A novel potassium deficiency-induced stimulon in *Anabaena torulosa*

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Potassium deficiency enhanced the synthesis of fifteen proteins in the nitrogen-fixing cyanobacterium *Anabaena torulosa* and of nine proteins in *Escherichia coli*. These were termed potassium deficiency-induced proteins or PDPs and constitute hitherto unknown potassium deficiency–induced stimulons. Potassium deficiency also enhanced the synthesis of certain osmotic stress-induced proteins. Addition of K^+ repressed the synthesis of a majority of the osmotic stress-induced proteins and of PDPs in these bacteria. These proteins contrast with the dinitrogenase reductase of *A. torulosa* and the glycine betaine-binding protein of *E. coli*, both of which were osmo-induced to a higher level in potassium-supplemented conditions. The data demonstrate the occurrence of novel potassium deficiency-induced stimulons and a wider role of K^+ in regulation of gene expression and stress responses in bacteria.

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1. Introduction

Potassium (K^+) is an essential macronutrient for the growth of most organisms. In bacteria, potassium plays an important role in the maintenance of intracellular pH (Booth 1985) and cell turgor. Recovery of cell turgor after an osmotic upshock has been shown to be K^+-dependent in both, the Gram positive (Whatmore *et al* 1990) and the Gram negative (Laimins *et al* 1981; Epstein 1986; Dinnbier *et al* 1988) bacteria as well as in cyanobacteria (Reed and Stewart 1985). Osmotic adjustments during such a recovery involve an initial phase of rapid K^+ uptake followed by accumulation of organic osmolytes to increase the internal osmoticum (Dinnbier *et al* 1988; Higgins *et al* 1987; Csonka and Hanson 1991). K^+ is required for osmo-induction of genes involved in osmo-protectant accumulation, such as the *otsA*, encoding osmo-regulated trehalose synthetase (Giaver *et al* 1988) and the *proU* operon, encoding an uptake system for glycine betaine and proline (Sutherland 1986; Rajkumari *et al* 1996). Potassium glutamate has been implicated in the transcriptional induction of the osmo-induced *proU* operon (Ramirez *et al* 1989; Prince and Villarejo 1990). Activity of certain cellular enzymes (Suelter 1970) and heat shock proteins, such as Hsp70 (Palleros *et al* 1993; Feifel *et al* 1996), and Hsp60 (Viitanen *et al* 1990) also require K^+.

The role of K^+ in general protein synthesis has been investigated. Formation of pre-ribosomal particles was detected in K^+-depleted cells (Ennis and Lubin 1965; Harold and Baarda 1968). Protein synthesis was impaired in a mutant of *Bacillus subtilis* that was unable to retain intracellular K^+ (Willis and Ennis 1968). More specifically, the peptidyl transferase reaction during protein synthesis was found to be K^+-dependent (Monro 1967; Maden and Monro 1968). These data were recently corroborated by the analysis of crystal structure of 50S subunit of ribosome that revealed the presence of K^+ ion interacting with guanine residues of 23S rRNA (Nissen *et al* 2000).

In the photosynthetic, nitrogen-fixing cyanobacterium *Anabaena torulosa*, K^+ starvation caused impairments in photosynthesis, nitrogen fixation, protein synthesis and consequently inhibited growth (Alahari and Apte 1998). Intracellular levels of certain proteins increased during

Keywords. *Anabaena*; *Escherichia coli*; potassium regulation; stress proteins

Abbreviations used: GBBP, Glycine betaine-binding protein; ISPs, ionic stress-induced proteins; KdpATPase, K^+-dependent ATPase; OSPs, osmotic stress-induced proteins: PDI, potassium deficiency-induced; PDPs, potassium deficiency-induced proteins.

early stages of K^+ starvation. This was surprising since the only known K^+ deficiency-induced bacterial genes/proteins are the three subunits (KdpA, B and C) of the K^+-dependent ATPase (KdpATPase), product of the osmo-responsive *kdp* operon in *Escherichia. coli* (Epstein and Davis 1970; Laimins *et al* 1981) and several other bacteria.

In this paper, we report on the modification of protein synthesis patterns in *A. torulosa* and in *E. coli* subjected to K^+ deficiency and hyperosmotic stress. *In vivo* radiolabelling of proteins was used to monitor immediate alteration in gene expression under different growth conditions. The results revealed the occurrence of hitherto unknown potassium deficiency-induced stimulons in both these bacteria. The data also elucidate a wider role for K^+ in regulation of stress responses in these bacteria.

2. Materials and methods

2.1 *Cultures and growth conditions*

A. torulosa culture from the laboratory collection was grown and maintained in combined nitrogen-free BG-11 medium (Castenholz 1988) under continuous aeration ($2\ l\ min^{-1}$) and illumination ($2{\cdot}5\ mW\ cm^{-1}$) from white fluorescent lamps at 25° ± 2°C. The medium was modified to obtain either (i) BG-11/K0 medium wherein K_2HPO_4 (175 μM) was replaced by equimolar Na_2HPO_4 or (ii) BG-11/K5 medium which is BG-11/K0 containing 5 mM KCl. These media will be referred to as K0 and K5 respectively in this paper. For K^+ starvation, three-day old K5-grown cells were harvested by centrifugation (5000 *g* for 5 min), washed three times with three volumes of K0 each and inoculated in K0. For detection of osmotic stress-induced proteins, two-day old cultures from K5 or K0 media were concentrated to a density of approximately $10\ \mu g\ ml^{-1}$ chlorophyll *a* and incubated overnight with aeration in their respective media. These were subjected to osmotic (0·2 M sucrose) or salt (0·1 M NaCl) upshock for 30 min before *in vivo* radiolabelling of proteins. Nitrogenase activity was measured by the acetylene reduction assay as described earlier (Apte *et al* 1987). For detection of the dinitrogenase reductase under osmotic stress, nitrogen-fixing cultures were inoculated in K5 or K0 media containing 0·2 M sucrose for three days and then harvested by centrifugation.

E. coli strain MC4100 (Δ*arg-lac, U169 rpsL150 relA1 araD139 flbB301 deoC1 ptsF25*; M Casadaban, University of Illinois, Chicago, USA) was grown in the K115 medium (Laimins *et al* 1981) in an incubator shaker at 37°C and 150 rpm. The K115 medium was modified to obtain K0 medium by replacing K_2HPO_4 and KH_2PO_4 with equimolar concentrations of the corresponding sodium salts. For K^+ starvation, K115 preculture was diluted 1 : 1000 in K0 and incubated overnight. This was diluted 1 : 50 in fresh K0 medium and incubated on shaker for 6 h before subjecting to osmotic stress and *in vivo* radiolabelling of proteins. Hyperosmotic or hypersaline stresses were applied as 0·4 M sucrose or 0·2 M NaCl respectively, for 30 min or for 2 h as indicated. For detection of the *proX* product under osmotic stress, K115 or K0 overnight cultures were inoculated in corresponding media or the same media supplemented with 0·4 M NaCl and incubated for 4 h.

2.2 *Protein extraction, radiolabelling, electrophoresis and immunodetection*

In vivo radiollabeling with [^{35}S]methionine, preparation of whole filament protein extracts and their resolution by SDS-PAGE were carried out as described earlier for *Anabaena* cultures (Apte and Bhagwat 1989). Briefly, 50 μCi ml^{-1} of [^{35}S]methionine (specific activity 3000Ci $mmol^{-1}$) was added and labelling was terminated after 5 min by rapid centrifugation and washing of cells with respective media. Cell lysates were obtained by resuspending cell pellets in Laemmeli's buffer (Laemmeli 1970) and boiling for 5 min. For *E. coli*, [^{35}S]methionine was added at 10 μCi ml^{-1} and radiolabelling was terminated after 1 min followed by processing of the cell pellets as above. Cyanobacterial protein extracts were resolved on 5–14% gradient SDS-polyacrylamide gels, while 10% SDS-PAGE was used for *E. coli* extracts. For immunodetection of the dinitrogenase reductase (NifH) or the glycine betaine-binding protein (GBBP), protein extracts (150 μg total protein) were resolved by 10% SDS-PAGE and electroblotted on nylon membranes as described earlier (Alahari *et al* 2001). Corresponding specific antisera were used at 1 : 2000 dilution and cross-reacting bands were detected using 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium chloride (Roche Diagnostics, Germany) as recommended by the manufacturer.

3. Results

3.1 *Potassium deficiency-induced proteins in* A. torulosa

As reported earlier (Alahari and Apte 1998), potassium deficiency had adverse effects on growth and metabolism of *A. torulosa*. Changes in gene expression due to K^+ deficiency were examined in terms of protein synthesis patterns. *In vivo* radiolabelling of proteins with [^{35}S]methionine followed by resolution of the protein extracts by 1-D SDS-PAGE and autoradiography was adequate to discern K^+-regulated gene expression during K^+ starvation and/or osmotic stress. Figure 1 shows *in vivo* radiolabelled proteins of *A. torulosa* cells from K0 medium compared to

those from K5 medium on successive days post-inoculation. Alteration of protein synthesis ensued within 24 h. Qualitatively and quantitatively, the most elaborate changes were apparent on day 2 of starvation (lanes 3 and 4). Synthesis of eight proteins was found to be repressed and that of fifteen proteins significantly enhanced in the K^+-deficient cultures. A few other proteins were also induced to varying levels on days 1 and 3. While most of the qualitative alterations persisted, the magnitude of expression of potassium deficiency-induced proteins (PDPs) decreased by day 3 (lane 6) probably as a consequence of general carbon and nitrogen starvation and cessation of metabolic functions as reported earlier (Alahari and Apte 1998). Such extensive modification of protein synthesis by K^+ deficiency has not been reported so far in bacteria.

3.2 *Osmotic stress-induced proteins under potassium deficiency in* A. torulosa

K^+ is known to be essential for the expression of several osmoresponsive genes in heterotrophic bacteria. Require-

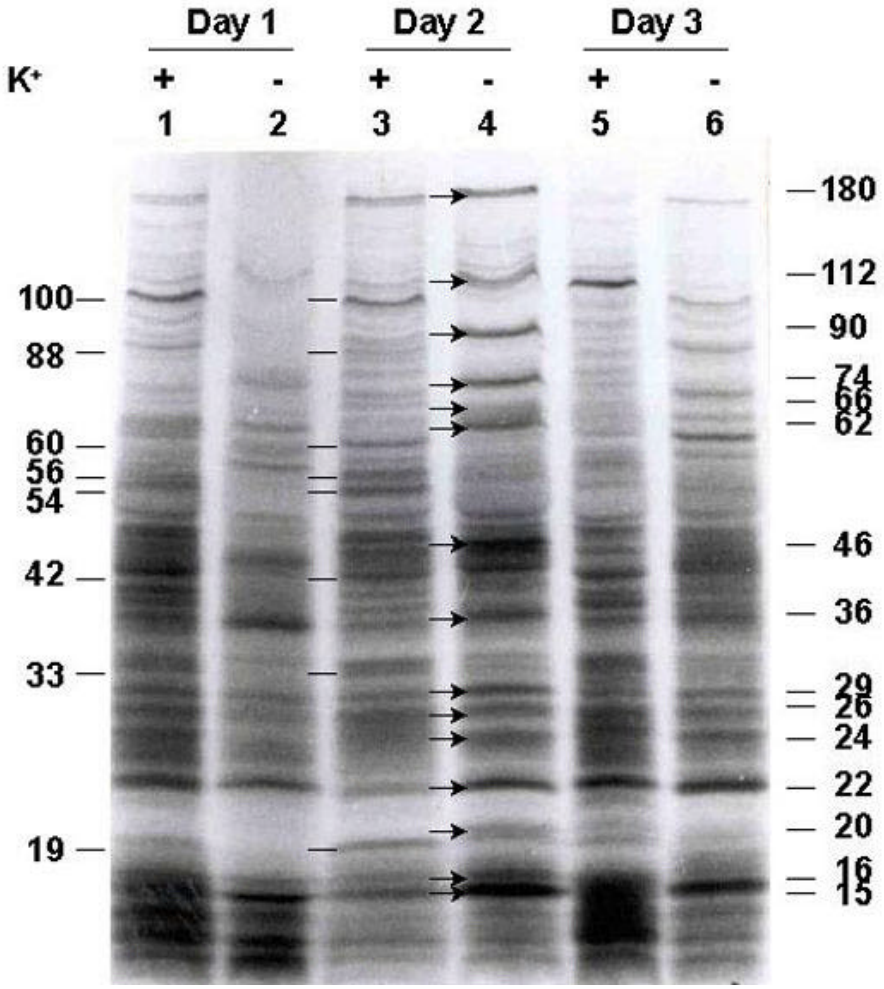

Figure 1. Potassium deficiency-induced proteins (PDPs) in *A. torulosa*. Autoradiogram of electrophoretically resolved protein extracts of cells from K5 (lanes 1, 3 and 5) or K0 (lanes 2, 4 and 6) media on day 1 (lanes 1 and 2), day 2 (lanes 3 and 4) or day 3 (lanes 5 and 6) post-inoculation is shown. Proteins were radiolabelled *in vivo* with [^{35}S]methionine for 5 min and the extracts were resolved by 5–14% gradient SDS–PAGE. Equal trichloroacetic acid (TCA)-precipitable radioactivity was loaded per lane. Lines indicate proteins whose synthesis was repressed on day 2 while induced proteins are indicated by arrows. The relative molecular mass (kDa) of the repressed and induced proteins is given on the left and right hand side respectively. The '+' and '–' refer to the K5 or K0 growth conditions respectively.

ment of K^+ for induction of osmoresponsive genes was therefore investigated critically in *A. torulosa*. Cultures from K5 or K0 media were subjected to a hyperosmotic (0·2 M sucrose) or a hypersaline (0·1 M NaCl) shock for 30 min on day 3 post-inoculation. Proteins induced in response to these stresses were visualized by *in vivo* radiolabelling (figure 2). Hypersaline stress includes an osmotic and an ionic component. Proteins induced by both salt or sucrose comprise the set of osmotic stress-induced proteins (OSPs) while those induced exclusively by salt were termed ionic stress-induced proteins (ISPs). In general, the number and intensity of OSPs in K^+-deficient condition were significantly higher compared to those in K^+-supplemented condition. While cells grown in K5 medium showed a moderate induction of eight OSPs, at least fifteen OSPs were strongly induced in cells from K0 medium.

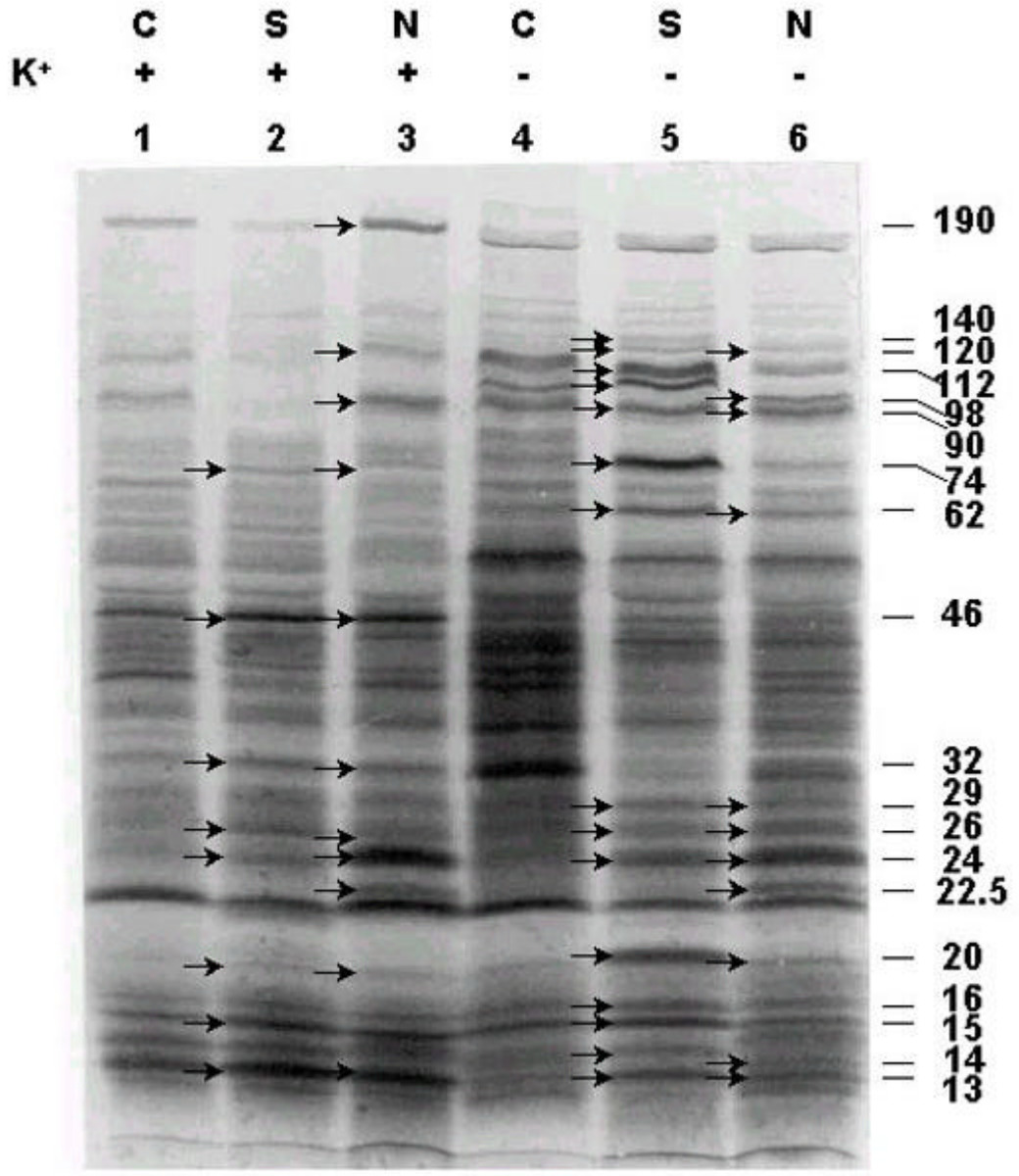

Figure 2. Osmotic and ionic stress-induced proteins (OSP and ISPs) in *A. torulosa*. Autoradiogram of electrophoretically resolved protein extracts of three-day old cultures from K5 (lanes 1–3) or K0 (lanes 4–6) media (see §2) that were subjected to 0·2 M sucrose (S, lanes 2 and 5) or 0·1 M NaCl (N, lanes 3 and 6) upshock for 30 min and radiolabelled *in vivo* at the end of the stress period. Corresponding unstressed control cultures (C, lanes 1 and 4) were also included for comparison. Other details are as described in figure 1.

Some of the prominent OSPs (20, 62, 74, 90, 110 and 112 kDa) were not induced significantly in the presence of 5 mM K^+. Only six of the OSPs were induced in both the growth conditions. In contrast, the induction of ISPs by NaCl upshock were not affected by the availability of K^+. The major ISPs (22·5, 24 and 98 kDa) were strongly induced under both the growth conditions. Few of the high molecular weight PDPs appeared to be further induced by osmotic and ionic stress. As summarised in table 1, osmotic and salt stress appeared to evoke unique as well as overlapping responses of protein synthesis in *Anabaena*, which were further regulated by the availability of K^+.

3.3 *Repression of PDP/OSPs by addition of potassium in* A. torulosa

Specificity of K^+ in regulation of the PDP/OSP induction in *A. torulosa* was examined further. A three-day old K0 culture was subjected to 0·2 M sucrose upshock for 30 min to visualize the PDP and OSP expression by *in vivo* radiolabelling (figure 3). This was followed by addition of 5 mM KCl to the culture. Synthesis of the prominent PDP/OSPs was dramatically repressed within 30 min of addition of K^+, although the culture continued to be exposed to the hyperosmotic stress. Synthesis of the 13 and 15 kDa OSPs appeared to be independent of the added K^+. In the absence of added K^+, synthesis of most of these proteins continued well beyond 1 h of osmotic upshock (data not shown). Thus, addition of K^+ had a strong repressive effect on the synthesis of most of the PDPs/OSPs.

3.4 *Potassium deficiency and osmotic stress-regulated proteins in* E. coli *MC4100*

The KdpATPase expression is the only reported case of a K^+ deficiency triggered operon in *E. coli*. The presence of PDPs and the effect of K^+ starvation on OSP/ISP syntheses were therefore examined in detail in *E. coli* strain MC4100. Figure 4a shows *in vivo* radiolabelled proteins from K115 and K0 cultures (lanes 3 and 4), revealing a set of nine PDPs in the range of 22–78 kDa after 6 h of incubation. Exposure to osmotic (0·4 M sucrose) or ionic (0·2 M NaCl) stresses for 30 min caused enhanced synthesis of five proteins in the K115 culture and of seven proteins in the K0 culture. There appeared to be no proteins induced specifically in response to NaCl stress (ISPs) in *E. coli*. Interestingly, the OSPs seen under K115 conditions were different from those seen under K0 conditions with only the 50 kDa protein being induced by both the stresses regardless of the availability of K^+.

Figure 4b shows the effect of addition of K^+ to an *E. coli* culture that had been incubated in K0 for 6 h and then subjected to 0·4 M sucrose upshock for 30 min. KCl was added to the osmotically stressed culture as well as to an unstressed K0 culture to visualize the individual effects of K^+ on PDP and OSP synthesis. Proteins were radiolabelled *in vivo* 2 h after addition of K^+. Synthesis of the major PDPs and OSPs was repressed by K^+ (figure 4b, lanes 1 and 4).

Thus, like *A. torulosa*, *E. coli* also expressed several PDPs and the syntheses of PDPs and OSPs were influenced by the presence of K^+ in the medium (table 1). Sutherland *et al* (1986) have earlier shown a lack of OSP induction in cells of *Salmonella typhimurium* if 1 mM KCl was added simultaneously with an osmotic upshock of 0·44 M sucrose. Our results showing K^+-enforced immediate repression of OSPs/ISPs in *E. coli* are in agreement with these data.

3.5 *Requirement of K^+ for expression and osmoinduction of dinitrogenase reductase of* A. torulosa *and GBBP of* E. coli

The dual control of stress protein synthesis by K^+ deficiency and osmotic upshock was examined for two repre-

Table 1. Potassium deficiency-induced proteins (PDPs) and osmotic/ionic stress-induced proteins (OSPs/ISPs) in *A. torulosa* and *E. coli*.

Strain	K^+-dependent OSPs[a]	K0-OSPs[b]	K^+-independent OSPs[c]	ISPs[d]	PDPs[e]
A. torulosa	32 and 46	14, 16, 29, 62, 90, 110, 112, 120 and 140	13, 15, 20, 24, 26 and 74	22·5, 24 and 98	15, **16**, 20, 22, **24**, **26**, **29**, 36, 46, 62, 66, **74**, **90**, 112 and 180
E. coli	44, 55, 65 and 78	20, 23, 27, 31, 32 and 86	50	Same as OSPs	22, **27**, **31**, 50, 55, 60, 70, 74 and 78

Numbers indicate relative molecular mass in kDa.
[a]OSPs induced specifically in high-K^+ medium. [b]OSPs induced specifically under K^+ deficiency. [c]OSPs induced under both K5 and K0 and growth conditions. [d]ISPs induced exclusively by NaCl and not affected by K^+. [e]Numbers in bold face indicate PDPs that were further induced by osmotic stress.

sentative proteins known to be osmoinduced in the bacteria being studied. Nitrogenase activity and synthesis of dinitrogenase reductase, the product of *nifH* gene, have been shown to require K^+ (Alahari and Apte 1998; Apte and Alahari 1994) and enhanced by osmotic stress in *Anabaena* strains (Fernandes *et al* 1993; Fernandes and Apte 2000). Similarly, the *proU* operon of *E. coli* is osmoinduced (Gowrishankar 1985; Dattananda *et al* 1991) and is known to be dependent on potassium for its transcriptional induction (Prince and Villarejo 1990). Antisera to dinitrogenase reductase and to GBBP, product of the *proX* gene of the *proU* operon, were employed to assess the intracellular levels of these proteins in osmotically stressed cultures from either K^+-supplemented or K^+-deficient media (figure 5). The results show that the intracellular steady-state level of both these proteins was increased after an osmotic upshock and was significantly higher in the presence of K^+ than in its absence. We have shown earlier that the KdpATPase is expressed in K0 conditions in both these bacteria (Alahari *et al* 2001). The K0 media used here contain about 15 μM K^+ and it is possible that potassium procured via the Kdp system may be sufficient to support transcriptional osmo-induction of the *nifH* and *proU* genes although not sufficient to support growth of the bacteria. Thus, unlike the OSPs that were repressed by presence of K^+ (figures 3 and 4b), the steady-state levels of dinitrogenase reductase in *A. torulosa* and GBBP in *E. coli* were higher in the presence of K^+.

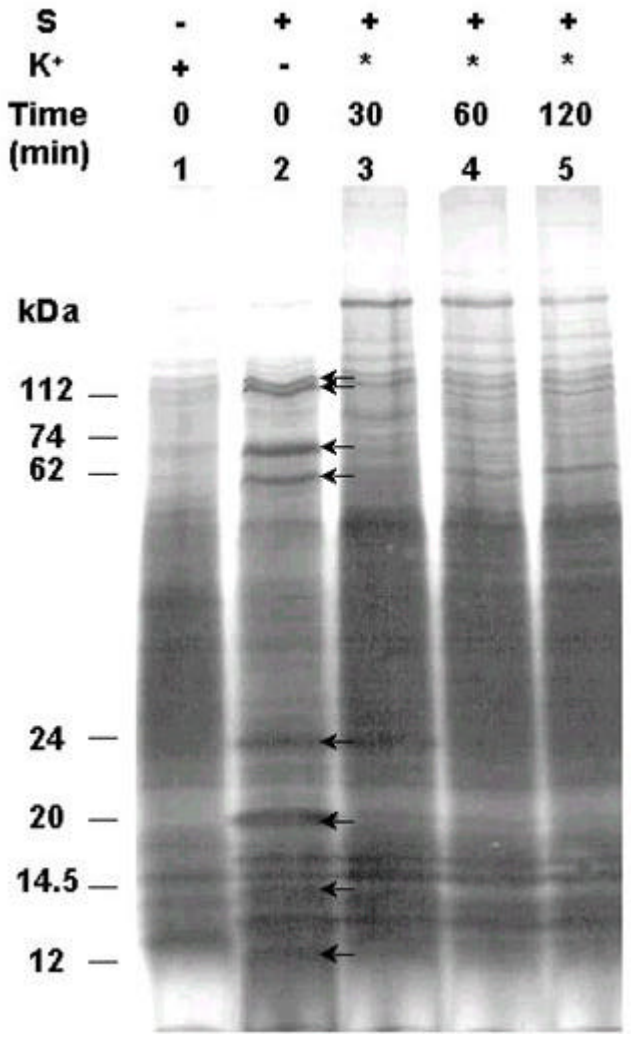

Figure 3. Repression of OSPs by the addition of K^+ in *A. torulosa*. Autoradiogram of electrophoretically resolved protein extracts is shown. A three-day old K0 culture was subjected to osmotic stress by addition of 0·2 M sucrose for 30 min (lanes 2–5). KCl (5 mM) was then added to the stressed cells (*, lanes 3–5). Proteins were radiolabelled *in vivo* 30 min (lane 3), 60 min (lane 4) or 120 min (lane 5) after addition of K^+. Protein extract from a three-day old K5 culture is included for comparison (lane 1). Arrows indicate PDP/OSPs whose synthesis was repressed upon addition of K^+. Other details are as described in figure 1.

4. Discussion

The results reported here demonstrate the occurrence of a novel set of PDPs in the nitrogen-fixing cyanobacterium *A. torulosa* and in *E. coli*. One-dimensional electrophoresis and high level of detection by autoradiography have been adequate to unambiguously demonstrate the occurrence of novel PDPs and their regulation by K^+ and osmotic stress. The number of such PDPs is large enough for this set to be considered as a potassium deficiency-induced (PDI) stimulon. A majority of the proteins belonging to this stimulon appear to sense dual signals of potassium deficiency and hyperosmotic stress i.e. they exhibit an obligatory requirement for K^+ deficiency for induction and their expression is further enhanced by hyperosmotic stress (table 1). A few proteins of the stimulon are induced by K^+ deficiency but not by hyperosmotic stress. The dependence of PDP/OSP expression on K^+ deficiency is strict; addition of K^+ strongly and rapidly represses their expression both in the presence and in the absence of an osmotic stress.

K^+ deficiency is expected to cause turgor loss as also happens in the case of a hyperosmotic shock. However, the members of the PDI stimulon sharply contrast with some of the well-known osmoresponsive genes in bacteria that require the obligatory presence of potassium for their osmoinduction and are inhibited or not expressed in the absence of K^+. The latter sense the dual signals of hyperosmotic stress and presence of K^+ simultaneously, while the PDI stimulon genes respond primarily to K^+ deficiency and also to osmotic stress. In both *A. torulosa* and in *E. coli*, the distinction of PDP expression (figures 2 and 4) from the K^+ requiring osmoresponsive expression of NifH and GBBP respectively is evident from figure 5.

We have shown earlier that compared to *E. coli* where NaCl and sucrose have identical effects on expression of osmoresponsive genes, *Anabaena* strains show differential effects of ionic and osmotic stressors (Fernandes *et al* 1993). This is further substantiated by the data in table 1, which show that all the OSPs and ISPs are identical in *E. coli*, but not in *A. torulosa* which synthesizes at least three unique ionic stress proteins (22·5, 24 and 98 kDa). Such differential effects on gene expression have also been seen in DNA microarray analysis in *Synechocystis* sp. PCC 6803 (Kanesaki *et al* 2002). Interestingly, many PDPs and OSPs overlap in their expression in *Anabaena*, while the expression of the ISPs is independent of the availability of K^+.

Thus, the osmoresponsive bacterial genes seem to clearly belong to three categories (i) those requiring K^+ and represented by the well known osmoresponsive genes, such as *proU*, *otsAB* and *betABT* in *E. coli* or *nifH* in *Ana*

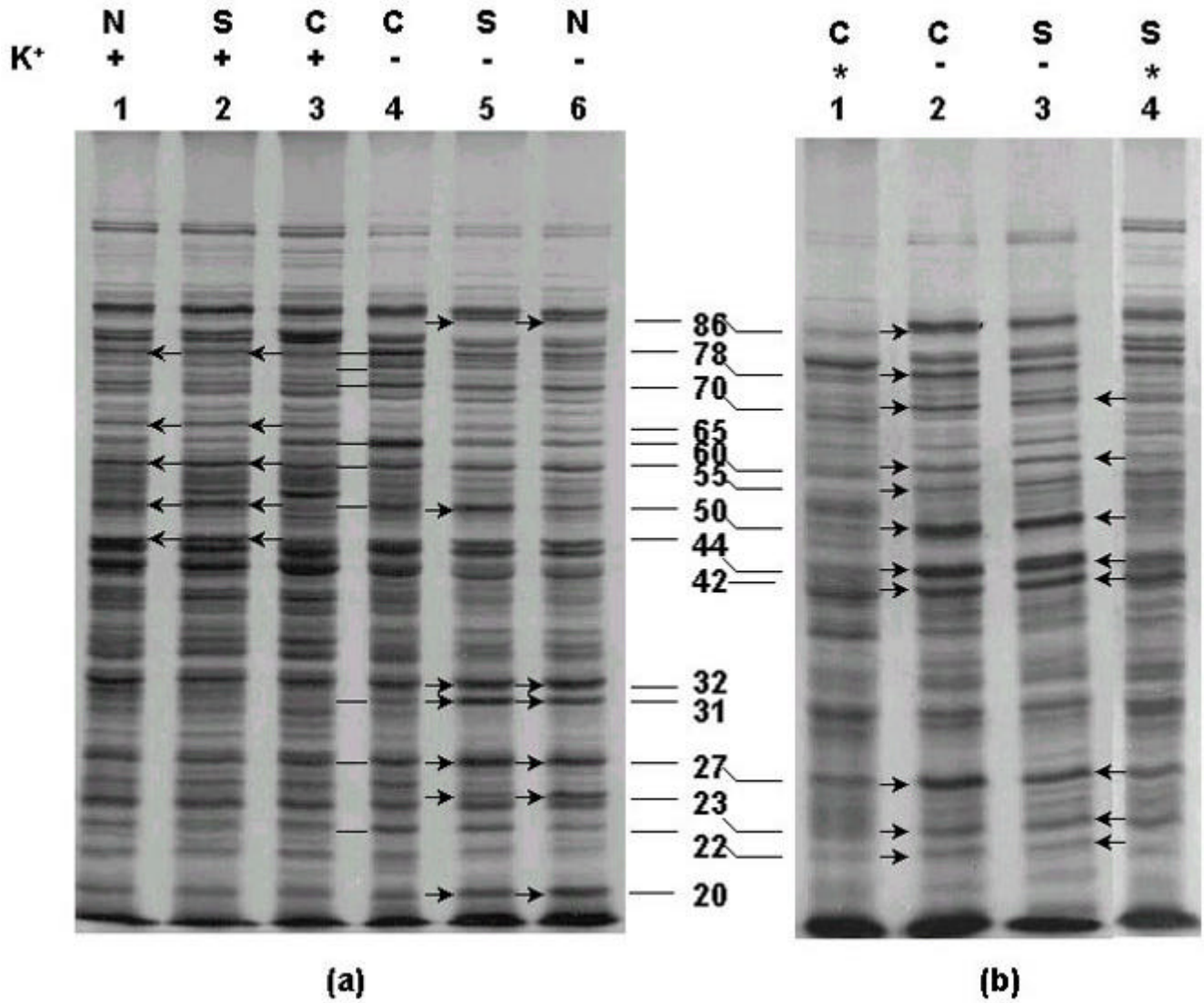

Figure 4. Effect of external K^+ on OSP and PDP syntheses in *E. coli* MC4100. Autoradiograms of *in vivo* radiolabelled proteins of *E. coli* MC4100 are shown. (**a**) Cultures from K115 (lanes 1–3) or K0 (lanes 4–6) media were subjected to 0·4 M sucrose stress (S, lanes 2 and 5) or 0·2 M NaCl stress (N, lanes 1 and 6) for 30 min or incubated unstressed (C, lanes 1 and 4) and then radiolabelled with [^{35}S]methionine for 1 min. Proteins were resolved by 10% SDS-PAGE. Arrows indicate proteins whose synthesis was induced by hyperosmotic stress, while lines indicate proteins induced by potassium deficiency. The relative molecular mass (kDa) of the induced proteins is indicated. (**b**) Cells from K0 medium were subjected to 0·4 M sucrose (S) upshock for 30 min (lanes 3 and 4). KCl (5 mM) was then added to an aliquot of osmotically stressed cells (*, lane 4) and to an aliquot of unstressed control (C) cells (*, lane 1). All aliquots were incubated for the next 2 h and then radiolabelled for 1 min. Proteins whose synthesis was repressed by addition of K^+ are indicated by arrows. Other details were as in (**a**).

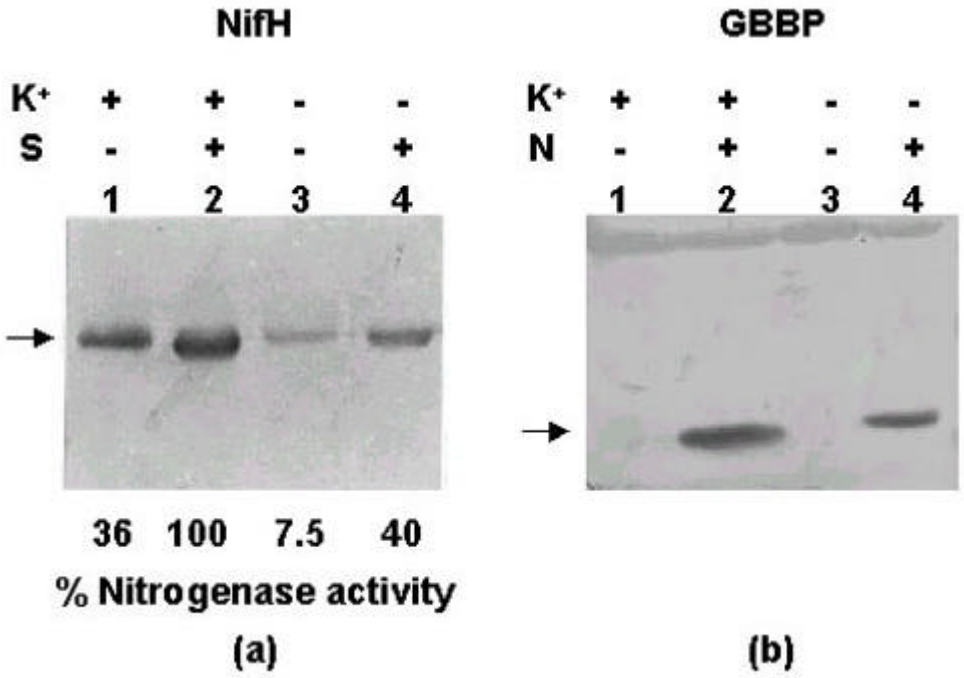

Figure 5. Dual regulation of NifH and GBBP by osmotic stress and external K^+. (**a**) Protein extracts from three-day old nitrogen-fixing cultures of *A. torulosa* from K5 (lanes 1 and 2) or K0 (lanes 3 and 4) media or the same media supplemented with 0·2 M sucrose (S, lanes 2 and 4) were resolved by 10% SDS-PAGE and electroblotted. The Western blot was allowed to react with anti-dinitrogenase reductase antiserum. Nitrogenase activity measured by acetylene reduction assay is given as percentage of the activity of the K5 unstressed control cells. The 100% value corresponds to 13·2 µmol ethylene evolved mg protein^{-1} min^{-1}. (**b**) Protein extracts of 6 h old *E. coli* MC4100 cultures from K115 (lanes 1, 2) or K0 (lanes 3, 4) media or the same media supplemented with 0·5 M NaCl (N, lanes 2 and 4) were resolved and electroblotted as above. Blot was allowed to react with anti-GBBP antiserum.

baena spp; (ii) those expressing strictly under K^+ depri vation and represented thus far solely by the *kdp* operon; and (iii) a small minority of OSPs whose expression is indifferent to the presence or absence of K^+ in the medium (table 1). The present data show that the class (ii) inclu des many hitherto unknown genes/proteins in addition to those of the *kdp* operon.

Two important features of PDI stimulon, regulation of expression and identity of the constituent genes require attention in order to understand their role in stress adaptation. The present study has addressed the issue of PDP regulation. The data show that PDPs are primarily triggered by turgor loss that occurs both due to K^+ deficiency as well as hyperosmotic stress and is aggravated by combined K^+ deprivation plus hyperosmotic stress and is aggravated by combined K^+ deprivation plus hyperosmotic stress conditions. Further, the strong and immediate repression of the PDI stimulon by exogenous K^+ makes its regulation closely resemble that of the *kdp* operon. It is tempting to speculate, that the two-component KdpD (sensor kinase)/KdpE (transcriptional activator) regulatory system (or another analogous system) may be responsible for regulating PDP expression in addition to *kdp* expression in these bacteria. Interestingly, cyanobacteria possess a truncated version of KdpD and seem to lack KdpE (Ballal *et al* 2002). The precise molecules and mechanisms effecting regulation of expression of the PDPs in these organisms remain enigmatic.

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