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Pleiotropic effects of potassium deficiency in a heterocystous, nitrogen-fixing cyanobacterium, *Anabaena torulosa*

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Omission of potassium from the growth medium caused multiple metabolic impairments and resulted in cessation of growth of the filamentous, heterocystous, nitrogen-fixing cyanobacterium *Anabaena torulosa*, during both diazotrophic and nitrogen-supplemented growth. Prominent defects observed during potassium deprivation were: (i) the loss of photosynthetic pigments, (ii) impairment of photosynthetic functions, (iii) reduced synthesis of dinitrogenase reductase (Fe-protein), (iv) inhibition of nitrogenase activity, and (v) specific qualitative modifications of protein synthesis leading to the repression of twelve polypeptides and synthesis and accumulation of nine novel polypeptides. The observed metabolic defects were reversible, and growth arrested under prolonged potassium deficiency was fully restored upon re-addition of potassium. Such pleiotropic effects of potassium deficiency demonstrate that apart from its well-known requirement for pH and turgor homeostasis, K^+ plays other vital specific roles in cyanobacterial growth and metabolism.

Keywords: potassium deficiency, cyanobacteria, nitrogen fixation, photosynthesis, *Anabaena torulosa*

INTRODUCTION

In bacteria, potassium (K^+) is a major cellular cation, with concentrations ranging from 0·2 to 0·6 M (Epstein & Schultz, 1965). Such high intracellular levels of K^+ are maintained by the activities of multiple uptake systems (Harold & Kakinuma, 1986; Altendorf & Epstein, 1993; Schrempf *et al.*, 1995; Stumpe *et al.*, 1996). K^+ plays a major role in the maintenance of intracellular pH (Booth, 1985) and cell turgor (Epstein, 1986; Whatmore & Reed, 1990; Csonka & Hanson, 1991) in bacteria. K^+ deficiency has been found to decrease protein synthesis (Harold & Baarda, 1968) and to influence the activity of certain cellular enzymes (Suelter, 1970). More specifically, intracellular K^+ has been found to positively regulate the expression of certain osmo-responsive genes, such as *proU* (Prince & Villarejo, 1990), *otsA* (Giaever *et al.*, 1988) and others in enteric bacteria. Recently K^+ has also been implicated in osmoregulatory functions (Meury & Kohiyama, 1993) and in regulation of heat-shock response (Palleros *et al.*, 1993) in bacteria.

For the last several years our laboratory has been exploring the stress and adaptive responses of photosynthetic nitrogen-fixing *Anabaena* strains to a variety of environmental conditions ranging from nutrient deficiency (Apte & Thomas, 1983, 1984) to salinity, osmotic and heat stresses (Apte *et al.*, 1987; Apte & Bhagwat, 1989; Bhagwat & Apte, 1989; Fernandes *et al.*, 1993; Iyer *et al.*, 1994). K^+ plays a vital role in stressful environments, both as an extracellular signal and as an intracellular metabolic regulator. Information on the effects of this cation on cyanobacterial growth and metabolism would, therefore, be of value for a better understanding of the molecular basis of stress tolerances and for agricultural applications of these microbes as biofertilisers in stressful environments.

A requirement of K^+ has been known for cyanobacteria since the 1940s (see Wolk, 1973). All cyanobacterial culture media include varying amounts of K^+, ranging from 0·1 mM in ASM to 5·74 mM in Kratz and Myers medium (Castenholz, 1988). The mechanism of K^+ transport (Reed *et al.*, 1981), induction of K^+ transport by turgor perturbations (Reed & Stewart, 1985) and accumulation of K^+ during osmotic adjustments (Miller *et al.*, 1976; Reed *et al.*, 1984, 1985) have been described in cyanobacteria. But apart from these studies, there have to our knowledge been no investigations into the nature of the cyanobacterial requirement for K^+. The present work was undertaken to investigate the role played by K^+ in the cellular physiology of a photo-

synthetic, nitrogen-fixing *Anabaena* strain. The results show that, in addition to a possible turgor loss, K^+ deprivation causes multiple metabolic impairments, during both nitrogen-supplemented and diazotrophic growth.

METHODS

Organism and growth conditions. A filamentous, heterocystous, nitrogen-fixing, brackish-water cyanobacterial strain, *Anabaena torulosa* (Apte & Thomas, 1980), was used in axenic condition. Stock cultures were maintained in the standard BG-11 medium without combined nitrogen (Castenholz, 1988). The medium was modified to obtain either BG-11/K0 (in which K_2HPO_4 was replaced by equimolar Na_2HPO_4) or BG-11/K5 (BG-11/K0 containing 5 mM KCl) media. When required, 3 mM NH_4Cl and 5 mM MOPS were added to either BG-11/K0 or BG-11/K5 media for combined nitrogen supplementation. The initial pH of all media was adjusted to 7·0.

K^+ starvation was achieved by harvesting 3-d-old BG-11/K5-grown cells (by centrifugation at 5000 *g* for 5 min) followed by washing three times, each with 5 vols BG-11/K0 medium. Washed cells were inoculated in BG-11/K0 and BG-11/K5 media at a density of 0·6–1·0 μg chlorophyll *a* ml^{-1}. All cultures were grown photoautotrophically under continuous illumination (2·5 mW cm^{-2}) from white fluorescent tubes and under aeration (2 l min^{-1}) at 25±2 °C.

Measurement of growth and nitrogenase activity. Growth was usually measured in terms of chlorophyll *a* content as described by Mackinney (1941), or occasionally as turbidity (OD_{750}) or total protein content (determined by the Lowry method). Nitrogenase activity was estimated in 2 ml culture aliquots by the standard acetylene reduction assay, as described earlier (Apte *et al.*, 1987). Standard ethylene (Applied Science Laboratories) was used for determination of ethylene in the samples.

Estimation of photosynthetic pigments, photoevolution of oxygen and photofixation of carbon. Relative levels of photosynthetic pigments were estimated from the absorption spectra of dilute suspensions of whole filaments in the visible range (400–750 nm). The spectra were recorded in a Hitachi 150-20 UV/visible double-beam spectrophotometer, where the turbidity of cultures could be adjusted to zero to obtain spectra from comparable cell densities. A_{680}, A_{620} and A_{495} were taken as *in vivo* measures of chlorophyll *a*, phycocyanin and carotenoids, respectively, as described earlier (Apte & Thomas, 1983).

For measurements of photosynthesis, 1·5 ml culture aliquots (5 μg chlorophyll *a* ml^{-1}) were transferred to an oxygraph chamber (Gilson) maintained with stirring at 25 °C. Samples were illuminated with monochromatic light (650 nm, 500 W cm^{-2}), passing through a heat filter of 1 M $CuSO_4$ solution, to record the O_2 evolved. The oxygraph was calibrated by a standard reaction with glucose/glucose oxidase and catalase. Light-dependent uptake of bicarbonate was measured with 1 ml culture aliquots (5 μg chlorophyll *a* ml^{-1}) incubated under white light (2·5 mW cm^{-2}) at 25 °C with 1 μCi (37 kBq ml^{-1}) $NaH^{14}CO_3$ (specific activity 51 mCi $mmol^{-1}$, 1·89 GBq $mmol^{-1}$) with shaking (150 r.p.m.) for 15 min, as described earlier (Apte & Thomas, 1983).

Determination of protein synthesis rates. One-millilitre culture aliquots were pulse-labelled with [^{35}S]methionine (specific activity >30 TBq $mmol^{-1}$) added at 1·5 MBq ml^{-1}, for 5 min. Radiolabelling was terminated by transferring cells to ice followed by rapid centrifugation (1 min) in a microcentrifuge. Cells were washed three times with BG-11 medium and once with sterilized distilled water. Solubilization and extraction of proteins, and estimation of the radioactivity incorporated into proteins, have been described earlier (Apte & Bhagwat, 1989).

Protein electrophoresis, Western blotting and immunodetection. Preparation of protein samples and electrophoresis were carried out as described earlier (Apte & Bhagwat, 1989). Cellular proteins were resolved by electrophoresis on denaturing gradient (5–14%, w/v, acrylamide) SDS-polyacrylamide slab gels. Gels were fixed and stained with Coomassie brilliant blue G-250. Alternatively, proteins were resolved by SDS-PAGE on 10% (w/v) gels and transferred to positively charged nylon membranes (Boehringer Mannheim) by electroblotting at 0·2 A for 16–18 h in a transfer buffer containing 125 mM Tris/HCl pH 8·6 and 192 mM glycine. The blot was washed with buffer 1 [50 mM Tris/HCl pH 7·4, 150 mM NaCl and 1% (w/v) blocking reagent (casein acid hydrolysate]. It was then incubated with 1:3000 dilution of an anti-dinitrogenase reductase (Fe-protein) antibody in buffer 1 at 4 °C for 16–18 h. The antiserum consisted of rabbit IgG raised against a composite mixture of purified Fe-proteins from *Rhodospirillum rubrum*, *Azotobacter vinelandii* and *Rhizobium japonicum*. Immunodetection of the anti-Fe-protein antibody was carried out using anti-rabbit IgG conjugated to alkaline phosphatase followed by colour development with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (X-phos) (all obtained from Boehringer Mannheim), according to the manufacturer's recipe.

RESULTS

In initial experiments the standard BG-11 growth medium, which contains 0·175 mM K^+, was found to be limited in the availability of this cation. Addition of up

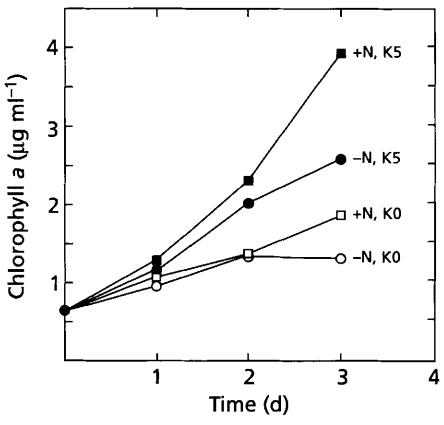

Fig. 1. Effect of K^+ on the growth of *A. torulosa* in media free of combined nitrogen (−N, circles) or supplemented with NH_4Cl (+N, squares). In both conditions, filaments were grown either in the absence (K0, open symbols) or presence (K5, filled symbols) of 5 mM KCl. Growth was measured as the content of chlorophyll *a*. Each value is the mean of three replicates; variation from the mean was less than 10%. The results are representative of three similar independent experiments.

Table 1. Pleiotropic effects of K^+ deficiency in *A. torulosa*

All determinations (except heterocyst frequency) were carried out 3 d after inoculation in the respective medium. Growth was calculated as increase in chlorophyll *a* density over that present at the time of inoculation. Numbers in parentheses represent values for K^+-deficient cultures expressed as a percentage of the respective K^+-supplemented controls.

	Growth medium:			
	Plus N		Minus N	
	BG-11/K5	BG-11/K0	BG-11/K5	BG-11/K0
Growth [μg chlorophyll *a* (ml culture)$^{-1}$]	3·29	1·23 (37)	1·93	0·65 (34)
Heterocyst frequency (% of total cells)*	–	–	8·80	8·45
Nitrogenase activity [μmol C_2H_2 reduced (mg protein)$^{-1}$ min^{-1}]	–	–	10·1	1·60 (16)
Photosynthetic pigments†				
Chlorophyll *a* (A_{680})	0·160	0·054 (34)	0·235	0·075 (32)
Phycocyanin (A_{620})	0·135	0·044 (33)	0·190	0·060 (32)
Carotenoids (A_{495})	0·140	0·060 (43)	0·210	0·149 (71)
Photosynthetic functions				
Photoevolution of O_2 [μmol (mg protein)$^{-1}$ min^{-1}]	350	193 (55)	750	263 (35)
Photofixation of CO_2 [10^{-7} × c.p.m. $NaH^{14}CO_3$ (mg protein)$^{-1}$ h^{-1}]	9·50	1·61 (17)	7·40	2·44 (33)
Protein synthesis [10^{-5} × c.p.m. [^{35}S]methionine (μg protein)$^{-1}$ min^{-1}]	ND	ND	1·20	0·78 (65)

ND, Not determined.

* Heterocyst frequency was determined from >2000 cells counted under the microscope 30 h after inoculation.

† Values estimated from absorption spectra of very dilute suspensions of whole filaments.

to 5 mM K^+ (as KCl) to BG-11 enhanced growth (data not shown). Therefore, in all experiments BG-11/K5 served as the K^+-supplemented control medium. The residual K^+ contamination in BG-11/K0 was found to be less than 10 μM, by atomic absorption spectrometry.

Effect of K^+ on growth

Growth was retarded during K^+ limitation under both nitrogen-deficient and nitrogen-supplemented conditions (Fig. 1). The extent of inhibition caused by K^+ starvation in nitrogen-deficient (34%) and nitrogen-supplemented (37%) media was comparable (Table 1). The requirement of K^+ during nitrogen-fixing (diazotrophic) growth was investigated in detail.

Prolonged starvation of K^+ did not affect the viability of nitrogen-fixing *A. torulosa* filaments (Fig. 2). Microscopic examination of BG11/K0 cultures did not reveal any cell lysis. Addition of 5 mM KCl to BG-11/K0 cultures, after 2, 4 or 6 d of K^+ starvation, rapidly revived growth and fully restored it to the level of BG11/K5 cultures. The revival rate was dependent on the extent of previous starvation, i.e. the longer the

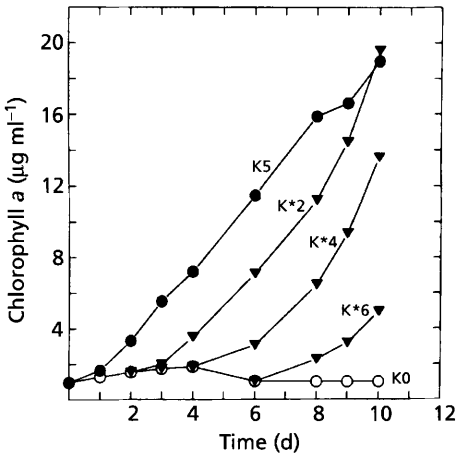

Fig. 2. Revival of growth of K^+-starved *A. torulosa* filaments upon re-addition of K^+. KCl (5 mM) was added (▼) to combined nitrogen-free BG-11/K0 cells (○) after 2 (K*2), 4 (K*4) and 6 (K*6) d of K^+ starvation. Growth of cells in combined nitrogen-free BG-11/K5 (●) is included for comparison. The values are means of three replicates; variation from the mean was less than 10%. The results are representative of three similar independent experiments.

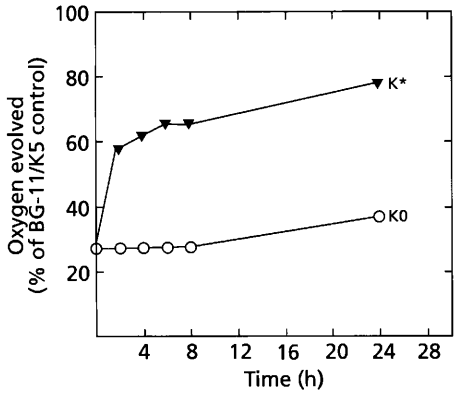

Fig. 3. Effect of K^+ on the photoevolution of oxygen in nitrogen-fixing *A. torulosa* filaments. Oxygen evolved by the BG-11/K0 cells (K0, ○) was compared with that evolved by the BG-11/K0 cells to which 5 mM KCl was added at 0 h (K*, ▼) and expressd as a percentage of the value for BG-11/K5 filaments. Values corresponding to 100% (BG-11/K5 control) were 945, 941, 941, 920, 889 and 750 nmol O_2 (mg protein)$^{-1}$ min^{-1} at 0, 2, 4, 6, 8 and 24 h, respectively. The values represent means of two replicates and the variation from the mean was less than 10%. The results are representative of two similar independent experiments.

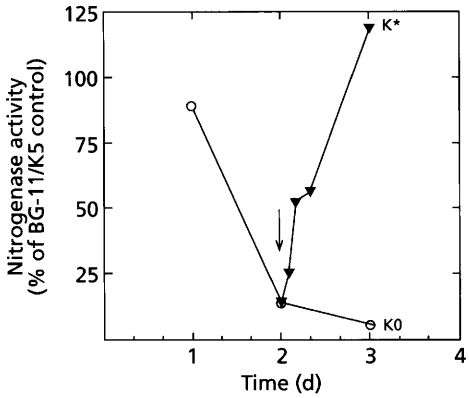

Fig. 4. Effect of K^+ on nitrogenase activity in *A. torulosa*. Nitrogenase activity of BG-11/K0 cells (K0, ○) was compared with that of BG-11/K0 cells to which 5 mM KCl was added at the time indicated by the arrow (K*, ▼) and expressed as a percentage of the value for BG-11/K5 filaments. Values corresponding to 100% (BG-11/K5 control) on days 1, 2 and 3 were 8·7, 11·3 and 9·5 μmol ethylene (mg chlorophyll *a*)$^{-1}$ h^{-1}, respectively. The 100% (BG-11/K5 control) values corresponding to the activity of the K* culture at 2, 4 and 8 h after addition of K^+ (shown by the arrow) were 11·4. 11·8 and 10·8 μmol (mg chlorophyll *a*)$^{-1}$ h^{-1}, respectively. Values are means of three replicates and the variation from the mean was less than 10%. The results are representative of four independent experiments.

starvation, the slower the revival. Results obtained using protein content or turbidity as measures of growth were very similar to those in Figs 1 and 2 (data not shown).

Effects of K^+ on morphology and differentiation

Cells of *A. torulosa* were distinctly smaller under K^+ deficiency, although the length of the filaments, and the number, frequency and morphology of the heterocysts, were unaffected (Table 1). In both the K^+-deficient and the K^+-supplemented filaments, heterocyst differentiation reached a peak around 30 h (8–9% heterocysts as compared to 4% at inoculation) (Table 1). The heterocysts appeared normal and, like those formed under K^+-sufficient conditions, they lacked the red fluorescence of phycobiliproteins typical of the vegetative cells. The inhibition of diazotrophic growth by K^+ starvation was therefore apparently not due to any defects in the development of or the quality of the heterocysts.

Effects of K^+ on photosynthesis

K^+ deficiency lowered the content of all major photosynthetic pigments, viz. chlorophyll *a*, phycocyanin and carotenoids, and inhibited the photoevolution of oxygen as well as the photofixation of carbon, both in the presence and in the absence of combined nitrogen in the growth medium (Table 1). In nitrogen-fixing cultures, the photoevolution of oxygen partially recovered (to about 65% of the controls) within 2–4 h upon addition of 5 mM KCl to K^+-deficient cells (Fig. 3). However, subsequent recovery of photosynthetic oxygen evolution and carbon fixation was slow (75–80% in 24 h; Fig. 3); complete (100%) recovery occurred only after 3–4 d (data not shown).

Effects of K^+ on nitrogen fixation

Nitrogenase activity in *A. torulosa* was severely inhibited by K^+ deficiency (Fig. 4). Two days of K^+ starvation reduced the activity to only 15–20% of that in K^+-sufficient cells and by day 3 the activity was negligible. Addition of K^+ to BG-11/K0 cultures, at the time indicated by the arrow in Fig. 4, restored nitrogenase activity in a time-dependent manner: 60% recovery within 8 h and >100% recovery within 24 h (Fig. 4). A similar experiment conducted 3 d after initiation of K^+ starvation also showed revival of nitrogenase activity, albeit at a slower rate (data not shown).

Western blotting of protein extracts and immunodetection of dinitrogenase reductase (Fe-protein of nitrogenase complex) identified a single 33 kDa polypeptide whose levels were considerably reduced after 48–72 h of K^+ starvation (Fig. 5). Addition of 5 mM KCl caused no significant increase in the Fe-protein content in 8 h (Fig. 5, lane 4), although within this time the nitrogenase activity recovered to 60% of the control (Fig. 4). This can be ascribed to a comparable recovery of photosynthesis (Fig. 3) and consequent increased availability of reductant, ATP and carbon skeletons. On the other hand, 24 h after addition of K^+ the level of Fe-protein (Fig. 5, lane 7) far exceeded the observed nitrogenase activity (Fig. 4). This may be due to limitations on the availability of reductant and ATP for

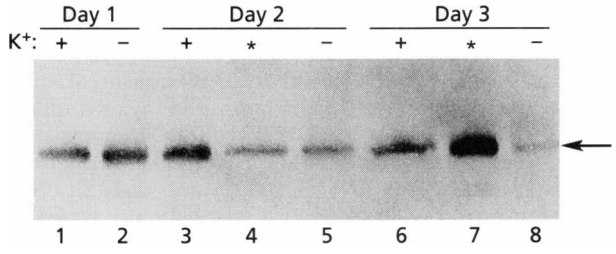

Fig. 5. Effect of K^+ on the content of dinitrogenase reductase (Fe-protein of nitrogenase complex) in *A. torulosa*. Solubilized protein extracts containing 150 µg protein were resolved by SDS-PAGE on 10% (w/v) gels and electroblotted onto nylon membranes. The 33 kDa polypeptide (arrowed) was immunodetected using a primary anti-dinitrogenase reductase (rabbit IgG) antibody followed by a secondary anti-rabbit IgG coupled to alkaline phosphatase. The + and − respectively denote the presence (BG-11/K5) or the absence (BG-11/K0) of 5 mM KCl in the growth medium; the * indicates addition of 5 mM KCl to BG-11/K0-grown cells on day 2. Samples in lanes 4 and 7 were taken 8 and 24 h, respectively, after addition of K^+.

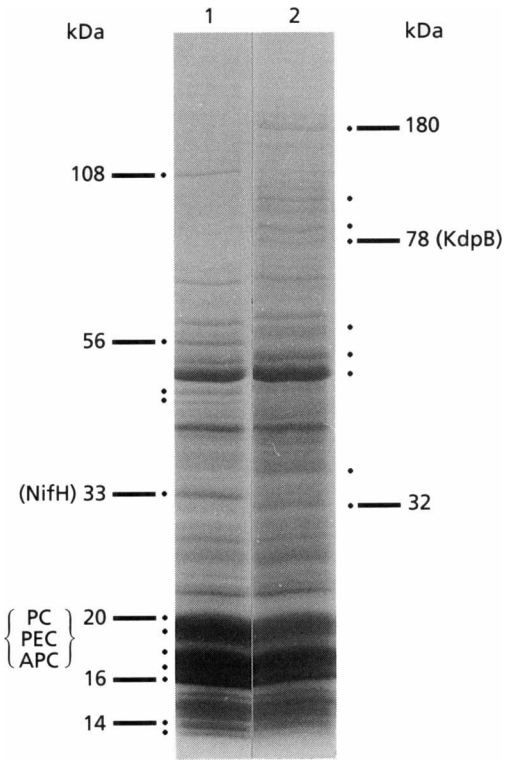

Fig. 6. Modification of protein synthesis in *A. torulosa* during K^+ starvation. Protein samples (100 µg), from cells grown in BG-11/K5 (lane 1) or BG-11/K0 (lane 2) for 3 d were resolved by SDS-PAGE on a 5–14% (w/v) gradient gel and visualized by Coomassie staining. Prominent polypeptides whose levels were either decreased (13·5, 14·0, 16·0, 17·0, 18·0, 19·5, 20·0, 33·0, 48·0, 50·0, 56·0 and 108·0 kDa species) or enhanced (32·0, 36·0, 52·0, 54·0, 58·0, 78·0, 82·0, 90·0 and 180·0 kDa species) by K^+ starvation are marked with dots on the left and the right, respectively. The numbers indicate the molecular masses determined using standard proteins (SDS-6H and SDS-7 kits from Sigma) that were co-electrophoresed on the gel. Some of the identified proteins are given in parentheses. PC, phycocyanin; PEC, phycoerythrocyanin; APC, allophycocyanin.

nitrogen fixation, since such cultures exhibited only partial recovery of photosynthesis (Fig. 3).

Effects of K^+ on protein synthesis

K^+ starvation decreased the efficiency of cyanobacterial protein synthesis. Under nitrogen-fixing conditions, the rate of [^{35}S]methionine incorporation into proteins was reduced by 35% after 3 d of K^+ starvation (Table 1). After 5 and 7 d of K^+ starvation, the rate of protein synthesis decreased by 54% and 68% respectively (data not shown). In addition, the proteins synthesized under K^+ deprivation appeared to be markedly different from those synthesized by K^+-supplemented cells (Fig. 6). Synthesis of 12 polypeptides was adversely affected and their levels declined after 3 d of K^+ starvation. One of the repressed proteins (33 kDa) was identified as dinitrogenase reductase (NifH) (Fig. 5). The five most abundant *Anabaena* polypeptides (16–20 kDa) (Fig. 6) appeared as bluish-grey bands on gels even without staining. These have earlier been shown to belong to the α/β-subunits of phycocyanin, allophycocyanin and phycoerythocyanin, which together constitute the phycobilisomes of *Anabaena* (Wood & Haselkorn, 1980). The levels of these polypeptides decreased under K^+ starvation (Fig. 6), in agreement with the loss of phycocyanin and photosynthetic functions shown in Table 1. The synthesis of at least nine polypeptides was selectively enhanced and these accumulated in K^+-starved filaments. A 78 kDa polypeptide (earlier reported as 90 kDa, due to a miscalculation) was previously shown to cross-react with an antibody against the KdpB protein of the K^+-dependent ATPase of *Escherichia coli* (Apte & Alahari, 1994). The identity of the other eight polypeptides is unknown.

DISCUSSION

Based on information mostly obtained from heterotrophs, the major role(s) assigned to K^+ in bacteria is the maintenance of cellular turgor and intracellular pH. Much less is known about the role of K^+ in the physiology and metabolism of photoautotrophic and/or diazotrophic bacteria, which have life-styles strikingly different from those of heterotrophs. In particular, the role(s) of K^+ in the vital processes of photosynthesis and nitrogen fixation, if any, have not been adequately investigated. In the photosynthetic nitrogen-fixing cyanobacterium *Anabaena torulosa*, K^+ deficiency caused pleiotropic effects (Table 1).

As in other bacteria, K^+ appears to have some broad-based general effects on the cellular physiology of *Anabaena* strains. Involvement of K^+ in maintenance of pH and membrane potential was reported earlier in *Anabaena* strains (Reed *et al.*, 1981). A possible loss of turgor during K^+ starvation is suggested by the significantly decreased cell size of *A. torulosa* filaments under these conditions. Further functions of K^+ suggested by the present study include its involvement in cyanobacterial photosynthesis, nitrogen fixation and particularly in regulation of protein synthesis. Whether

these effects on the major metabolic activities of *Anabaena* are related to or are independent of the involvement of K^+ in turgor maintenance is not clear at present.

The cyanobacterial requirement of K^+ appeared to be distinctly different from that for Na^+, reported earlier in *Anabaena* strains. While Na^+ was needed in trace amounts (25 μM) specifically during diazotrophic growth (Apte & Thomas, 1980), K^+ was required as a macronutrient (5 mM) during both nitrogen-fixing and nitrogen-supplemented growth (Fig. 1). Na^+ deprivation did not influence the synthesis of nitrogenase proteins (Apte & Thomas, 1984, 1985), while K^+ deprivation decreased the synthesis of dinitrogenase reductase (Fig. 5). Impairments of photosynthesis caused by Na^+ deficiency were indirect effects of nitrogen starvation since they could be reversed by addition of nitrogenous compounds (Apte & Thomas, 1983). In contrast, K^+ deprivation adversely affected photosynthesis, irrespective of the availability/unavailability of combined nitrogen during growth (Table 1). The primary lesions caused by Na^+ deficiency were reduced transport of inorganic phosphate (Apte & Thomas, 1985) and bicarbonate (Maeso *et al.*, 1987). The observed multiple effects of K^+ deficiency make it somewhat difficult to identify the primary target(s) of K^+ deprivation in *A. torulosa*.

All the defects caused by K^+ starvation were fully reversed upon supplementation of K^+, demonstrating that they derived from K^+ deficiency. Absence of cell lysis in K^+-deficient cultures and the rapid and near-complete revival of K^+-replete cells suggested that K^+ deprivation was not lethal. Reversal of all physiological/biochemical activities and growth occurred even after prolonged K^+ starvation (Fig. 2), indicating that the effects of K^+ deprivation were bacteriostatic rather than bactericidal. After readdition of K^+, nitrogen fixation recovered concomitantly with photosynthesis and was fully restored within 24 h. Complete recovery of photosynthesis was rather slow, probably because it involved biosynthesis of nitrogenous photosynthetic pigments (chlorophyll *a*, phycocyanin), which in turn depended on the availability of fixed nitrogen.

K^+ deprivation caused profound alterations, both qualitative and quantitative, in the nature of proteins synthesized. Prominent among the repressed proteins were the dinitrogenase reductase (NifH or Fe-protein) (Fig. 5) and major phycobiliproteins (Fig. 6). The functional significance of other modifications in protein synthesis, especially those induced by K^+ deprivation (Fig. 6), is not clear at present. One of the induced proteins (78 kDa) appears to belong to a K^+-dependent ATPase. In *E. coli* such a Kdp-ATPase facilitates rapid accumulation of K^+ aimed at osmotic/turgor adjustments (Epstein, 1992). It seems likely, therefore, that some of the proteins induced by K^+ deficiency may be involved in procurement of K^+ and subsequent adaptation of *A. torulosa* to K^+ starvation.

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