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Evidence for turgor-sensitive K^+ influx in the cyanobacteria *Anabaena variabilis* ATCC29413 and *Synechocystis* PCC6714

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The effects of varying turgor pressure upon K^+ uptake and accumulation have been investigated in *Anabaena variabilis* ATCC29413 and *Synechocystis* PCC6714 by measuring K^+ influx and internal concentration in cells subjected to hyperosmotic treatment. Upon addition of NaCl, sorbitol or sucrose to the bathing medium, an immediate rapid increase in cellular K^+ influx was observed for up to 20 min following solute addition. Intracellular K^+ concentration also increased during this period. Dark treatment reduced the steady-state K^+ influx and also decreased K^+ uptake in hyperosmotically-treated cells. In the presence of the energy-transfer inhibitor, *N,N'*-dicyclohexylcarbodiimide (DCCD), there was no appreciable net cellular K^+ influx. These observations are consistent with the operation of a primary active, energy-dependent K^+ uptake system that responds directly to changes in cell turgor in both cyanobacteria. This K^+ uptake precedes the longer-term osmotic adjustment resulting from low-molecular weight organic solute accumulation in these cyanobacteria.

Introduction

The ionic relations of cyanobacteria are poorly understood, in contrast to those of many other bacteria [1,2]. The characteristics of transport and accumulation of the monovalent cation K^+ have been studied in greatest detail, although data are available for only a limited number of strains, including *Synechocystis* sp. *(Anacystis nidulans)* [3,4], *Anabaena flos-aquae* [5] and *Anabaena variabilis* [6,7]. Early studies [3] suggested that K^+ was in passive electrochemical equilibrium across the plasma membrane of *Synechocystis* sp. cells grown in a high-K^+ medium (20.5 mmol · dm^{-3}). Similar conclusions were arrived at by Paschinger [4], using inhibitors to demonstrate that K^+ uptake was governed by an interior-negative membrane potential, in accordance with the Nernst equation [8]. Using chemiosmotic terminology [9], K^+ transport in *Synechocystis* sp. was regarded by Paschinger [4] as passive uniport (secondary active transport), as a result of primary active extrusion of H^+ [4]. Such a mechanism can account for a 10-fold accumulation of K^+ in cells with a plasma membrane potential of −60 mV and up to 100-fold at −120 mV [8].

Subsequent studies using *A. variabilis* grown in a high-K^+ medium (5.0 mmol · dm^{-3}) clearly demonstrated the existence of a primary active K^+ transport system, with no close (obligate) link between K^+ transport and changes in plasma membrane potential [6]. Thus, the ATPase inhibitor DCCD was found to hyperpolarise the cell, while K^+ fluxes were dramatically reduced by the same treatment. These observations are consistent with an energy (ATP)-dependent, active K^+ transport system. Transport of K^+ in *A. variabilis* was

Abbreviation: DCCD, *N,N'*-dicyclohexylcarbodiimide.

also shown to be sensitive to the *trans* K^+ concentration (thus, K^+ efflux was reduced in the absence of external K^+), a feature common to many primary active transport systems operating near to thermodynamic equilibrium [10]. Furthermore, passive uniport would be unable to account for the observed high K^+ levels in cyanobacteria grown in low-K^+, freshwater-based media (less than 1 mmol · dm^{-3}) [5,11,12].

Allison and Walsby [5] have shown that K^+ accumulation in *A. flos-aquae* is light-stimulated. They found that the increase in internal K^+ content due to light-stimulated K^+ uptake was sufficient to collapse enough of the gas vesicles within cells of this cyanobacterium to destroy its buoyancy, suggesting that active K^+ transport may have a fundamental role to play in the control of buoyancy in this organism.

The present study was carried out to investigate further the K^+ transport processes of two cyanobacteria, the euryhaline unicell *Synechocystis* PCC6714 [13] and the heterocystous, filamentous form *Anabaena variabilis* [6,7]. We have studied the effects of changes in external osmotic potential (and, consequently, cell turgor) on K^+ uptake and accumulation in both organisms, to see whether changes in turgor affect K^+ transport, as in some other cell types [14].

Materials and Methods

Cyanobacteria and growth conditions. An axenic isolate of *Anabaena variabilis* ATCC29413 was obtained from the American Type Culture Collection, Rockville, MD, U.S.A. by Mr. A. Darling of this Department; *Synechocystis* PCC6714 was obtained in axenic culture from the culture collection of the Institut Pasteur, Paris, France.

A. variabilis was grown in BG-11_0 medium at pH 8.2 [15] containing additional KCl at 5 mmol · dm^{-3}; the basal growth medium for *Synechocystis* PCC6714 (BG-11, containing $NaNO_3$ at 1.5 g · dm^{-3} [15]) was also supplemented with KCl at 5 mmol · dm^{-3}. Cells were grown in 500-cm^3 flasks containing 250 cm^3 medium, at 23°C under continuous illumination (at a photon flux density of 45 μmol · m^{-2} · s^{-1}). Cells were harvested in late exponential phase, at biovolume densities approaching 1 mm^3 · cm^{-3}.

Measurement of intracellular volume. The intracellular volume of *A. variabilis* was determined using [U-^{14}C]sucrose and ^3H_2O, as described previously [16]. Silicone oil microcentrifugation was used to separate cells from their bathing medium [6]. Cell volumes of *Synechocystis* PCC6714 were determined using a particle size analyzer (Coulter Electronics Ltd., Luton, U.K., model ZB, fitted with a C1000 channelyzer unit) linked to an Acorn microcomputer to provide direct estimation of mean cell volume. K^+ influx rates have been calculated for *Synechocystis* PCC6714 in terms of plasma membrane surface area, by assuming a spherical cell shape (with a surface area/cell volume ratio of 2650 $m^2 \cdot dm^{-3}$). The plasma membrane surface area/cell volume ratio of *A. variabilis* (1661 $m^2 \cdot dm^{-3}$), determined by Reed et al. [6] was used to correct all other data.

Influx determinations. K^+ flux rates were calculated from measurements of the exchange rates of radioisotopic $^{42}K^+$ which was provided by the Scottish Universities Research and Reactor Centre, East Kilbride, U.K. All samples were counted in 5-cm^3 aliquots of Packard Instruments (Illinois, U.S.A.) scintillation cocktail 229 using a Packard Instruments 300 liquid scintillation spectrometer, with automatic quench correction (sample channels ratio). All data were corrected for decay of $^{42}K^+$ during counting, since $^{42}K^+$ has a half-life of 12.36 h.

Influx measurements were carried out by incubating cells in growth medium (5.0 mmol · dm^{-3} KCl) containing radioactive tracer (at up to 10.0 GBq · mol^{-1}) for varying periods of time, up to 1h, after which the cells were separated from the medium by microcentrifugation (Sarstedt Microcentrifuge, Leicester, U.K.) at 12000 × *g* through Dow Corning 550 silicone oil/dinonylphthalate (50 : 50, v/v) into perchloric acid/water (40 : 60, v/v), as before [16].

Measurement of intracellular K^+ concentration. Internal K^+ levels were measured in *A. variabilis* and *Synechocystis* PCC6714 by pre-incubating cells in radioisotopic basal growth medium (either BG-11 or BG-11_0 plus 5 mmol · dm^{-3} KCl, containing $^{42}K^+$ at up to 10.0 GBq · mol^{-1}) for 24 h, to ensure exchange and equilibration of intra- and extracellular K^+, since half-times for exchange fall within the range 2–3 h [6]. Cells were then sep-

arated from their bathing medium by silicone oil microcentrifugation and the data corrected for the presence of extracellular K^+ within the cell wall matrix using values from short-term (60 s) influx studies (see Fig. 1). Similar procedures were used in all experiments where the extracellular osmotic potential was subsequently varied by the addition of organic or inorganic solutes.

Changes in external osmotic potential and cell turgor. Decreases in cell turgor were obtained by adding one of the following solutes to a suspension of cyanobacterial cells in basal growth medium (5.0 mmol · dm^{-3} KCl): NaCl, at either 100 mmol · dm^{-3} or 200 mmol · dm^{-3}, sorbitol at 150 mmol · dm^{-3} or sucrose at 150 mmol · dm^{-3}, to increase the external osmolality by 186, 369, 154, or 157 mosmol · kg^{-1}, respectively. Organic solutes were used in addition to inorganic, ionic solutes to determine the effects of osmotic, as well as ionic changes in the composition of the medium upon K^+ transport and accumulation.

All chemicals, including the inhibitor, DCCD, were obtained from Sigma, Poole, U.K. Experiments were carried out at 20°C under constant illumination (at a photon fluence rate of 45 μmol · $m^{-2} \cdot s^{-1}$) unless otherwise stated in the text.

Results

Effects of increasing external osmolality on K^+ influx

Fig. 1a shows that tracer influx in cells of *A. variabilis* maintained in growth medium (5.0 mmol · dm^{-3} KCl) is biphasic, with an initial, rapid uptake complete in 60 s, corresponding to an extracellular (cell wall) component, while the second, slower phase represents translocation of K^+ across the plasma membrane and into the cell interior [6]. Under steady-state conditions (i.e., no net K^+ flux), the second component of K^+ influx can be represented by a single, exponential function [6,17], since the organism behaves as a single, uniformly mixed compartment with respect to tracer exchange. In short-term experiments, as in Fig. 1a, the second, slower phase of uptake is approximately linear, and semi-log transformation

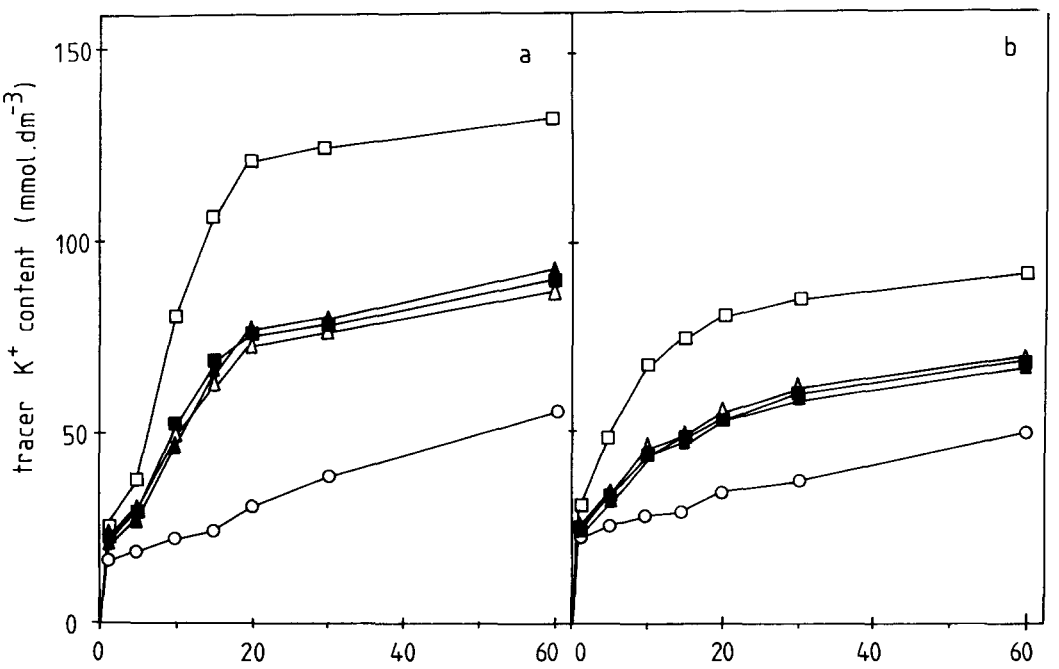

Fig. 1. Uptake of K^+ (as $^{42}K^+$) in cells of (a) *A. variabilis* ATCC29413 and (b) *Synechocystis* PCC6714 transferred from basal growth medium (containing KCl at 5.0 mmol·dm^{-3}) to a medium supplemented with either 100 mmol·dm^{-3} NaCl (■), 200 mmol·dm^{-3} NaCl (□), 150 mmol·dm^{-3} sorbitol (△) or 150 mmol·dm^{-3} sucrose (▲). Steady-state K^+ influx kinetics are also shown for cells in basal growth medium (○). Values represent the mean of four replicates.

of the data [17] is not required prior to calculation of plasma membrane K^+ influx. In steady-state, the cellular K^+ influx will be balanced by an efflux of K^+ of equal magnitude, leading to no net change in internal K^+ concentration, despite the circulation of K^+ across the plasma membrane. Given that the half-time for equilibration of K^+ in *A. variabilis* is approx. 2–3 h [6], while the doubling time of cells in late exponential growth phase is more than 36 h, the criteria of steady-state kinetics [17] can be reasonably applied to the data shown in Fig. 1 for cells in basal growth medium. Fig. 1a also shows the effects of increasing the external osmolality upon K^+ influx in *A. variabilis*. It is clear that, in all cases where additional solutes were added to the basal growth medium, there was a transient, rapid increase in tracer K^+ uptake. This enhanced phase of uptake was completed within a 20-min period following adjustment of the external osmolality, and the slopes of all influx curves are approximately parallel from 20–60 min. Fig. 1a also demonstrates that the extent of change in tracer K^+ uptake during the period 0–20 min varied as a function of the osmolality of the bathing medium, with 200 mmol $\cdot dm^{-3}$ NaCl (369 mosmol $\cdot kg^{-1}$) producing the greatest increase in K^+ influx, while the other three treatments (at 154–186 mosmol $\cdot kg^{-1}$) resulted in a smaller change, being approx. 50% of that observed in 200 mmol $\cdot dm^{-3}$ NaCl.

Fig. 1b shows similar data for the unicell *Synechocystis* PCC6714, with a somewhat larger initial, extracellular component and a slower second (cellular) phase of influx than was observed for *A. variabilis* (Fig. 1a). However, despite quantitative differences in cellular K^+ influx rates, the same trends were apparent upon addition of solutes to the bathing medium, with an appreciable rapid increase in tracer influx during the period up to 15 min (Fig. 1b). As for *A. variabilis*, the greatest increase in K^+ influx was initiated by the addition of 200 mmol $\cdot dm^{-3}$ NaCl, with 100 mmol $\cdot dm^{-3}$ NaCl, 150 mmol $\cdot dm^{-3}$ sorbitol and 150 mmol $\cdot dm^{-3}$ sucrose all producing smaller increases in K^+ uptake within the same time period.

K^+ influx rates (calculated on a surface-area basis using the values shown in Fig. 1) over the period 1–15 min are presented in Table I. In all treatments, the flux rates for *Synechocystis* PCC6714 were less than 60% of the corresponding values for *A. variabilis*; the trend is more pronounced than in Fig. 1 due to the smaller cell size of *Synechocystis* PCC6714, with a consequent increase in the surface area/cell volume ratio. However, both cyanobacteria show similar increases in K^+ influx in response to solute addition, with a 6-fold stimulation of initial K^+ uptake in *A. variabilis* and a 3-fold increase in *Synechocystis* PCC6714 upon transfer to a medium containing 200 mmol $\cdot dm^{-3}$ NaCl. Cells of *A. variabilis* incubated in the other three media showed a 2–3-fold increase in K^+ influx, while *Synechocystis* PCC6714 produced a 2-fold change under similar conditions (Table I).

TABLE I

INITIAL K^+ INFLUX RATES CALCULATED FROM $^{42}K^+$ UPTAKE STUDIES USING *A. VARIABILIS* ATCC29413 AND *SYNECHOCYSTIS* PCC6714

Values were calculated from the data shown in Fig. 1 (1–20 min).

Addition (mmol $\cdot dm^{-3}$)	K^+ influx rate (nmol $\cdot m^{-2} \cdot s^{-1}$)	
	A. variabilis	*Synechocystis* PCC6714
Basal medium	7.8	4.0
100 NaCl	29.6	8.9
200 NaCl	53.2	16.9
150 sorbitol	27.4	9.9
150 sucrose	30.7	8.9

Samples of *A. variabilis* and *Synechocystis* PCC6714 which were preincubated for 60 min in a medium containing 200 mmol $\cdot dm^{-3}$ NaCl showed no transient increase in cellular K^+ uptake (cf Figs. 2 and 1). On the contrary, a reasonable agreement was observed between untreated and preincubated cells of *A. variabilis* and *Synechocystis* PCC6714 (Fig. 2), although in both cases, preincubation in NaCl-containing medium led to an apparent increase in the initial extracellular component. This is presumably due in part at least to cell shrinkage in response to hyperosmotic treatment.

Effects of external osmolality on internal K^+ concentration

Table II shows data for the intracellular K^+

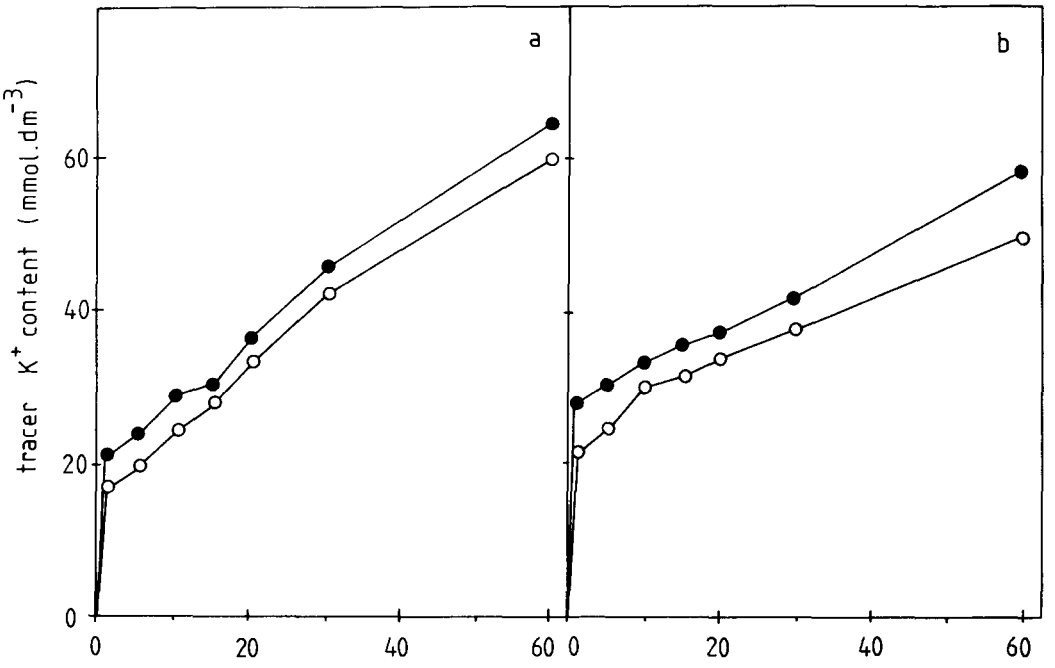

Fig. 2. Effect of preincubation for 60 min in medium containing 200 mmol·dm^{-3} NaCl (●) upon K^+ ($^{42}K^+$) uptake in (a) *A. variabilis* and (b) *Synechocystis* PCC6714. Values (four replicates) are also shown for cells in basal growth medium (containing 5.0 mmol·dm^{-3} KCl) with no added NaCl (○).

concentration of *A. variabilis* and *Synechocystis* PCC6714, obtained using radioisotopic equilibration techniques [6], with appropriate correction for extracellular K^+ (Fig. 1: 60 s values). *A. variabilis* and *Synechocystis* PCC6714 maintained in basal growth medium showed, respectively, a 32-fold and 35-fold accumulation of K^+. Upon treatment with either NaCl, sorbitol or sucrose for 20 min, the internal K^+ levels were found to increase by up to 75 mmol·dm^{-3} in *A. variabilis* and 57 mmol·dm^{-3} in *Synechocystis* PCC6714. This suggests that the increases in K^+ influx shown in Fig. 1 result in a net K^+ accumulation within cells of these cyanobacteria, since there was a quantitative agreement between the enhanced uptake of K^+ noted during the period 0–20 min following transfer to hyperosmotic media as shown in Fig. 1 and the increases in intracellular K^+ concentration noted in Table II.

TABLE II

INTRACELLULAR K^+ CONCENTRATIONS CALCULATED FROM $^{42}K^+$ EQUILIBRATION STUDIES USING *A. VARIABILIS* ATCC29413 AND *SYNECHOCYSTIS* PCC6714

All cells were preequilibrated in $^{42}K^+$-containing basal medium for 24 h. NaCl, sorbitol or sucrose (as appropriate) were added 20 min prior to assay. Values are shown as mean ± S.D. (four replicates).

Addition (mmol·dm^{-3})	Intracellular K^+ (mmol·dm^{-3} (cell volume))	
	A. variabilis	*Synechocystis* PCC6714
Basal medium	162.2 ± 8.3	174.3 ± 1.8
100 NaCl	202.3 ± 3.7	201.1 ± 2.8
200 NaCl	236.9 ± 2.5	231.5 ± 6.8
150 sorbitol	209.8 ± 3.2	208.9 ± 1.1
150 sucrose	197.5 ± 6.9	201.4 ± 6.5

Effects of darkness and the energy-transfer inhibitor DCCD

Fig. 3a shows the effects of dark treatment upon tracer K^+ influx in *A. variabilis*. Darkness reduced the steady-state K^+ influx rate in cells maintained in basal growth medium by up to 60%, as in previous experiments [6]. A similar reduction in K^+ influx was also observed in cells of *Synechocystis* PCC6714 upon transfer to darkness (Fig.

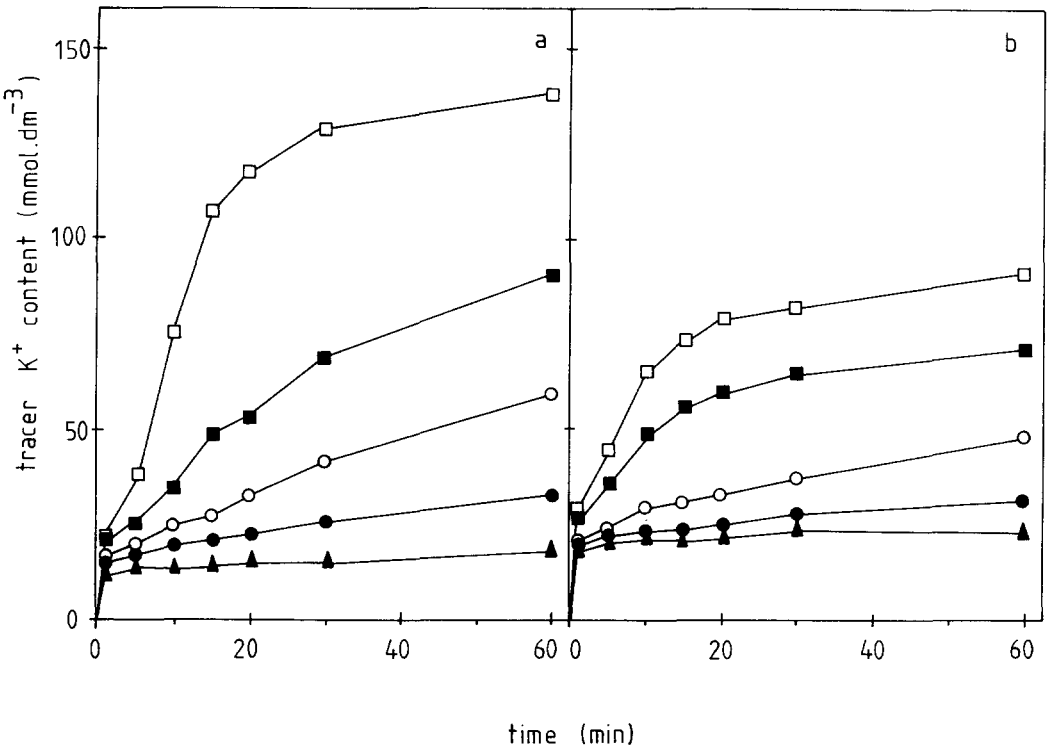

Fig. 3. Effects of light and darkness upon K^+ uptake in (a) *A. variabilis* and (b) *Synechocystis* PCC6714. Values (four replicates) are shown for cells maintained in basal growth medium (containing 5.0 mmol·dm^{-3} KCl) in the light (○) and dark (●) and also for cells transferred to 200 mmol·dm^{-3} NaCl in the light (□) and dark (■). The effects of the energy-transfer inhibitor DCCD (▲) are also shown.

3b). Dark treatment of cells transferred to a medium containing 200 mmol · dm^{-3} NaCl resulted in a reduction in the rate of K^+ uptake when compared to illuminated cells exposed to hyperosmotic treatment. However, dark-treated cells of *A. variabilis* and *Synechocystis* PCC6714 in 200 mmol · dm^{-3} NaCl still showed an enhanced rate of uptake when compared to cells in basal growth medium, although the response is most pronounced in *Synechocystis* PCC6714 (Fig. 3b).

Fig. 3 also shows the effects of DCCD, an energy-transfer inhibitor that blocks ATP generation and consumption by binding specifically to ATPases [18]. DCCD at 100 μmol · dm^{-3} is known to be a potent inhibitor of cyanobacterial K^+ transport and accumulation [6]. Cells of *A. variabilis* and *Synechocystis* PCC6714 pretreated for 10 min in DCCD at 100 μmol · dm^{-3} and then transferred to a medium containing 200 mmol · dm^{-3} NaCl showed little evidence of net cellular uptake of K^+ (Fig. 3), following the initial, rapid phase of uptake into the cell wall matrix.

Discussion

Both *A. variabilis* and *Synechocystis* PCC6714 have been shown to respond in a similar manner to hyperosmotic treatment, with substantial increases in tracer K^+ influx upon addition of either NaCl, sorbitol or sucrose to the bathing medium, suggesting an osmotic rather than an ionic response (Table I). Increasing external osmolality thus results in an increase in internal K^+ concentration (Table II), a feature which is consistent with the observed transient increase in K^+ uptake (Fig. 1). Raising the external osmolality will lead to an equivalent reduction in the cell turgor (hydrostatic) pressure with a tendency for water to leave the cell in response to a decrease in external water potential [19].

The present study shows that K^+ influx in *A. variabilis* and *Synechocystis* PCC6714 behaves as a turgor-sensitive process, increasing dramatically when turgor is reduced. A turgor-sensitive K^+ influx mechanism, leading to an increase in internal K^+ concentration in response to an increase in external osmolality may act to restore the necessary positive turgor for sustained cell expansion growth. On the basis of the data shown in Table II, increases in internal K^+ concentration would appear to be insufficient to restore cell turgor to its original level. However, (1) the data take no account of counterion movement and it is likely that intracellular levels of Cl^- and/or other anions may increase together with K^+, to maintain charge balance. (2) The osmotic volume of these cells is unknown and the data shown in Table II have been calculated in terms of total cellular volume (*Synechocystis* PCC6714) or cellular water content (*A. variabilis*). If the nonosmotic volume of the cells is large as in many other nonvacuolate cells [20], then the effective increase in intracellular K^+ concentration may be considerably greater than is shown in Table II. (3) Cells do not always show full restoration of turgor following a change in external water status [21]. (4) Any penetration of extracellular solutes under hyperosmotic conditions [22,23] will also serve to reduce the osmotic gradient across the plasma membrane, thus reducing the net change in turgor for a given change in external osmolality.

Similar phenomena to those described in the present study have also been reported for walled algal cells. Thus, a large increase in the net salt influx occurs when turgor pressure in the 'giant-celled' algae *Valonia* and *Halicystis* is abolished by inserting a capillary into the cell interior [24], with a 10-fold increase in the rates of K^+ and Cl^- influx under these conditions. Subsequent experiments have clearly shown that the transplasmalemma K^+ fluxes of several algal and bacterial cells are sensitive to hydrostatic pressure, increasing in response to decreased turgor and decreasing when turgor is raised [25–27], although some algal cells show no turgor-sensitive K^+ influx [20,28]. Turgor-sensitive systems have been cited as evidence for osmotic adjustment and turgor regulation using ionic solutes (i.e., restoration of turgor following a change in external water potential) in 'giant-celled' algae [26,28]. The effects of turgor changes on K^+ influx in the cyanobacteria *A. variabilis* and *Synechocystis* PPC6714 can likewise be interpreted in terms of a negative feedback signal involved in turgor homeostasis in an environment of fluctuating osmolality.

Turgor-sensitive increases in K^+ influx in both *A. variabilis* and *Synechocystis* PCC6714 are adversely affected by dark-incubation, in keeping with active ion-transport processes in many other photosynthetic cells [9,24]. Furthermore, the absence of any substantial cell K^+ influx in the presence of 100 $\mu mol \cdot dm^{-3}$ DCCD also supports the concept of primary active, energy-dependent (and turgor-sensitive) K^+ uptake.

The present study suggests that rapid increases in intracellular K^+ concentration will occur as a direct consequence of hyperosmotic treatment, using either inorganic or organic solutes (Table II). Previous studies of osmotic adjustment in *A. variabilis* and *Synechocystis* PCC6714 have shown that the intracellular low molecular weight carbohydrates sucrose [13] and glucosylglycerol [13], respectively, are increased in response to increased external osmolality. The latter studies were carried out using cells maintained in media of high osmotic strength for at elast 48 h and short-term changes in ion levels were not considered. However, we have also shown previously that cells of the euryhaline unicell *Synechocystis* PCC6803 (a glucosylglycerol-accumulating strain with similar osmotic responses to *Synechocystis* PC 6714) grown in freshwater-based and seawater-based media contain similar amounts of both Na^+ and K^+ (at 35 and 150 $mmol \cdot dm^{-3}$, respectively [29]).

These data suggest that short-term recovery of turgor in cyanobacteria may involve active K^+ uptake, while longer-term acclimation and osmotic adjustment is achieved by varying the internal low molecular weight carbohydrate pool.

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