Expression and possible role of stress-responsive proteins in *Anabaena*

S K APTE*, T FERNANDES, H BADRAN and A BALLAL
Cell Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India
**Corresponding author (Fax, 91-22-556 0750).*

Nitrogen-fixing *Anabaena* strains offer appropriate model systems to study the cellular and molecular responses to agriculturally important environmental stresses, such as salinity, drought and temperature upshift. Sensitivity to stresses results primarily from reduced synthesis of vital cellular proteins such as phycocyanin and dinitrogenase reductase leading to impairment of photosynthesis and nitrogen fixation. Exposure to stresses induces the synthesis of a large number of general stress proteins and a few unique stress-specific proteins through transcriptional activation of stress-responsive genes. Using a subtractive RNA hybridization approach a large number of osmoresponsive genes have been cloned from *Anabaena torulosa*. The expression of general stress proteins has been shown to form the basis of adaptation and cross-protection against various stresses in *Anabaena*. Prominent among such proteins are the K^+-scavenging enzyme, KdpATPase, and the molecular chaperone, GroEL. Unlike heterotrophs, carbon starvation does not appear to evoke a global stress response in *Anabaena*. Supplementation of combined nitrogen or K^+ improves inherent tolerance of *Anabaena* strains to many environmental stresses.

1. Introduction

Among the agriculturally important abiotic stresses, soil salinity, drought and heat rank as the most detrimental and cause serious loss of crop productivity all over the world. As a result, the actual yield potential of most crop plants is rarely realized even under the best of agricultural practices (Boyer 1972). Although stresses hamper the survival and growth of living forms, most organisms have evolved mechanisms to combat stressful situations. Their ability to survive stresses is usually the result of possession and selective expression of certain novel genes. Information on such genes and their regulation is an important pre-requisite for devising future strategies aimed at building stress tolerant bacteria and crop plants (Bohnert and Jensen 1996).

As organisms which originated millions of years ago (Brock 1973) and have successfully lived through several environmental stresses on this planet, cyanobacteria offer excellent opportunities for studying diverse stress responses. Of particular interest is the ability of heterocystous cyanobacteria like *Anabaena*, to tolerate stresses such as nutrient deficiency, salinity, drought and temperature upshift (Apte *et al* 1997). Agriculturally important capabilities of photosynthesis and nitrogen fixation and their phylogenetic relationship with plant chloroplasts make cyanobacteria appropriate model systems for studying plant responses to environmental stresses. In view of their natural abundance in tropical climate, such studies are considered especially relevant for biotechnological exploitation of cyanobacteria as nitrogen biofertilisers for rice cultivation under stressful environments (Singh 1950).

Our laboratory has been investigating the physiological, biochemical and molecular genetic aspects of growth, differentiation, photosynthesis and nitrogen fixation in *Anabaena* strains for the last two decades (Apte 1992, 1993; Apte and Nareshkumar 1996). In recent years, our studies have focussed on the analysis of cellular and molecular basis of stress and adaptive responses of *Anabaena* strains to environmental abiotic stresses that adversely affect crop productivity (Apte *et al* 1997).

Keywords. *Anabaena*; salinity; drought; heat shock; interactive effects; stress proteins

This article aims to review the significant recent results from our laboratory which have contributed to the elucidation of the prime mechanisms underlying cyanobacterial responses to major agricultural stresses.

2. Materials and methods

2.1 *Organisms and growth conditions*

Anabaena torulosa (Apte and Thomas 1980), a brackish-water halotolerant strain, and *Anabaena* sp. strain L-31 (Thomas 1970), a fresh-water osmotolerant form, were used in axenic condition. The cyanobacteria were grown in combined nitrogen-free BG-11 medium (Castenholz 1988) under continuous illumination (2·5 mW cm^{-2}) and shaking (150 rpm) at 25°C ± 2°C. Salinity or osmotic stress was applied, as NaCl or sucrose respectively, at the specified concentrations while heat shock was imparted at 42°C (Bhagwat and Apte 1989). Stress tolerance was estimated in terms of growth and nitrogen fixation, as described earlier (Apte *et al* 1987).

2.2 *Analysis of stress proteins*

Stress proteins were detected by *in vivo* pulse-radiolabelling of stressed cells with [^{35}S]methionine followed by extraction and resolution of proteins by 5–14% gradient SDS-PAGE and their visualization by autoradiography, as described earlier (Apte and Bhagwat 1989). Occasionally electrophoretograms were also developed by staining with Coomassie brilliant blue G-250. Western blotting of stress proteins and their immuno-detection were carried out as per the standard procedures (Sambrook *et al* 1989). The primary antibodies were detected using anti-rabbit IgG coupled to alkaline phosphatase (Boehringer Mannheim, GmBH, Germany) followed by colour development as per the manufacturer's recipe.

3. Tolerance of nitrogen-fixing *Anabaena* cultures to major environmental abiotic stresses

The photosynthetic, nitrogen-fixing cyanobacteria such as *Anabaena* are well equipped to handle deficiency of two most vital nutrients, namely carbon and nitrogen. Both these metabolic processes are, however, very sensitive to agriculturally important stresses (Apte *et al* 1997). Nutrient deficiencies, particularly those of nitrogen and potassium, decrease overall tolerance of cyanobacteria to different environmental stresses (Apte 1992, 1993). Combined nitrogen-supplemented or potassium-rich cyanobacterial cultures show higher levels of tolerance to most stresses (Apte *et al* 1987; Reddy *et al* 1989). The reasons for this are elaborated in the following sections.

3.1 *Halotolerance*

The two *Anabaena* strains studied, exhibit better halotolerance than plants. We have shown earlier that *A. torulosa* can grow and fix nitrogen up to salinity levels of 250 mM NaCl (Apte *et al* 1997) or 8·50 dS m^{-1} of soil conductivity (Apte and Thomas 1997). In comparison, most crop plants are sensitive to NaCl levels beyond 50 mM and fail to grow well at soil conductivity values above 2 dS m^{-1} (Serrano and Gaxiola 1994; Apte and Thomas 1997). The halotolerance of nitrogen-fixing *Anabaena* cultures does not match with that of *Escherichia coli* strains which can grow up to 0·6–0·8 M NaCl (Csonka and Hanson 1991). This is primarily because cyanobacterial nitrogen fixation is very sensitive to ionic stress *per se*. The diversion of cellular energy from N_2 fixation to Na^+ efflux during exposure to NaCl severely inhibits nitrogenase activity (Apte *et al* 1987). Combined nitrogen (nitrate or ammonium) very effectively curtails Na^+ influx (Reddy *et al* 1989). As a result combined nitrogen-supplemented *Anabaena* cultures attain the halotolerance levels of enteric bacteria (Apte *et al* 1987, 1997; Reddy *et al* 1989).

3.2 *Osmotolerance*

Cyanobacteria are known to be exceptionally drought tolerant. They can be stored desiccated for months or years without loss of viability. Rehydration is known to result in immediate reappearance of nitrogenase activity and revival of growth (Peat *et al* 1988). Nitrogen-fixing *Anabaena* sp. strain L-31 cultures show better tolerance to purely osmotic stress (sucrose) than to salinity or NaCl (ionic + osmotic) stress (Fernandes *et al* 1993). Unlike the ionic stress, there is no energy expenditure towards Na^+ efflux during osmotic stress and this energy conservation facilitates better diazotrophic growth. Nitrate-supplemented *Anabaena* cultures exhibit even higher osmotolerance than the diazotrophic cutures (Fernandes *et al* 1993; Iyer *et al* 1994). The reasons for this are elaborated in § 7.

3.3 *Thermotolerance*

Thermotolerance of *Anabaena* strains is comparable to that of plants and seems superior to that of enteric bacteria. Nitrogen-fixing *Anabaena* cultures do not grow at temperatures above 45°C (data not shown). However, unlike bacteria they can withstand prolonged periods of exposure to moderate heat stress (42°C continuously for 5 days) without serious loss of viability. Generally, bacterial nitrogen fixation is very sensitive to temperatures above 30°C since NifA, the transcriptional activator of *nif* genes, is highly thermolabile (Buchanan-Wollaston *et al* 1981). In most *Anabaena* strains, optimum tem-

perature for N_2 fixation ranges from 35°C–39°C, or even higher (Stacey *et al* 1977). Temperatures above 42°C inhibit cyanobacterial nitrogen fixation though less severely (complete inhibition after 6 h at 42°C) than in *Klebsiella pneumoniae* (Collins and Brill 1985).

4. Stress-regulated protein synthesis in *Anabaena*

Exposure of *Anabaena* cells to different abiotic stresses results in prominent modifications of the protein synthesis programme (Apte *et al* 1997). Using *in vivo* radiolabelling ([^{35}S]methionine pulse for 5 min at the end of the stress), we have detected selective rapid expression of several stress proteins in *Anabaena* strains (Apte and Bhagwat 1989; Bhagwat and Apte 1989; Iyer *et al* 1994). Synthesis of several other proteins is repressed. Pre-treatment of cells with rifampicin, prior to their exposure to these stresses, blocks the synthesis of most stress proteins (Apte *et al* 1997). Thus, the expression of almost all the abiotic stress-induced proteins in *Anabaena* appears to be brought about by transcriptional activation of stress-responsive genes.

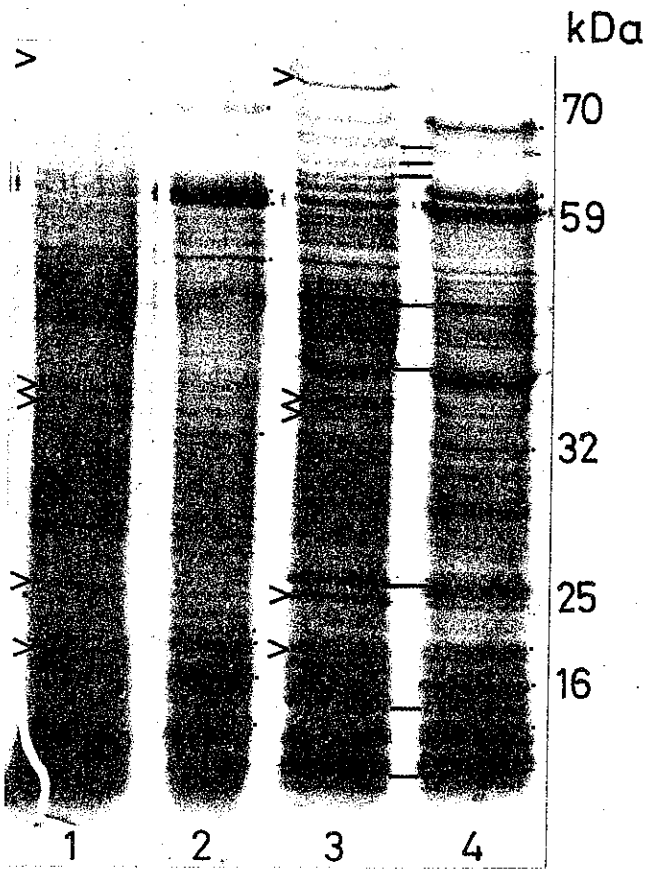

Figure 1. Effect of growth conditions on the expression of heat shock proteins in *Anabaena* sp. strain L-31. Cultures grown under aeration (3 l min^{-1}) (lanes 1 and 2) or under shaking (150 rpm) (lanes 3 and 4) for 3 days, were incubated either at 25°C (lanes 1 and 3) or 42°C (lanes 2 and 4) for 25 min. 1 ml culture aliquots were radiolabelled with [^{35}S]methionine (2·20 MBq ml^{-1}) for 5 min at their respective temperatures. Proteins were extracted and resolved by SDS-PAGE on 5–14% gradient gels followed by autoradiography, as described earlier (Apte and Bhagwat 1989). Equal TCA-precipitable radioactivity was loaded in all the lanes. Stress repressed proteins are marked by arrowheads and heat shock proteins by dots while (—) indicates novel proteins synthesized by shaker-grown cultures (lane 3) but not by aerated cultures (lane 1). The numbers on the right side denote the apparent molecular mass (in kDa) of different proteins.

Even apparently mild change in growth conditions significantly alters the protein synthesis pattern in *Anabaena* (figure 1). For example, compared to aerated cultures (lane 1), cultures grown with shaking (lane 3) synthesis several novel proteins (marked by horizontal lines), not synthesized by the aerated cultures. Shaker-grown cultures also exhibit higher rates of protein synthesis, especially of the heat shock proteins (compare the 16, 32, 59 and 70 kDa HSPs in lanes 2 and 4 of figure 1). The reasons for such qualitative and quantitative differences in the stress protein expression by the shaker-grown and aerated cultures are not clear but may result from differences in the availability of dissolved O_2, CO_2 or of other nutrients.

4.1 *Synthesis of unique versus general stress proteins*

In nitrogen-fixing *Anabaena* cultures, a 30 min exposure to salinity, osmotic or heat stresses evokes nearly identical gene expression (figure 2). Although each of these stresses induces the expression of about 10–15 polypeptides, many of them are commonly induced by more than one stress. Only three polypeptides are induced specifically by NaCl (termed the ionic stress proteins or ISPs) and two by heat stress (the heat shock proteins). At least four proteins are induced both by NaCl and sucrose but not by heat shock and these are called the osmotic stress proteins, or OSPs. About 8 polypeptides are commonly induced by heat, salinity as well as osmotic stress and these, hereafter, will be referred to as the general stress proteins or GSPs (figure 2). Thus, a large number of stress proteins described earlier as SSPs, OSPs or HSPs etc. (Apte and Bhagwat 1989; Bhagwat and Apte 1989; Iyer *et al* 1994), actually belong to the category of GSPs.

In most life forms, the stress proteins are generally expressed at low levels and for a short period. This precludes their visualization by less sensitive approaches such as Coomassie staining. Compared to radiolabelling, Coomassie staining detects no stress proteins within 30 min (data not shown) and detects only one HSP (59 kDa), one ISP (21·5 kDa) and 8 OSPs in nitrogen-fixing cultures exposed for 24 h to stress (figure 3). Among the stress proteins of *Anabaena*, therefore, only

OSPs appear to accumulate at levels which allow their detection even by Coomassie staining.

The synthesis of heat shock proteins has not been adequately investigated in heterocystous nitrogen-fixing cyanobacteria. Our studies indicate that, in general, the heat shock response in *Anabaena* qualitatively resembles that in eubacteria (figure 2). Since most HSPs are constitutively synthesized at low levels, we have examined the effect of growth conditions on HSP expression. Figure 1 shows that all of the 8 prominent HSPs observed in the aerated cultures are also synthesized in *Anabaena* grown under shaking, but at significantly elevated rates. Thus, the growth conditions may influence the quantity, but not the quality, of HSPs in *Anabaena*.

4.2 *Regulation of stress protein synthesis by nutritional deficiency*

Commonality between stress responses is known in many life forms and for several stresses. In particular the heat shock response overlaps with a variety of stress responses in different organisms (Morimoto *et al* 1992), including *Anabaena* (figure 2). This is probably because most stresses result in unfolding, denaturation and aggregation of proteins and, therefore, result in the induction of heat shock response (Hecker *et al* 1996). Another general stress stimulus is nutrient starvation (Hengge-Aronis 1993). In *E. coli*, carbon starvation is a global inducer of stress protein synthesis and numerous (> 40) novel starvation induced proteins of *E. coli* overlap with proteins synthesized under heat shock, high osmolarity, nitrogen and phosphate starvation, exposure to heavy metals and other stresses (Hengge-Aronis 1993). A 13·5 kDa "universal stress protein" is expressed under several different stress conditions because all of them interfere with the flow of carbon in the central catabolic pathway of carbon breakdown and thereby alter carbon utilization abilities of *E. coli* (Nystrom and Neidhardt 1993). In the Gram positive *Bacillus* too, a large number of general stress proteins are known to be induced in response to different stresses (Volker *et al* 1994).

We have examined the influence of nutrient deficiencies, such as that of carbon and nitrogen, on stress protein synthesis in *Anabaena* (figures 2–4). Photoautotrophically-grown *Anabaena* cultures were starved for carbon, by treatment with the PS-II inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or by dark incubation, before subjecting them to heat shock. Barring

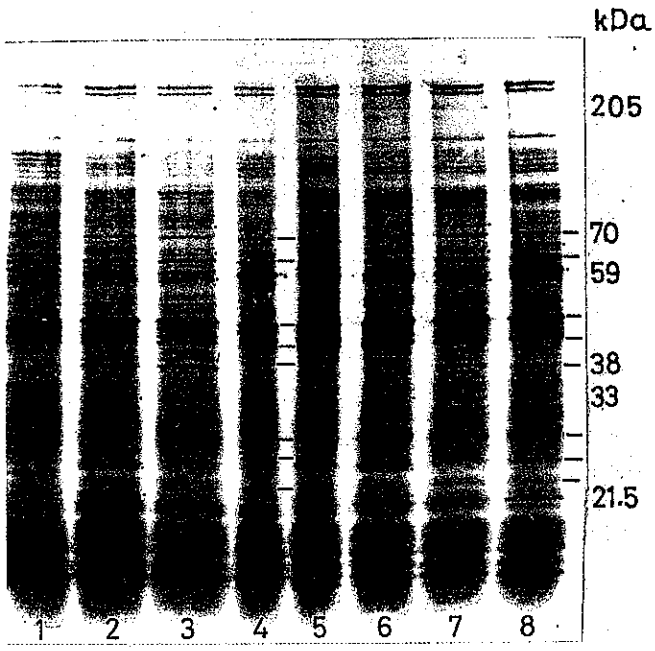

Figure 2. Synthesis of stress proteins by *Anabaena* sp. strain L-31, grown with different nitrogen sources. Cultures were grown for 3 days in BG-11 medium either with (lanes 1–4) or without (Lanes 5–8) 10 mM KNO_3. Stress was applied for 30 min either as 150 mM NaCl (lanes 2 and 6) or 350 mM sucrose (lanes 3 and 7) or temperature upshift to 42°C (lanes 4 and 8). Cells were radiolabelled during the last 5 min of stress period. The dots to the right side of lanes indicate ISPs (lanes 2 and 6), OSPs (lanes 3 and 7) or HSPs (lanes 4 and 8) while (—) indicate the GSPs. Other details are as described in figure 1.

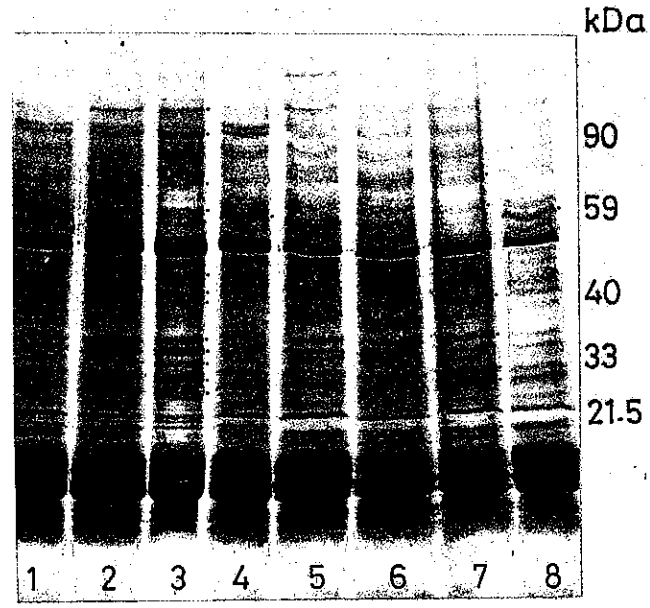

Figure 3. Accumulation of stress proteins by *Anabaena* sp. strain L-31 grown either in the presence or absence of combined nitrogen. Cells grown as described in figure 2 were stressed for a period of 24 h. Proteins were extracted and resolved as described in figure 1 and detected by staining with Coomassie brilliant blue G-250. Other details are as described in figure 2.

a single (42 kDa) carbon starvation protein (CSP), no major differences in HSP expression compared to the light grown controls are observed under these conditions (figure 4). Thus, unlike *E. coli, Anabaena* exhibits no major carbon starvation-induced proteins. Further, carbon starvation also does not modify stress protein expression in *Anabaena*.

The most commonly experienced nutrient starvation by *Anabaena* strains in nature is nitrogen deficiency. Comparison of stress proteins expressed in nitrogen-supplemented or nitrogen-deficient cultures (figures 2 and 3) show that qualitatively the stress proteins synthesized under the two conditions are quite similar. The only major difference noted is in the levels of OSPs which accumulate to even higher levels in nitrogen-supplemented cultures (figure 3, lane 3). We have shown earlier that nitrate induces OSP expression and this is related to the enhanced osmotolerance exhibited by nitrogen-supplemented *Anabaena* cultures (Iyer *et al* 1994).

5. Cloning and expression of stress-responsive genes

Several earlier attempts by our laboratory to clone osmoresponsive genes of *Anabaena* using DNA : DNA hybridizations with cloned osmoresponsive *E. coli* genes (*ots AB, betABT, kdpABC* etc.) did not succeed. As has been revealed by the recently published genome sequence of the cyanobacterium *Synechocystis* PCC 6803, the homology at the nucleotide level between the cyanobacterial and *E. coli* genes is rather poor. In a few cases, such as that of *kdpABC*, the protein product (KdpATPase) could be easily immunodetected on Western blots of membrane proteins but Southern blots revealed only very faint bands (Apte and Alahari 1994). When subjected to stress, *Anabaena* strains accumulate more or less the same intracellular compatible osmolytes, such as K^+, sugars, sugar alcohols and quaternary amines (Borowitzka *et al* 1980; Reed *et al* 1984; Reed and Stewart 1985) as does *E. coli* (Csonka and Hanson 1991). The presence and stress induced expression of the corresponding genes has, therefore, never been in doubt.

Since almost all of the stress-induced proteins in *Anabaena* were found to be transcriptionally activated (the same is the case in enteric bacteria also), we devised a strategy for cloning genes differentially expressed during stress. Employing a subtractive RNA hybridization strategy, we have successfully cloned the salinity stress responsive genes from *Anabaena* strains (Apte and Haselkorn 1990). Some of these have been found to express in *E. coli* using the same signals that induce their expression in *Anabaena*. For example a cosmid clone (pATCOSK6) when expressed in *E. coli* strain HB101 displays two specific ISPs and one OSP in response to exposure of the transgenic *E. coli* to NaCl or sucrose stress (Apte and Haselkorn 1990; Apte *et al* 1997).

We have now initiated attempts to clone stress responsive *Anabaena* genes either by (i) PCR amplification using primers based on *Synechocystis* "stress-responsive" gene sequences (such as *kdpABC*), or (ii) functional complementation of defined "stress-sensitive" (temperature-/osmo-sensitive) *E. coli* mutants. These approaches are in progress.

6. Identification of stress proteins in *Anabaena*

Identification of cyanobacterial stress proteins has not kept pace with such efforts in other bacteria, because very few groups are pursuing such an exercise. Stresses

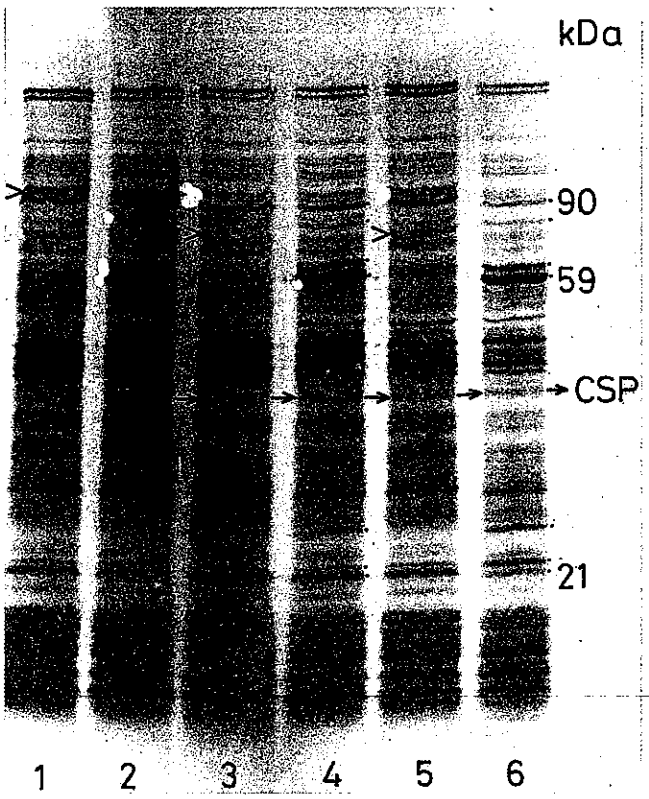

Figure 4. Effect of carbon starvation on the synthesis of heat shock proteins in *Anabaena* sp. strain L-31. Three day old cultures were incubated either in light (lanes 1 and 2) or in dark (lanes 3 and 4) or with 1 μM DCMU (lanes 5 and 6) and grown either at 25°C (lanes 1, 3 and 5) or at 42°C (lanes 2, 4 and 6) for 30 min. Radiolabelling, electrophoresis and autoradiography of proteins was carried out as described in figure 1. The arrowheads indicate stress-repressed and dots indicate the heat shock proteins. A single carbon starvation-induced protein (CSP) detected is shown.

repress the synthesis of several metabolically important proteins. Nitrogen deficiency lowers the content of the blue phycobiliprotein, phycocyanin (Wood and Haselkorn 1980). Similar reduction in phycocyanin levels (and thereby in the photosynthesis) is also seen under salinity, osmotic and heat stresses (Apte *et al* 1997). Using immunodetection approach our laboratory has found that salinity, but not osmotic, stress represses the synthesis of dinitrogenase reductase thereby resulting in the loss of nitrogen fixation capabilities (Fernandes T A and Apte S K, unpublished results). This may be the major reason why nitrogen-fixing *Anabaena* cultures show high sensitivity to salinity and other stresses.

We have recently initiated characterization of several stress-induced proteins in *Anabaena*. A membrane-bound K^+ deficiency-induced protein (which is also osmo-induced) has been identified as belonging to the KdpB subunit of a K^+-dependent ATPase (Apte and Alahari 1994). In enteric bacteria, this enzyme has been shown to play an important role in initial osmotic adjustments during exposure to salinity/osmotic stresses (Altendorf and Epstein 1993). In *Anabaena* its expression is induced by K^+ deficiency and osmotic stress. The possible role this protein may play in desiccation tolerance of *Anabaena* is being investigated further. A 40 kDa OSP that accumulates in many *Anabaena* strains has been identified as cyanodehydrin by another laboratory (Close and Lammers 1993). Although its biological function is not yet known, the dehydrins have been shown to be very important for halo/osmotolerance in crop plants, especially cereals (Xu *et al* 1996).

Immunodetection approach has also identified a major *Anabaena* GSP as the molecular chaperone GroEL. Interestingly, the GroEL proteins show considerable molecular heterogeneity in *Anabaena*. Figure 5 shows at least two major (59 and 61 kDa) and two minor (58 and 62 kDa) polypeptides which cross-react with the antiserum raised against *E. coli* GroEL protein. The 61 kDa form seems to be constitutively present in *Anabaena* cells while the 59 kDa polypeptide is distinctly induced upon heat shock (figure 5). This is the first time multiple molecular forms of GroEL have been reported in any biological system. The situation is reminiscent of that in another nitrogen-fixing bacterium *Bradyrhizobium japonicum*, where 5 different *groESL* operons, though not as many proteins, have been shown (Fischer *et al* 1993). Whether the *Anabaena* GroEL-like polypeptides (figure 5) represent products of different genes remains to be ascertained.

7. Possible role of stress proteins in cyanobacterial stress tolerances

Generally two kind of stress tolerance mechanisms operate in bacteria. Some of them are expressed at all times and are used to combat certain frequently encountered stresses. Low level constitutive expression of major HSPs belongs to this category. On the other hand, most of the stress responses are adaptive responses which remain shut off under normal conditions of growth and are expressed in a need based manner only during the stress. These are exemplified by selectively enhanced expression of various stress proteins in response to exposure to one or more stresses.

Recent data from our laboratory clearly show that OSPs play a major role in osmotolerance of *Anabaena* strains (Iyer *et al* 1994). This conclusion is based on the following facts: (i) OSPs are rapidly expressed in a dose-dependent manner (Apte and Bhagwat 1989) and are accumulated by cells during prolonged osmotic stress (figure 3), (ii) pre-adaptation of cultures to sub-lethal levels of sucrose (which fully induces OSPs) enhances osmotolerance to lethal levels of osmotic stress and (iii) conditions which induce OSPs (NaCl, nitrate) or repress OSPs (NH_4^+) respectively enhance or reduce osmotolerance (Iyer *et al* 1994). The OSPs, thus, form the molecular basis of adaptation and cross-protection to cyanobacteria against osmotic stress. They do not appear to play a major role in tolerance to the ionic stress. The ionic stress tolerance mainly depends on the ability of cells to exclude Na^+ from the cytoplasm (Apte and Thomas 1986) and can be enhanced further by modifying environmental conditions which curtail Na^+ influx (Apte *et al* 1987; Reddy *et al* 1989).

The observed overlap between cyanobacterial responses to salinity/osmotic stresses and heat shock has opened up possibilities of testing whether the GSPs play a role in stress tolerance of *Anabaena*. The fact that GroEL is the most abundant cyanobacterial chaperone and is induced by most stresses (GSP) (figures 1–3) suggests

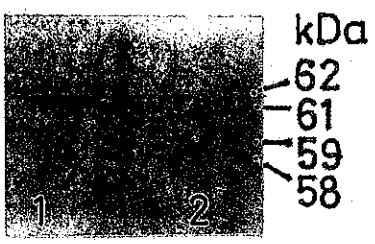

Figure 5. Effect of heat shock on the levels of GroEL proteins in *Anabaena* sp. strain L-31. 3-day old cultures were incubated for 3 h either at 25°C (lane 1) or at 42°C (lane 2). Proteins were extracted and resolved by SDS-PAGE on 10% gels followed by electroblotting onto positively charged nylon membranes. Immunodetection was carried out with antiserum raised against the purified GroEL protein of *E. coli*. The primary antibody was detected using an anti-rabbit IgG coupled to alkaline phosphatase followed by colour development. Apparent molecular mass of the polypeptides detected are indicated.

that Hsp60 family proteins may play important role in the regulation of general stress response in cyanobacteria. Certain other HSPs, for example the DnaK, have also been implicated in osmotic adaptation in bacteria (Meury and Kohiyama 1993).

A large overlap has also been observed in stress protein synthesis in response to K^+ deficiency and osmotic stress (Apte and Alahari 1994). K^+ is also likely to play a major role in regulating biochemical activities of certain HSPs like DnaK (Palleros *et al* 1993). Since K^+ is involved in the maintenance of bacterial cell turgor, it is tempting to speculate that turgor perturbations caused by osmotic stress/K^+ starvation may provide the general stimulus for OSP syntheses in *Anabaena*. It would be very rewarding, therefore, to examine signal transduction pathways linked to gene expression during desiccation caused by K^+ deprivation, ionic/osmotic stresses or heat shock.

Acknowledgements

We wish to thank Prof. K Altendorf, University of Osnabruck, Germany (anti-KdpB), Prof. C Gross, University of California, San Francisco, USA (anti-GroEL) and Prof. P Ludden, University of Wisconsin, Madison, USA (anti-dinitrogenase reductase) for kindly providing us the various antisera used in this work.

References

Altendorf K and Epstein W 1993 Kdp-ATPase of *Escherichia coli*; *Cell Physiol. Biochem.* **4** 160–168

Apte S K 1992 Molecular biology of cyanobacterial nitrogen fixation: recent advances; *Indian J. Microbiol.* **32** 103–126

Apte S K 1993 Cyanobacterial nitrogen fixation; molecular genetic aspects; *Proc. Indian Natl. Sci. Acad.* **B59** 367–386

Apte S K and Alahari A 1994 Role of alkali cations (K^+ and Na^+) in cyanobacterial nitrogen fixation and adaptation to salinity and osmotic stress; *Indian J. Biochem. Biophys.* **31** 267–279

Apte S K and Bhagwat A A 1989 Salinity stress induced proteins in two nitrogen-fixing *Anabaena* strains differentially tolerant to salt; *J. Bacteriol.* **171** 909–915

Apte S K, Fernandes T A, Iyer V and Alahari A 1997 Molecular basis of tolerance to salinity and drought stresses in photosynthetic nitrogen-fixing cyanobacteria; in *Plant molecular biology* (eds) K K Tewari and G S Singhal (New Delhi: Narosa Publishing House) pp 258–268

Apte S K and Haselkorn R 1990 Cloning of salinity stress induced genes from salt tolerant nitrogen-fixing cyanobacterium *Anabaena torulosa*; *Plant Mol. Biol.* **15** 723–733

Apte S K and Nareshkumar G 1996 A model for cell type-specific differential gene expression during heterocyst development and the constitution of aerobic nitrogen fixation ability in *Anabaena* sp. strain PCC 7120; *J. Biosci.* **21** 397–411

Apte S K, Reddy B R and Thomas J 1987 Relationship between sodium influx and salt tolerance of nitrogen-fixing cyanobacteria; *Appl. Env. Microbiol.* **53** 1934–1939

Apte S K and Thomas J 1980 Sodium is required for nitrogenase activity in cyanobacteria; *Curr. Microbiol.* **3** 291–293

Apte S K and Thomas J 1986 Membrane electrogenesis and sodium transport in filamentous nitrogen-fixing cyanobacteria; *Eur. J. Biochem.* **154** 395–401

Apte S K and Thomas J 1997 Possible reclamation of coastal soil salinity using halotolerant nitrogen-fixing cyanobacteria; *Plant Soil* **189** 205–211

Bhagwat A A and Apte S K 1989 Comparative analysis of proteins induced by heat-shock, salinity and osmotic stress in the nitrogen-fixing cyanobacterium *Anabaena* sp. strain L-31; *J. Bacteriol.* **171** 5187–5189

Bohnert H J and Jensen R G 1996 Strategies for engineering water stress tolerance in plants; *Trends Biotechnol.* **14** 89–97

Borowitzka L J, Demmerle S, Mackay M A and Norton S 1980 Carbon-13 nuclear magnetic resonance study of osmoregulation in blue-green algae; *Science* **210** 650–651

Boyer J S 1972 Plant productivity and environment; *Science* **218** 443–448

Brock T D 1973 Evolutionary and ecological aspects of cyanophytes; in *The biology of blue-green algae* (eds) N G Carr and B A Whitton (Oxford: Blackwell) pp 487–500

Buchanan-Wollaston V, Cannon M C, Beynon J L and Cannon F C 1981 Role of *nifA* gene product in the regulation of *nif* expression in *Klebsiella pneumoniae*; *Nature (London)* **294** 776–778

Castenholz R W 1988 Culturing of cyanobacteria; *Methods Enzymol.* **167** 68–93

Close T and Lammers P J 1993 An osmotic stress protein of cyanobacteria is immunologically related to plant dehydrins; *Plant Physiol. (Bethesda)* **101** 773–779

Collins J J and Brill W J 1985 Control of *Klebsiella pneumoniae nif* mRNA synthesis; *J. Bacteriol.* **162** 1186–1190

Csonka L N and Hanson A D 1991 Prokaryotic osmoregulation: genetics and physiology; *Annu. Rev. Microbiol.* **45** 569–606

Fernandes T A, Iyer V and Apte S K 1993 Differential responses of nitrogen-fixing cyanobacteria to salinity and osmotic stresses; *Appl. Environ. Microbiol.* **59** 899–904

Fischer H M, Babst M, Kaspar T, Acuna G, Arigoni F and Hennecke H 1993 One member of a *groESL*-like chaperonin multigene family in *Bradyrhizobium japonicum* is co-regulated with symbiotic nitrogen fixation genes; *EMBO J.* **12** 2901–2912

Hecker M Schumann W and Volker U 1996 Heat-shock and general stress response in *Bacillus subtilis*; *Mol. Microbiol.* **19** 417–428

Hengge-Aronis R 1993 Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli*; *Cell* **72** 165–168

Iyer V, Fernandes T A and Apte S K 1994 A role of osmotic stress-induced proteins in the osmotolerance of a nitrogen-fixing cyanobacterium, *Anabaena* sp. strain L-31; *J. Bacteriol.* **176** 5868–5870

Meury J and Kohiyama M 1993 Role of heat-shock protein DnaK in osmotic adaptation of *Escherichia coli*; *J. Bacteriol.* **173** 4404–4410

Morimoto R I, Sarges K D and Abravaya K 1992 Transcriptional regulation of heat-shock genes; *J. Biol. Chem.* **267** 21987–21990

Nystrom T and Neidhardt F C 1993 Isolation and properties of a mutant of *Escherichia coli* with an insertional inactivation of the *uspA* gene, which encodes a universal protein; *J. Bacteriol.* **175** 3949–3956

Palleros D R, Reid K L, Shi L, Welch W J and Fink A L 1993 ATP-induced protein-Hsp70 complex dissociation requires K^+ but not ATP hydrolysis; *Nature (London)* **365** 664–666

Peat A, Powell N and Potts M 1988 Ultrastructural analysis of

the rehydration of desiccated *Nostoc commune* HUN (cyanobacteria) with particular reference to the immunolabelling of NifH; *Protoplasma* **146** 72–80

Reed R H, Richardson D L, Warr S R C and Stewart W D P 1984 Carbohydrate accumulation and osmotic stress in cyanobacteria; *J. Gen. Microbiol.* **130** 1–4

Reed R H and Stewart W D P 1985 Evidence for turgor sensitive K^+ influx in cyanobacteria *Anabaena variabilis* ATCC 29413 and *Synechocystis* PCC 6714; *Biochim. Biophys. Acta* **812** 155–162

Reddy B R, Apte S K and Thomas J 1989 Enhancement of cyanobacterial salt tolerance by combined nitrogen; *Plant Physiol. (Bethesda)* **89** 204–210

Sambrook J, Fritsch E F and Maniatis T 1989 Detection and analysis of proteins expressed from cloned genes; in *Molecular cloning: A laboratory manual* (New York: Cold Spring Harbor Laboratory Press) 2nd edition, pp 18.60–18.74

Serrano R and Gaxiola R 1994 Microbial models and salt stress tolerance in plants; *Crit. Rev. Plant Sci.* **13** 121–138

Singh R N 1950 Reclamation of 'usar' lands in India through blue-green algae; *Nature (London)* **165** 325–326

Stacey G, Van Baalen C and Tabita 1977 Isolation and characterisation of a marine *Anabaena* sp. capable of rapid growth on molecular nitrogen; *Arch. Microbiol.* **114** 197–201

Thomas J 1970 Absence of the pigments of photosystem II of photosynthesis in heterocysts of a blue-green alga; *Nature (London)* **228** 181–183

Volker U, Engelmann S, Maul, Riethdorf S, Volker A, Schmid R, Mach H and Hecker M 1994 Analysis of the induction of general stress proteins of *Bacillus subtilis*; *Microbiology* **140** 741–752

Wood N B and Haselkorn R 1980 Control of phycobilin proteolysis and heterocyst differentiation in *Anabaena*; *J. Bacteriol.* **141** 1375–1385

Xu D, Duan X, Wang B, Hong B, Ho T D and Wu R 1996 Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice; *Plant Physiol.* **110** 249–257