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# $Na^+$ uptake and extrusion in the cyanobacterium *Synechocystis* PCC6714 in response to hypersaline treatment. Evidence for transient changes in plasmalemma $Na^+$ permeability

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**The kinetics of uptake and loss of $Na^+$ have been studied using radioisotopic tracer techniques in cells of the cyanobacterium *Synechocystis* PCC6714 exposed to hyperosmotic stress. Cells transferred from a freshwater-based medium to NaCl at 300–1000 mmol · $dm^{-3}$ showed net $Na^+$ uptake during the first 2 min following transfer, with the intracellular $Na^+$ level at 2 min increasing as a direct function of the external NaCl concentration. Further incubation of cells in low-level hypersaline media (350–500 mmol · $dm^{-3}$ NaCl) led to a marked reduction in cell $Na^+$ within 20 min, indicating an efficient active $Na^+$ extrusion system. In contrast, cells maintained in more extreme hypersaline media showed little (750 mmol · $dm^{-3}$ NaCl) or no (1000 mmol · $dm^{-3}$ NaCl) net $Na^+$ extrusion following upshock. Cells grown in a saline medium (with NaCl at 500 mmol · $dm^{-3}$) showed a greatly reduced net $Na^+$ uptake after 2 min in media containing higher levels of NaCl. However, net $Na^+$ uptake was also observed when these cells were downshocked to media containing 50–200 mmol · $dm^{-3}$ NaCl. This is the first demonstration of downshock-induced $Na^+$ accumulation in a microbial cell. Time-courses for $Na^+$ extrusion in cells downshocked from 500 mmol · $dm^{-3}$ to 100 mmol · $dm^{-3}$ NaCl were similar to those for cells upshocked from freshwater to 500 mmol · $dm^{-3}$ NaCl, requiring approximately 30 min to reach their lowest values. Net $Na^+$ extrusion in upshocked cells was found to be markedly sensitive to the external $K^+$ concentration, with limited net $Na^+$ extrusion in the absence of external $K^+$ and maximal reductions in cell $Na^+$ in media containing $K^+$ at 1–10 mmol · $dm^{-3}$. Temperature was also shown to affect uptake and loss of cell $Na^+$ during upshock: cells maintained at 5°C showed no capacity for net $Na^+$ extrusion, while higher temperatures (up to 35°C) led to a progressive reduction in the amount of cell $Na^+$ at 2 min following upshock and also in the rate of net $Na^+$ extrusion after this time.**

## Introduction

Many cyanobacteria can adjust to hypersaline treatment (upshock) by increasing the intracellular level of a species-specific low molecular weight organic solute. The carbohydrates glucosylglycerol [1], sucrose [2] and trehalose [3], together with the quaternary ammonium compound glycine betaine [4] have been identified as major osmotica in salt-stressed cyanobacteria from freshwater, marine and hypersaline habitats, with a single organic osmoticum forming the majority of the total low molecular weight organic solute fraction in most cases [5].

Intracellular ion levels have also been measured in several cyanobacteria grown in low-salinity and high-salinity media. In a range of isolates from freshwater, marine and hypersaline environments, including *Synechocystis* PCC6803 [6], *Nodularia harveyana* [7], *Aphanothece halophytica*, *Coc-*

*cochloris elabens* (*Synechococcus* spp.) and *Dactylococcopsis salina* [4], the internal levels of the monovalent cations $K^+$ and $Na^+$ vary only marginally in response to the salinity of the growth medium. In general, the trend is towards higher intracellular $K^+$ and lower intracellular $Na^+$ concentrations than in the growth medium, especially when cells are grown in media of elevated salinity [4,8]. Thus the euryhaline unicell *Synechocystis* PCC6803 contains $K^+$ at approximately 150 mmol $\cdot dm^{-3}$ (expressed on a total cell volume basis) while intracellular levels of $Na^+$ are approximately 35 mmol $\cdot dm^{-3}$ when grown in either freshwater- or seawater-based media [6]. Such studies, using cells that have been pre-equilibrated for at least 24–48 h in hyperosmotic and hypoosmotic media prior to experimental assay, have given no indication of any involvement of intracellular ions in the long-term osmotic adjustment processes of these cyanobacteria. However, other studies have shown that $K^+$ may be involved in osmoacclimation in the halotolerant cyanobacterium *Aphanothece halophytica* (*Synechococcus* sp.) with high intracellular $K^+$ levels (up to 1 mol $\cdot dm^{-3}$), varying as a function of the external salt concentration [9]. Further indications that changes in internal ion levels may occur in osmotically-stressed cyanobacteria have also been obtained for the sucrose-accumulating freshwater unicell *Synechococcus* PCC6311 [10]. In this organism, intracellular $Na^+$ levels rose rapidly following upshock from a freshwater-based medium to a medium containing NaCl at either 300 mmol $\cdot dm^{-3}$ or 600 mmol $\cdot dm^{-3}$, appearing to reach a level close to that of the external medium within 30 min. Intracellular $Na^+$ then declined more slowly over the following 16 h, reaching a lower equilibrium level within approx. 40 h. These studies used flame emission spectrophotometric techniques to measure the internal $Na^+$ levels of pelleted cells, with a correction for external $Na^+$ present in the adhering medium obtained by using [$^{14}$C]inulin. This procedure may overestimate intracellular $Na^+$ since no account is taken of the cation exchange properties of the cell wall matrix [11]. In addition, substances of high molecular weight, including [$^{14}$C]inulin, may not fully penetrate the cell wall to the plasmalemma, leading to further inaccuracies in the measurement of intracellular solute levels [12]. The additional problems of measuring low internal $Na^+$ levels against a background of high extracellular $Na^+$ without adequate rinsing procedures is a further potential source of error [4,9].

We have investigated the effects of upshock upon $Na^+$ transport and accumulation in the unicellular cyanobacterium *Synechocystis* PCC6714. This euryhaline freshwater isolate grows well in a wide range of salinities, up to and exceeding that of full-strength seawater (full-strength seawater is approx. 500 mmol $\cdot dm^{-3}$ NaCl) and previous studies have shown that glucosylglycerol is involved in the maintenance of osmotic equilibrium and turgor generation in cells grown in seawater-based media [5]. However, changes in intracellular $K^+$ levels have also been observed in recent experiments, with cells showing a rapid net $K^+$ uptake in response to hyperosmotic treatment (addition of either NaCl, sorbitol or sucrose at concentrations up to 200 mmol $\cdot dm^{-3}$), indicating that a turgor-sensitive $K^+$ uptake system operates in this cyanobacterium [13], as in some heterotrophic bacteria [14]. In the present study, changes in intracellular ion levels have been monitored over a range of ionic conditions (varying external $Na^+$ or $K^+$ concentration) and temperatures, using radioactive tracer techniques to provide further detailed information on the effect of salinity change upon ion uptake and accumulation. The present results show an initial net $Na^+$ influx (within 2 min) in cells exposed to hyperosmotic and hypoosmotic stress beyond a critical value, followed by $K^+$-dependent, temperature-sensitive $Na^+$ extrusion, leading to a subsequent reduction in cell $Na^+$.

## Materials and Methods

*Cyanobacterium and growth conditions.* An axenic isolate of the cyanobacterium *Synechocystis* PCC6714 was obtained from the culture collection of the Institut Pasteur, Paris, France and was maintained in a modified BG11 medium [15] at pH 8.2, with a reduced $Na^+$ content (as $NaNO_3$) of 6 mmol $\cdot dm^{-3}$ and a $K^+$ content (as $K_2HPO_4$) of 0.5 mmol $\cdot dm^{-3}$. This medium will be referred to as $BG11_m$. Cells were grown in 12 $dm^3$ flasks containing 10 $dm^3$ of $BG11_m$ medium at 20°C under continuous illumination (at a photon fluence rate of 45 $\mu$mol $\cdot m^{-2} \cdot s^{-1}$). Cells were harvested

in later exponential phase, at biovolume densities approaching 1 $mm^3 \cdot cm^{-3}$.

Upshock experiments were carried out by adding NaCl, to give a range of hyperosmotic media from 50 $mmol \cdot dm^{-3}$ to 1000 $mmol \cdot dm^{-3}$. The effects of varying external $K^+$ concentration were studied by the addition of KCl to the medium, to give external $K^+$ concentrations up to 10 $mmol \cdot dm^{-3}$. Temperature control was achieved using thermostatically-operated water baths; in experiments where the cyanobacterium was subjected to changes in ambient temperature, cells were pre-incubated for 30 min at each temperature prior to upshock (500 $mmol \cdot dm^{-3}$ NaCl).

*Measurement of intracellular volumes.* Cell volumes of *Synechocystis* PCC6714 were determined using a particle size analyzer (Coulter Electronics Ltd., Luton, U.K., model ZB (industrial), fitted with a C1000 channelyzer unit) linked to an Acorn microcomputer to provide direct estimation of cell density, mean cell volume and biovolume density. A 50 $\mu m$ aperture (path length 60 $\mu m$) was used throughout.

In experiments where the cells were upshocked, membrane-filtered medium of identical composition and osmotic strength was used in the particle size analyzer, to ensure no change in cell volume during measurement. Care was taken to replace the entire contents of the aperture tube and associated glassware with the experimental medium, to ensure osmotic and ionic homogeneity during counting. Cells in basal medium ($BG11_m$) and in low-NaCl media (up to 100 $mmol \cdot dm^{-3}$ NaCl) were counted and sized in the minimum amount of electrolyte required for adequate functioning of the Coulter counter system, i.e. 9 $g \cdot dm^{-3}$ NaCl. For each value of mean cell volume, six replicate samples were taken, with 10000–18000 cells per sample volume (50 $mm^3$).

*Radioisotopic measurement of $Na^+$ exchange.* $Na^+$ uptake and loss were studied using the radioisotope $^{24}Na^+$ (obtained from the Scottish Universities Research and Reactor Centre, E. Kilbride, U.K.). All samples were counted in 5-$cm^3$ aliquots of scintillation cocktail 229 (Packard Instruments, IL, U.S.A.) using a Packard Instruments 300 liquid scintillation spectrometer, with automatic quench correction (samples channels ratio). All data were corrected for decay of $^{24}Na^+$ during counting since $^{24}Na^+$ has a half-life of 15 h.

Preliminary time course experiments were carried out using cells maintained in $BG11_m$ medium containing $^{24}Na^+$ (at specific activities up to 5 $GBq \cdot mol^{-1}$) for 18 h to allow exchange and equilibration of intracellular and extracellular $Na^+$ [4]. Cells were susbsequently upshocked by the addition of radioisotopically-labelled NaCl containing $^{24}Na^+$ at the same specific activity and then incubated in experimental media for varying times, after which the cells were separated from the medium by a rapid filtration procedure since silicone oil microcentrifugation techniques proved unsatisfactory due to problems associated with changes in medium density upon upshock [16]. Aliquots (0.5–2.0 $cm^3$) of cell suspensions were removed from the experimental flasks and transferred to 'nuflow' cellulose acetate membrane filters (Oxoid Ltd., Basingstoke, U.K.) of pore size 0.45 $\mu m$ and diameter 25 mm. Adhering medium was then removed from the cells by vacuum filtration. Cells were then rinsed rapidly with identical medium, free of added $^{24}Na^+$, to remove any extracellular radioisotope present in the remaining medium and associated with cation exchange groups on the cell wall. Preliminary experiments using cells that had been killed by pre-treatment in 50% dimethyl sulphoxide for 30 min showed that over 98% of the extracellular $^{24}Na^+$ fraction was removed by rinsing with 12–15 $cm^3$ of non-radioactive medium over a period of 25–30 s. All subsequent experiments were carried out using the same filtration and rinsing procedures, although pre-equilibration in $^{24}Na^+$-containing $BG11_m$ medium was not required in all cases due to the low intracellular $Na^+$ content of such cells (see below).

Experiments were carried out in a Chandos Instruments (New Mills, U.K.) multi-place stirrer base, at 20°C under constant illumination (at a photon fluence rate of 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$) unless otherwise stated.

## Results

### *Effects of hyperosmotic stress upon $Na^+$ uptake and accumulation*

Fig. 1 shows a time-course for changes in cell $Na^+$ upon transfer of *Synechocystis* PCC6714 from $BG11_m$ medium containing $^{24}Na^+$ (18 h pre-in-

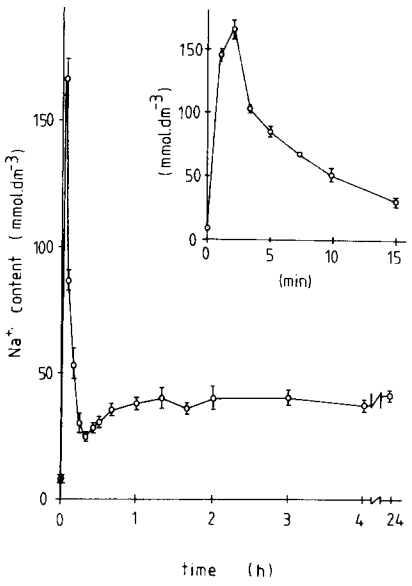


Fig. 1. Intracellular $Na^+$ content of *Synechocystis* PCC6714 transferred from freshwater $BG11_m$ to a medium containing 500 mmol·dm$^{-3}$ NaCl. Values (measured using $^{24}Na^+$ equilibration technique) represent the mean of three replicates (±S.D.); insert shows short-term changes (up to 15 min).

cubation) to a hyperosmotic medium containing NaCl (plus $^{24}Na^+$ at the same specific activity) at 500 mmol · dm$^{-3}$. The initial value for cell $Na^+$ in $BG11_m$ medium was low, being less than 10 mmol · dm$^{-3}$. However, upon upshock, cell $Na^+$ was observed to rise rapidly to a maximum value of 167 mmol · dm$^{-3}$ within 2 min. A less rapid decline in cell $Na^+$ was then observed over the next 18 min, reaching a value close to the original $Na^+$ concentration at 20 min. Subsequent incubation led to a further small increase in cell $Na^+$, with values up to 40 mmol · dm$^{-3}$ being recorded between 2 h and 24 h (Fig. 1). These values are significantly higher than the value at 20 min ($t$-test $P = 0.05$) suggesting that the increase in $Na^+$ content observed after 20 min was not due solely to experimental variation. Furthermore, the same pattern was also observed in all subsequent experiments at this concentration (see below).

Fig. 2a contains data for net $Na^+$ uptake in cells of *Synechocystis* PCC6714 transferred from $BG11_m$ medium to medium containing $^{24}Na^+$-labelled NaCl at 50–1000 mmol · dm$^{-3}$ for 2 min (see Fig. 1). Minimal uptake of external $Na^+$ was observed below 200 mmol · dm$^{-3}$. However, a direct relationship between $Na^+$ uptake and external $Na^+$ concentration was observed in more saline media (i.e. between 300 mmol · dm$^{-3}$ and 1000 mmol · dm$^{-3}$ NaCl), with the highest values for internal $Na^+$ (approx. 680 mmol · dm$^{-3}$, expressed on a total cell volume basis) being recorded in 1000 mmol · dm$^{-3}$ NaCl. Over this range, the cells showed minimal change in volume in response to hypersaline treatment (Fig. 2a).

Cells of *Synechocystis* PCC6714 grown in a saline medium ($BG11_m$ plus additional NaCl at 500 mmol · dm$^{-3}$) for a period of 10 days showed limited $Na^+$ uptake in hyperosmotic media (i.e. > 500 mmol · dm$^{-3}$ NaCl), with a 3-fold reduction in intracellular $^{24}Na^+$ level upon transfer to 1000 mmol · dm$^{-3}$ NaCl (Fig. 2b). However, these cells also showed net $Na^+$ uptake when exposed to hypoosmotic shock greater than −200 mmol ·

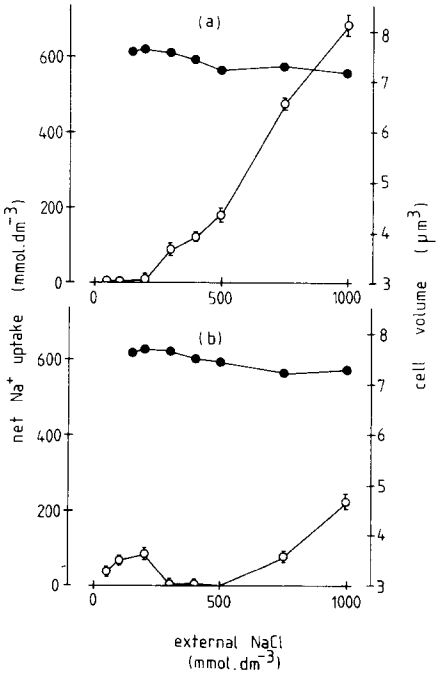


Fig. 2. Net uptake of $Na^+$ (as $^{24}Na^+$) and cell volume of *Synechocystis* PCC6714 grown in (a) freshwater $BG11_m$ and (b) $BG11_m$ plus NaCl at 500 mmol·dm$^{-3}$ after 2 min in a range of media from 500 to 1000 mmol·dm$^{-3}$ NaCl. Values for cell $Na^+$ uptake (○) represent the mean of three replicates (±S.D.); cell volume data (●) were obtained using six replicate measurements (S.D. < 0.2 $\mu m^3$ in all cases).

$dm^{-3}$, with significant $^{24}Na^+$ accumulation after a 2 min period in media containing NaCl at 50–200 mmol · $dm^{-3}$. Cell volume showed similar, small changes to freshwater-grown cells in response to upshock and downshock (Fig. 2b).

Uptake and loss of $^{24}Na^+$ were also studied in media containing NaCl at 350–1000 mmol · $dm^{-3}$ (Fig. 3). Cells in 350 mmol · $dm^{-3}$ and 500 mmol · $dm^{-3}$ NaCl (Fig. 3a) showed a similar pattern of $Na^+$ influx and efflux following upshock, although the levels of cell $Na^+$ were substantially lower in 350 mmol · $dm^{-3}$ NaCl than in 500 mmol · $dm^{-3}$ NaCl. Further increases in external $Na^+$ all resulted in higher values for intracellular $Na^+$ at 2 min with diminishing reductions in cell $Na^+$ concentration between 2 min and 60 min being observed at higher salinities. Thus cells transferred to 750 mmol · $dm^{-3}$ NaCl showed limited net $Na^+$ loss during this period while cells in 1000 mmol · $dm^{-3}$ were found to increase their intracellular $Na^+$ concentration up to 60 min (Fig. 3b). Cell volume changes were minimal during the time-courses shown in Fig. 3 (data not shown). It appears that the ability of cells to actively lower their intracellular $Na^+$ content following upshock is governed by the extent of hyperosmotic treatment.

*Uptake and loss of $Na^+$ in cells exposed to hypo-osmotic shock*

Fig. 2 showed that cells of *Synechocystis* PCC6714 grown in $BG11_m$ plus additional NaCl at 500 mmol · $dm^{-3}$ increased their internal level of $Na^+$ upon transfer to hypoosmotic media (i.e. with NaCl at 50–200 mmol · $dm^{-3}$). A time-course for this response is shown in Fig. 4 for cells transferred to 100 mmol · $dm^{-3}$ NaCl. A transient increase in internal $Na^+$ was observed within the first 2 min following downshock, reaching a maximum value of 64 mmol · $dm^{-3}$ (Fig. 4). Subsequent incubation in 100 mmol · $dm^{-3}$ NaCl led to a reduction in intracellular $Na^+$, reaching a new steady-state value in approx. 30 min. The response of cells grown in saline media to downshock into 100 mmol · $dm^{-3}$ NaCl is thus similar in many respects to the behaviour of freshwater-grown *Synechocystis* PCC6714 upon upshock into 350–500 mmol · $dm^{-3}$ NaCl (cf. Figs. 3 and 4).

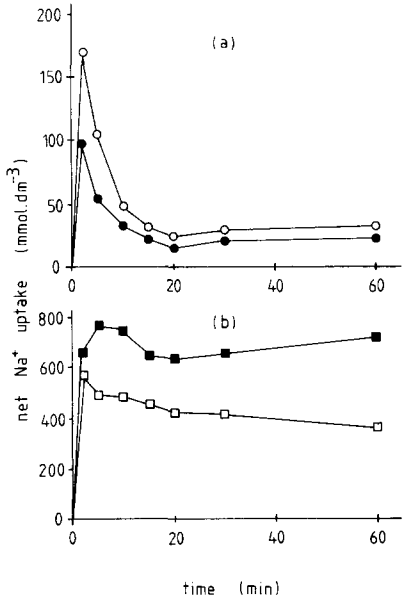


Fig. 3. Net $Na^+$ uptake in cells of *Synechocystis* PCC6714 transferred to a medium containing NaCl at 350–1000 mmol · $dm^{-3}$. Values (three replicates, S.D. < 15 mmol · $dm^{-3}$) are shown in (a) for 350 mmol · $dm^{-3}$ NaCl (●) and 500 mmol · $dm^{-3}$ NaCl (○); (b) contains data for 750 mmol · $dm^{-3}$ NaCl (□) and 1000 mmol · $dm^{-3}$ NaCl (■).

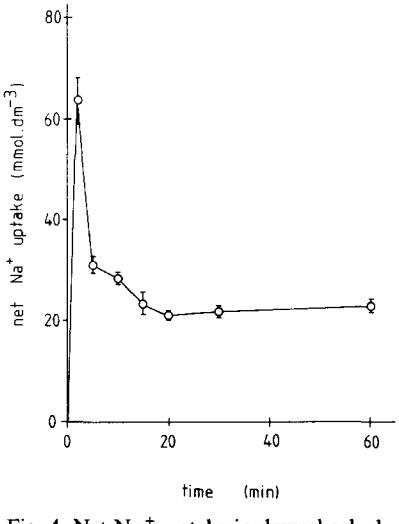


Fig. 4. Net $Na^+$ uptake in downshocked cells of *Synechocystis* PCC6714 transferred from $BG11_m$ plus NaCl at 500 mmol · $dm^{-3}$ to a medium containing 100 mmol · $dm^{-3}$ NaCl for up to 60 min. Values represent the mean of three replicates (± S.D.).

*Effects of varying external $K^+$ concentration upon $Na^+$ uptake and loss in response to hyperosmotic treatment*

Preliminary experiments demonstrated that the response shown in Fig. 1 for cells in 500 mmol · $dm^{-3}$ NaCl was insensitive to changes in external pH, with similar increases and decreases in cell $Na^+$ being observed at pH 6 and pH 9 (data not shown). However, the responses of *Synechocystis* PCC6714 were found to be sensitive to variations in external $K^+$ concentration (Fig. 5). Thus, when cells were upshocked in 500 mmol · $dm^{-3}$ NaCl in the absence of $K^+$ in their bathing medium, the initial rise in internal $Na^+$ up to 2 min was followed by a limited decrease in cell $Na^+$ up to 60 min. In contrast, cells that were upshocked in 500 mmol · $dm^{-3}$ NaCl medium containing $K^+$ at 1.0 or 10.0 mmol · $dm^{-3}$ showed similar major reductions in cell $Na^+$ between 2 and 20 min (Fig. 5). Cells transferred to 500 mmol · $dm^{-3}$ NaCl plus 0.5 mmol · $dm^{-3}$ $K^+$ (the $K^+$ concentration in $BG11_m$ medium, and in all other experiments, see Figs. 1–4) also showed substantial net $Na^+$ extrusion within 20 min, although the values at this $K^+$ concentration were found to be somewhat higher than in media containing 1.0 and 10.0 mmol · $dm^{-3}$ $K^+$. Thus external $K^+$ concentrations in excess of 0.5 mmol · $dm^{-3}$ were necessary for maximum net $Na^+$ extrusion following hyperosmotic treatment (500 mmol · $dm^{-3}$ NaCl).

*Effects of temperature upon $Na^+$ uptake and loss in response to hyperosmotic treatment*

Fig. 6 shows data for the internal $Na^+$ content of *Synechocystis* PCC6714 transferred from $BG11_m$ medium to a medium containing 500 mmol · $dm^{-3}$ NaCl at temperatures between 5°C and 35°C. At the lowest temperature (5°C) a steady increase in intracellular $Na^+$ uptake was observed following the initial rapid phase of $Na^+$ uptake (2 min), with maximum values for intracellular $Na^+$ being recorded after a 60 min incubation period at 5°C. Cells at 15–35°C all showed the same biphasic response, with increasing cell $Na^+$ up to 2 min and

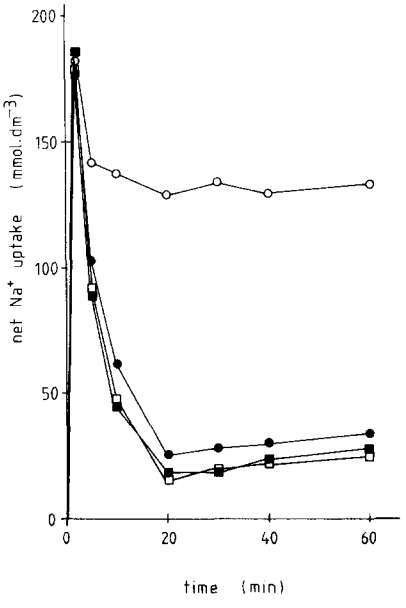


Fig. 5. Effects of varying external $K^+$ concentration upon $Na^+$ uptake and extrusion in *Synechocystis* PCC6714 transferred from fresh water $BG11_m$ to a medium containing NaCl at 500 mmol·$dm^{-3}$. Values (three replicates; S.D. < 8 mmol·$dm^{-3}$) are shown for external $K^+$ levels of 0.0 mmol·$dm^{-3}$ (○), 0.5 mmol·$dm^{-3}$ (●), 1.0 mmol·$dm^{-3}$ (□) and 10.0 mmol·$dm^{-3}$ (■).

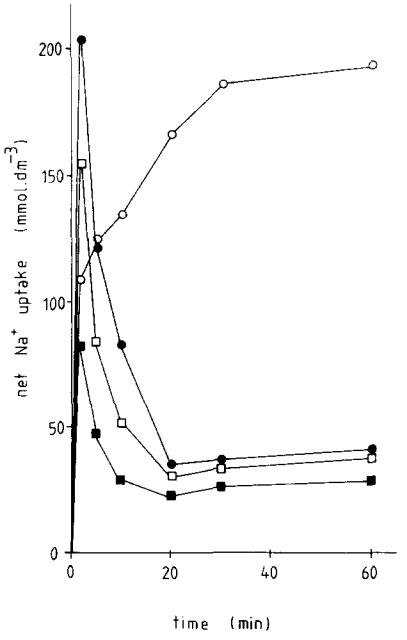


Fig. 6. Effects of temperature upon $Na^+$ uptake and extrusion in *Synechocystis* PCC6714 transferred from freshwater $BG11_m$ to a medium containing NaCl at 500 mmol·$dm^{-3}$. Values (three replicates; S.D. < 9 mmol·$dm^{-3}$) are shown for cells incubated at 5°C (○), 15°C (●), 25°C (□) and 35°C (■).

decreasing cell $Na^+$ between 2 and 20 min (with smaller, subsequent increases up to 60 min). However, maximal changes in intracellular $Na^+$ were observed at 15–20°C while minimal increases and decreases were recorded for cells incubated at 35°C. The values for intracellular $Na^+$ in cells transferred to 500 mmol · $dm^{-3}$ NaCl at 35°C (Fig. 6) are thus lower than those for cells upshocked in 350 mmol · $dm^{-3}$ NaCl at 20°C (Fig. 3).

## Discussion

The data in Fig. 1 show that intracellular $Na^+$ (as measured by $^{24}Na^+$ equilibration for 18 h) is low in freshwater-grown *Synechocystis* PCC6714 ($< 10$ mmol · $dm^{-3}$ $Na^+$ prior to upshock). This value was not exceeded in the long-term, even after 48 h incubation in radioactively-labelled $BG11_m$ medium (data not show). Thus steady-state equilibration was reached within 18 h. Low intracellular $Na^+$ concentrations are characteristic of non-vacuolate, eukaryotic algal cells [17,18] and *Synechocystis* PCC6714 contains $Na^+$ at levels comparable to the lowest values reported for algal cells. The low intracellular $Na^+$ content of *Synechocystis* PCC6714 is also consistent with current theories regarding solute compartmentation in photosynthetic cells, with vacuolar localization of (toxic) $Na^+$ and cytoplasmic accumulation of $K^+$ together with organic solutes [19] since cyanobacterial cells lack large vacuoles.

The data in Fig. 2a show that cells of *Synechocystis* PCC6714 reduced their volume by less than 10% upon transfer from a freshwater-based medium to medium containing NaCl at up to 1000 mmol · $dm^{-3}$. Such a response is in direct conflict with the assumption that cells transferred to hyperosmotic media behave as so-called 'ideal osmometers', shrinking to an extent that is directly proportional to the reciprocal of the external osmolality once a critical (minimum) osmolality is exceeded and cell turgor has been removed [20,21]. This study suggests that NaCl would not be a suitable solute to use in studies of the changes in cell volume that accompany increases in osmolality, due to net solute uptake at high external NaCl concentration (Fig. 2). Thus, changes in cell volume due to water flux from the cells in response to upshock were mitigated by solute transfer across the plasmalemma, since a substantial net $Na^+$ uptake was observed in hyperosmotic media (300–1000 mmol · $dm^{-3}$ NaCl). Below 300 mmol · $dm^{-3}$ there was little evidence of substantial $Na^+$ penetration of the cell interior and the cells would thus be expected to behave in the manner predicted by 'classical' osmotic theory [20] within this range. This may also explain why such phenomena have not been observed previously, using low-$Na^+$ media [22,23]. Above 300 mmol · $dm^{-3}$ NaCl, the direct relationship between net $Na^+$ uptake and external NaCl concentration will have served to minimize any decrease in cellular volume due to upshock (Fig. 2a), with cells transferred to 1000 mmol · $dm^{-3}$ showing the greatest proportional increase in net $Na^+$ uptake.

Acclimation of *Synechocystis* PCC6714 in a saline medium is also demonstrated by the data contained in Fig. 2, since cells grown in 500 mmol · $dm^{-3}$ NaCl accumulated less $^{24}Na^+$ than freshwater-grown cells upon transfer to extreme hypersaline media (i.e. $> 500$ mmol · $dm^{-3}$). Such effects may help to explain how the upper salinity tolerance limit of an organism can be increased by pre-treatment at an intermediate salinity [24]. Previous studies have concentrated on the changes in low molecular weight organic solutes that occur in cyanobacteria grown in saline media, with the inference that these compounds may act as 'compatible solutes' [25], protecting the cell from NaCl inactivation of enzyme activity and loss of metabolic function [26]. However, the present study suggests that acclimation may also be related to direct ionic effects and to changes in intracellular ion levels upon upshock.

The observation that cells grown in a saline medium (500 mmol · $dm^{-3}$ NaCl) showed net accumulation of $Na^+$ when downshocked to low-salinity media is a novel demonstration of the effects of hypoosmotic stress (Figs. 2 and 4). Previous studies have concentrated on intracellular ion increases during upshock and this phenomenon does not appear to have been reported previously. Net $Na^+$ uptake was induced by hypoosmotic shocks in excess of $-200$ mmol · $dm^{-3}$. This value compares favourably with the NaCl change required to initiate $Na^+$ uptake during hyperosmotic treatment (Fig. 2), suggesting that it is the extent

of osmotic (NaCl) shock, rather than its direction, which is responsible for the change in plasmalemma permeability, resulting in net $Na^+$ accumulation. The present study also suggests that transient disruption of cellular metabolism, photosynthetic activity, etc. during downshock may also be related to the penetration of $Na^+$ into the cell interior. Previous work using micro-celled algae has stressed osmotic rather than ionic effects during downshock [27].

Time-course data for cells upshocked to 350 mmol · $dm^{-3}$ and 500 mmol · $dm^{-3}$ NaCl show rapid entry of $Na^+$ within 2 min, indicating a large increase in membrane permeability to $Na^+$ during this period, followed by slower net efflux, leading to a lower post-upshock value at 20 min (Fig. 3a). These data can be used to estimate net $Na^+$ efflux rates over this time period of 28.2 nmol · $m^{-2}$ · $s^{-1}$ (expressed relative to plasmalemma surface area) [13] in 350 mmol · $dm^{-3}$ NaCl and 50.4 nmol · $m^{-2}$ · $s^{-1}$ in 500 mmol · $dm^{-3}$ NaCl. On a cell volume basis, the rates are comparable to the net $Na^+$ effluxes reported for $Na^+$-enriched cells of *Chlorella pyrenoidosa* upon transfer to $K^+$-enriched medium [28,29] at 5–8 mmol · $dm^{-3}$ · $min^{-1}$. However, the net efflux of $Na^+$, complete within 20 min in 350–500 mmol · $dm^{-3}$ NaCl is considerably faster than that reported by Blumwald et al. [10] for the cyanobacterium *Synechococcus* PCC6311, where intracellular $Na^+$ remained high for up to 16 h. Rapid net $Na^+$ extrusion following upshock-induced increases in internal $Na^+$ has also been observed in the euryhaline eukaryotic alga *Dunaliella tertiolecta* [30] with new steady-state values being established within 30 min. Downshocked cells of *Synechocystis* PCC6714 also extrude $Na^+$ within a similar time period (at a rate of approx. 14.6 nmol · $m^{-2}$ · $s^{-1}$; Fig. 4).

Cells of *Synechocystis* PCC6714 showed an upper limit for net $Na^+$ extrusion of approx. 750–1000 mmol · $dm^{-3}$ NaCl (Fig. 3b). Since *Synechocystis* PCC6714 is able to grow in seawater-based media and in hypersaline media up to this level of NaCl, there may be a direct link between growth and the capacity to actively extrude $Na^+$ upon upshock.

The observation that net extrusion of $Na^+$ is sensitive to external $K^+$, with little evidence of sustained $Na^+$ efflux in media with no added $K^+$ (Fig. 5) suggests that some form of exchange between $Na^+$ and $K^+$ is involved. Batterton et al. [31] have provided evidence that a ($Na^+/K^+$)-ATPase may exist in cyanobacterial cells, although the activities quoted in their study of *Anacystis nidulans* (*Synechococcus* sp.) suggest that such a component would form a relatively minor fraction of the total ATPase activity. The insensitivity of net $Na^+$ extrusion to changes in external pH also suggests that the response does not rely upon a $Na^+/H^+$ antiport system, such as that proposed for *Anacystis nidulans* (*Synechococcus* sp.) [17, 23, 32] and *Synechococcus* PCC6311 [33].

The effects of added external $K^+$ upon net $Na^+$ extrusion in *Synechocystis* PCC6714 are comparable to the reports of net $Na^+$ efflux upon addition of $K^+$ in $Na^+$-enriched *C. pyrenoidosa* [28] where $K^+$-depleted cells accumulated $Na^+$ to high intracellular levels (in excess of 75 mmol · $dm^{-3}$). Addition of $K^+$ at 3 mmol · $dm^{-3}$ led to a rapid decrease in cell $Na^+$, reaching less than 10 mmol · $dm^{-3}$ within 90 min. This time-course is comparable to the data shown in Fig. 1 for *Synechocystis* PCC6714 in 500 mmol · $dm^{-3}$ NaCl. Similarly, cells of the unicellular alga *Dunaliella tertiolecta* show transient upshock-induced $Na^+$ uptake, followed by rapid, $K^+$-sensitive net $Na^+$ extrusion [30].

Previous studies have shown that $K^+$ influx in *Synechocystis* PCC6714 is greatly stimulated (by a factor of up to 3-fold) upon addition of NaCl at 200 mmol · $dm^{-3}$ to the bathing medium, with a consequent increase in intracellular $K^+$ content within 20 min [13]. These changes indicate that a turgor-sensitive $K^+$ transport system operates in this cyanobacterium since, at concentrations up to 200 mmol · $dm^{-3}$, net $Na^+$ accumulation will be minimal (Fig. 2a). The possibility of a stoichiometric exchange of (intracellular) $Na^+$ and (extracellular) $K^+$ in more extreme hypersaline media remains to be tested and will be further complicated by the existence of turgor-sensitive $K^+$ uptake.

The effects of temperature upon $Na^+$ uptake and loss in hyperosmotic media have not hitherto been reported, with decreasing $Na^+$ accumulation as a function of increasing temperature (Fig. 6). The response is not related to loss of viability at high temperature, since *Synechocystis* PCC6714

has been grown routinely at 35°C in this laboratory and 37°C is the optimum temperature for growth of many cyanobacteria. Recent studies have also shown that carbohydrate accumulation in this strain is temperature-sensitive, with cells grown at high temperature showing considerable accumulation of a second carbohydrate, sucrose, in addition to glucosylglycerol [34]. The possibility that the effects of temperature upon ion and organic solute levels in *Synechocystis* PCC6714, are linked requires further investigation.

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