-1}$ in 10 mM-HEPES buffer, pH 8, and incubated for 24 h in the presence of 1 mM-CsCl and the specified concentrations of KCl or NaCl. The graph shows intracellular Cs^+ in the presence of KCl ($\circ$) and NaCl ($\bullet$). Mean values from three replicate determinations are shown $\pm$ SEM where these exceeded the dimensions of the symbols.

inhibition of Cs^+ accumulation resulted at the highest external concentration (50 mM) of K^+ or Na^+ used (Fig. 3).

Several other monovalent cations were examined for inhibitory effects on Cs^+ accumulation by *Synechocystis*

Fig. 4. Dependence of caesium accumulation on extracellular caesium concentration in *Synechocystis* PCC 6803. Cells were suspended to 5×10^7 ml⁻¹ in 10 mM-HEPES buffer, pH 8, supplemented with different amounts of CsCl. (a) Time course of Cs⁺ accumulation in external CsCl concentrations (mM) of: 0.2 (○); 0.4 (●); 0.8 (□); 1.5 (■) and 2.0 (△). (b, c) Intracellular Cs⁺ (●) and K⁺ (○) levels after 12 h (b) or 48 h (c) incubation. Mean values from three replicate determinations are shown $\pm$ SEM where these exceed the dimensions of the symbols.

Table 2. Effect of several monovalent cations on levels of intracellular K⁺ and Cs⁺ in *Synechocystis* PCC 6803

Cells were suspended to 5×10^7 ml⁻¹ in 10 mM-HEPES buffer, pH 8, and harvested after 24 h incubation. Test cells were incubated with 1 mM-CsCl. Mean values from three replicate determinations are shown $\pm$ SEM.

Metal cation added to external medium	Concn (mM)	Intracellular K ⁺ levels in control cells [nmol (10 ⁹ cells) ⁻¹]	Intracellular K ⁺ levels in test cells [nmol (10 ⁹ cells) ⁻¹]	Intracellular Cs ⁺ in test cells [nmol (10 ⁹ cells) ⁻¹]
None	–	284 $\pm$ 22	98 $\pm$ 4	540 $\pm$ 77
K ⁺	1	313 $\pm$ 6	100 $\pm$ 1	685 $\pm$ 2
Na ⁺	1	553 $\pm$ 28	195 $\pm$ 11	525 $\pm$ 10
Rb ⁺	1	23 $\pm$ 7	15 $\pm$ 4	670 $\pm$ 34
Li ⁺	1	342 $\pm$ 14	103 $\pm$ 3	718 $\pm$ 11
Tl ⁺	1	36 $\pm$ 3	24 $\pm$ 0	109 $\pm$ 11
None	–	387 $\pm$ 6	72 $\pm$ 3	529 $\pm$ 20
K ⁺	10	452 $\pm$ 12	250 $\pm$ 27	349 $\pm$ 26
Na ⁺	10	638 $\pm$ 26	442 $\pm$ 9	327 $\pm$ 48
Rb ⁺	10	12 $\pm$ 4	16 $\pm$ 6	261 $\pm$ 29
Li ⁺	10	655 $\pm$ 26	394 $\pm$ 15	284 $\pm$ 23
Tl ⁺	10	17 $\pm$ 0	14 $\pm$ 1	25 $\pm$ 5

PCC 6803 (Table 2). When K⁺, Na⁺, Rb⁺ or Li⁺ were added to cell suspensions at equimolar (1 mM) concentrations with Cs⁺, no inhibition of Cs⁺ accumulation was evident and in fact a slight stimulation resulted. Tl⁺, however, reduced Cs⁺ accumulation by approximately 80%. When the external monovalent cation concentration was increased to 10 mM, with that of Cs⁺ being maintained at 1 mM, inhibition of Cs⁺ accumulation resulted (Table 2). Tl⁺ again had the greatest effect and reduced Cs⁺ accumulation to negligible levels [approximately 25 nmol Cs⁺ (10⁹ cells)⁻¹ after 24 h]. K⁺, Na⁺,

Li⁺ and Rb⁺ at 10 mM had lesser inhibitory effects and reduced Cs⁺ accumulation by approximately 50%. In general a reduction in cellular K⁺ was correlated with increased cellular Cs⁺ (Table 2). This was not the case, however, when Rb⁺ was added to the external medium. This cation, like Tl⁺, caused the loss of virtually all intracellular K⁺ when no Cs⁺ was present and hence the addition and subsequent accumulation of Cs⁺ had little further effect on cellular K⁺ levels under these conditions. In contrast, intracellular K⁺ levels in the presence of either Na⁺, Li⁺ or K⁺ were greater than control levels.

Fig. 5. Influence of pH on caesium accumulation in *Synechocystis* PCC 6803. Cells were suspended to $5 \times 10^7 \text{ ml}^{-1}$ in different buffers (10 mM) (see text) adjusted to the specified pH values. Cells were analysed for intracellular Cs^+ (●) following 24 h incubation in the presence of 1 mM-CsCl. Mean values from three replicate determinations are shown $\pm$ SEM where these exceeded the dimensions of the symbols.

This was particularly evident at the higher concentrations of Na^+ or Li^+ studied (10 mM).

Dependence of Cs^+ accumulation on external Cs^+ concentration in *Synechocystis* PCC 6803

The initial rate of Cs^+ accumulation increased with increasing external Cs^+ concentrations (Fig. 4a, b). At the lowest Cs^+ concentration used (0.2 mM), Cs^+ accumulation reached an approximately constant level over the 48 h incubation period whereas at Cs^+ concentrations between 0.4 and 1.5 mM, cellular Cs^+ reached a maximal value at about 24 h and then decreased. At 2 mM- Cs^+ , the highest concentration tested, a decrease in cellular Cs^+

was evident after 12 h (Fig. 4a). When levels of intracellular Cs^+ or K^+ , attained after 12 h incubation, were plotted against external Cs^+ concentration, a linear relationship was evident over the range of Cs^+ concentrations examined ($\leq 2 \text{ mM}$) (Fig. 4b). For cells incubated for 24 h, this relationship deviated from linearity as the cells incubated in Cs^+ concentrations $\geq 0.8 \text{ mM}$ began to lose accumulated Cs^+ (Fig. 4a, c). After 48 h, cells incubated in either 0.8, 1.5 or 2 mM-CsCl contained similar amounts of Cs^+ (Fig. 4c). Loss of intracellular K^+ was correlated with Cs^+ accumulation, although the latter again exceeded K^+ loss (Fig. 4b, c).

Energy- and pH-dependence of Cs^+ accumulation by *Synechocystis* PCC 6803

Cs^+ accumulation by *Synechocystis* PCC 6803 was reduced upon incubation of cells in the light in the presence of $10 \mu\text{M}$ -3(3,4-dichlorophenyl)-1,1-dimethyl-urea (DCMU), at low temperature (4°C) in the light, or at 22°C in the dark, as compared with control cells incubated in the light (Table 3). DCMU (an inhibitor of photosystem II) and dark incubation had the greatest inhibitory effect on Cs^+ accumulation, the reduction being approximately 80% and 92% respectively, as compared with control cells incubated in the light. Low temperature also inhibited Cs^+ accumulation, although here the amount of Cs^+ accumulated was only reduced by approximately 28% (Table 3).

Cs^+ accumulation was markedly dependent on external pH (Fig. 5). Increased amounts of Cs^+ were accumulated as the pH of the external medium was increased, with maximal accumulation [approximately $1330 \text{ nmol } \text{Cs}^+ (10^9 \text{ cells})^{-1}$ after 24 h incubation] occurring at pH 10, the highest pH value used. At pH 6.5, only about $200 \text{ nmol } \text{Cs}^+ (10^9 \text{ cells})^{-1}$ was accumulated, an 85% reduction in the value observed at pH 10 (Fig. 5). Control experiments confirmed that Cs^+ accumulation was similar in different buffers used for any given pH value and there was no cell division over the 24 h incubation period.

Table 3. Energy-dependence of Cs^+ accumulation by *Synechocystis* PCC 6803

Cells were suspended to $5 \times 10^7 \text{ ml}^{-1}$ in 10 mM-HEPES buffer, pH 8, with 1 mM-CsCl in the specified treatments. Cells were harvested after 24 h incubation. Cs^+_{int} and K^+_{int} refer to intracellular Cs^+ and K^+ respectively. Mean values from three replicate determinations are shown $\pm$ SEM.

Treatment	Cs^+_{int} [nmol $(10^9 \text{ cells})^{-1}$]	K^+_{int} [nmol $(10^9 \text{ cells})^{-1}$]
Light, 22°C (control)	778 ± 16	31 ± 2
Dark, 22°C	66 ± 5	363 ± 4
Light + $10 \mu\text{M}$ -DCMU, 22°C	165 ± 13	404 ± 36
Light, 4°C	558 ± 50	120 ± 32

Discussion

Some early reports have demonstrated that Cs^+ accumulation can be inhibited by K^+ in microalgae and cyanobacteria (Williams & Swanson, 1958; Williams, 1960, 1970; Harvey & Patrick, 1967), although Williams (1960) reported that Cs^+ accumulation was not influenced by extracellular Na^+ in these aquatic microorganisms. This is in contrast to the results presented here for *Synechocystis* PCC 6803. A reduction in caesium accumulation and toxicity occurred when BG-11 medium was supplemented with elevated K^+ and Na^+ concentrations in addition to 1 mM- CsCl . The experiments clearly indicate that external K^+ and Na^+ play approximately equivalent roles in the inhibition of Cs^+ accumulation and indeed, in short-term experiments, Cs^+ accumulation was virtually eliminated in the presence of 50 mM- K^+ or Na^+ . The inhibitory effect of extracellular K^+ and Na^+ on caesium accumulation may have important implications for aquatic environments. The higher abundance of sodium in the marine environment (approximately 0.6 M) may explain why Cs^+ accumulation by *Synechococcus* sp. was not detected by Fisher (1985), who conducted uptake experiments in Mediterranean seawater. It is likely that elevated concentrations of caesium would be required in seawater for significant levels of Cs^+ accumulation to occur in *Synechocystis* PCC 6803, a cyanobacterium that characteristically grows on freshwater medium, but which can tolerate salt concentrations in excess of those that occur in seawater (Richardson *et al.*, 1983).

Substitution of intracellular K^+ with Cs^+ has been reported in bacteria (Nakamura *et al.*, 1982) and yeast (Borst-Pauwels, 1981). This study demonstrates that a reduction in cellular K^+ was associated with Cs^+ accumulation in *Synechocystis* PCC 6803, the extent of K^+ loss being proportional to the amount of Cs^+ accumulated. However, a clear stoichiometric relationship was not apparent and the amount of accumulated Cs^+ generally exceeded the amount of K^+ lost by the cells. It is not clear whether this was due to differential binding of Cs^+ and K^+ ions inside the cells resulting from some differences in the chemical properties of these ions. However, it is generally accepted that the alkali metals have low formation constants and a weak coordinating ability with organic ligands, the order being $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$ (Greenwood & Earnshaw, 1984), although K^+ and Cs^+ are known to bind to soil particles under certain conditions (Kirk & Staunton, 1989).

Growth experiments confirmed that, except for cells incubated in K^+ -depleted conditions, the lowest levels of intracellular K^+ occurred in cells which had accumulated the most Cs^+ and where the greatest growth inhibitory effects were observed. K^+ is known to be required by

microbes as an enzyme activator, an osmotic regulator and a regulator of internal pH, and it may also serve as an energy source in the form of a transmembrane K^+ gradient (Booth, 1985; Walderhaug *et al.*, 1987). It seems likely that an important mechanism of Cs^+ toxicity in *Synechocystis* PCC 6803 arises through replacement of cellular K^+ by Cs^+ and the inability of this ion to substitute for K^+ in metabolic processes. The evidence presented also indicates that Cs^+ accumulation had no further effect on the growth of K^+ -limited cells of this cyanobacterium.

In addition to K^+ and Na^+ , several other monovalent cations inhibited Cs^+ accumulation when supplied at a tenfold higher concentration than Cs^+ . However, Na^+ , Rb^+ , Li^+ and Tl^+ differed markedly in their individual effects on intracellular K^+ . Exposure to Rb^+ and Tl^+ caused the loss of most cellular K^+ while addition of Na^+ and Li^+ stimulated K^+ accumulation. Rb^+ and Tl^+ accumulation via the K^+ transport systems of microorganisms is well documented (Rhoads *et al.*, 1977; Damper *et al.*, 1979; Reed *et al.*, 1981b; Bakker, 1983). Similar K^+ transport systems of low specificity operating in *Synechocystis* PCC 6803 may account for some of the inhibitory effects exerted by Rb^+ and Tl^+ on Cs^+ accumulation. However, it should be noted that Tl^+ in particular can have severe adverse effects on the structural integrity of cell membranes which can result in loss of ions like K^+ (Norris *et al.*, 1976). The stimulatory effect of externally supplied Na^+ or Li^+ on intracellular K^+ levels implies that these cations interfere with the K^+ -transport system, and hence Cs^+ accumulation, through an alternative mechanism. One possibility is that Na^+ or Li^+ ions may bind to a second, non-transporting (modifier) site at the K^+ -carrier leading to higher affinity transport of K^+ and a reduced Cs^+ uptake. Stimulation of K^+ uptake is a common microbial response to salt stress (Reed, 1986) and it is likely that a similar response to Na^+ and Li^+ is induced in *Synechocystis* PCC 6803 which results in 'scavenging' for external K^+ and a concomitant reduction in Cs^+ transport. When Li^+ , Na^+ , K^+ or Rb^+ were supplied to cells at equimolar concentrations to Cs^+ , no reduction in Cs^+ accumulation resulted, in comparison to cells incubated with Cs^+ alone. These results are interesting as they imply that were Cs^+ to be taken up via the K^+ -transport system, as seems probable, then Cs^+ appears to have a higher affinity for this carrier than K^+ . Even the Kup system of *E. coli* which has a moderate affinity for Cs^+ still has a tenfold higher K_m value for Cs^+ than for K^+ (Bossemeyer *et al.*, 1989). Further detailed studies using potassium-depleted cells and/or radioisotopes would be required, however, to assess more fully the relative affinities of the monovalent cations for the K^+ -transport system of *Synechocystis* PCC 6803.

Caesium accumulation was directly proportional to the extracellular Cs^+ concentration over the range of concentrations studied (0.2–2.0 mM) following 12 h incubation. These results are similar to those of Williams & Swanson (1958), who demonstrated a close relationship between extracellular Cs^+ concentration and intracellular Cs^+ accumulation in *Chlorella pyrenoidosa*. However, these studies employed a lower range of external Cs^+ concentrations and the results deviated from linearity at Cs^+ concentrations above 0.04 mM. When Cs^+ accumulation over 48 h was examined using the same range of extracellular Cs^+ concentrations, cells incubated in the highest Cs^+ concentrations began to release Cs^+ at 24 h, and after 48 h incubation the amount of Cs^+ retained in cells incubated in 0.8, 1.5 and 2.0 mM- CsCl was approximately similar. Rothstein & Bruce (1958) reported some release of K^+ into the medium following uptake at high extracellular K^+ concentrations in yeast, although such released K^+ may be taken up again by the cells (White & Gadd, 1987). However, the proportion of accumulated Cs^+ released by *Synechocystis* PCC 6803 in this study is large and may be a response to an increased internal osmotic pressure resulting from more Cs^+ being accumulated than is compensated for by release of K^+ .

In common with cyanobacterial uptake of K^+ (Reed *et al.*, 1981a), Cs^+ accumulation was energy-dependent in *Synechocystis* PCC 6803 and dark incubation or DCMU treatment, to inhibit photosystem II-dependent non-cyclic electron transfer, had substantial inhibitory effects on Cs^+ accumulation. Cs^+ accumulation was also markedly influenced by external pH, with increasing Cs^+ accumulation at higher alkaline pH values. In *Anabaena variabilis*, a hyperpolarization of the membrane occurs when the pH of the external medium is increased from 6.0 to 8.0, which results in increased K^+ fluxes and internal K^+ concentration (Reed *et al.*, 1981a). As it seems likely that Cs^+ is accumulated via a K^+ transport system, then such a phenomenon would also account for increased Cs^+ accumulation. In the natural environment, cyanobacteria are commonly found in waters above pH 8, which gives them a competitive advantage over eukaryotic microalgae in alkaline waters (Paerl & Ustach, 1982). Furthermore, photosynthetic activity leads to depletion of the dissolved CO_2 pool, which results in an increase in pH (Kellar & Paerl, 1980). It is likely therefore that *Synechocystis* PCC 6803 can accumulate significant amounts of Cs^+ in its natural freshwater environment, and cyanobacteria may well play an important role in the cycling of ^{137}Cs and its mobilization into biological systems.

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