

BRIEF COMMUNICATION

Heat shock proteins in the terrestrial epilithic cyanobacterium *Tolypothrix byssoidea*

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The cyanobacterial crust occurring in desiccated state exposed to high temperature and solar radiation on the rock surface contained several low molecular mass (LMM) proteins (10.5, 13, and 25 kDa), water stress protein (wsp, 39 kDa), additional proteins (43 and 49 kDa), a chaperonin (58 kDa), and a stress-induced protein (84 kDa). When the crust was exposed to UV-C radiation, it counteracted the UV-B damage by overproduction of certain proteins and synthesis of two high molecular mass (HMM) proteins. Exposure of the crust to heat had an adverse effect on the survival of the organism there-in which was due to repression of few proteins. Unlike the modification pattern of protein synthesis in the cyanobacterium inhabiting the crust, the same organism grown in culture could tolerate heat by synthesizing two HMM proteins. Several proteins of diverse groups were repressed in the cyanobacterium in culture upon exposure to UV-C, which was counteracted by induction of three new polypeptides (chaperonin and HMMs), and overproduction of one 41 kDa protein.

Additional key words: epilithic crust, temperature, UV radiation.

The cellular stress response, or heat-shock response, is involved in protecting organisms from damage due to exposure to elevated temperature, ultraviolet (UV) radiation and xenobiotics. The stress response entails the rapid synthesis of a suite of proteins, referred to as stress proteins which are heat inducible (Craig 1985, Schlesinger 1986). The accumulation of stress proteins correlates with acquired tolerance, wherein exposure to a mild stress confers the ability to survive a subsequent but more severe stress that otherwise would be lethal to the organism (Lindquist 1986).

Cyanobacteria must readily adapt to environmental changes to survive. Heat shock proteins (hsp) were identified in *Synechococcus* sp. PCC 6301, *Anabaena* sp. L-31, *Anabaena torulosa*, *Anabaena* sp. PCC 7120, *Plectonema boryanum* PC 3606, *Synechococcus* sp. PCC 7942, and *Phormidium laminosum* (Borbely *et al.* 1985, Blondin *et al.* 1993, Fernandes *et al.* 1993, Apte *et al.*

1997). In *Synechococcus* PCC 6301, a temperature shift from 39 to 47 °C resulted in an increase of at least 16 proteins in four size classes, 11 - 24, 45 - 49, 61 - 79, and 91 kDa (Borbely *et al.* 1985). Four proteins (32, 65, 75, 92 kDa) were identified when *Anabaena* L-31 cultures were moved from 30 to 40 °C and several additional stress proteins (19, 23, 82 kDa) were synthesized during heat shock, high salinity, and osmotic stress (Bhagwat and Apte 1989, Fernandes *et al.* 1993). *Anabaena* sp. PCC 7120, *P. boryanum* PCC 6306, and *Synechococcus* sp. PCC 7942 produced 33, 35, and 19 kDa hsp, respectively, though the response to heat shock was consistent for all the three strains (Blondin *et al.* 1993). Chitnis and Nelson (1991) isolated the *Synechocystis* PCC 6803 genes, groEL and dnaK, which encode proteins of the chaperonin 60 and 70 kDa hsp families, respectively. However, the full spectrum of proteins synthesized in response to heat shock has not been identified or characterized.

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Abbreviations: HMM - high molecular mass; hsp - heat shock proteins; LMM - low molecular mass; wsp - water stress protein.

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When some organisms are exposed to elevated, sub-lethal temperatures, they acquire thermal resistance to previously lethal temperature (Marimoto *et al.* 1990). Cells that do not produce functional hsp cannot become thermo-tolerant (Lindquist and Craig 1988, Webb *et al.* 1990), and those that overproduce hsp usually show increased thermo-tolerance (Plesset *et al.* 1982, Laszlo and Li 1985).

The cyanobacterium *Tolypothrix byssoidea* occurs in desiccated state on the exposed rock surface of Sun temple, Konark forming characteristic blackish brown crust even during hot summer months when the temperature exceeds 50 °C coupled with high solar radiation (Roy *et al.* 1997). The organism survived even after 24 h of UV-C irradiation and showed prominent absorption in the UV-region of the spectrum due to presence of mycosporine amino acid-like substances and scytonemin in the sheath layer (Adhikary and Sahu 1998). I compared by polyacrylamide gel electrophoresis (PAGE), the heat shock response of the cyanobacterial crust freshly collected from exposed rock surfaces during summer season and of the corresponding organism, *T. byssoidea* growing in culture. The ability of the organism to develop tolerance to UV-C, γ -radiation and heat (50 °C) by induction and/or over-production specific proteins was also examined.

The blackish brown crust from the exposed rock surface of the Sun temple, Konark was collected in early afternoons of dry and clear days during April - May coinciding with the mid summer season. The crusts were soaked in distilled water for 4 to 6 h and then observed under microscope for presence of cyanobacteria. Subsequently a pinch of the washed crust was transferred to BG11 medium with or without combined nitrogen (Rippka *et al.* 1979) and to agar plates (1 %, m/v, Difco nutrient agar in the same mineral medium) and incubated at room temperature under fluorescent irradiation. Visible growth of the yellowish green filaments of *T. byssoidea* was observed after few days. The cyanobacterium was grown in conical flasks with cotton stoppers containing nitrogen free BG11 (Rippka *et al.* 1979) medium and incubated at 25 ± 1 °C in a culture room under fluorescent tubes at irradiance of 7.5 W m^{-2} . The cultures were continuously bubbled with air through a millipore filter. The filaments were homogenised with a glass tissue homogeniser and aliquots of the suspension were used in the experiments.

Stress induced proteins were detected by *in vivo* radiolabelling with [^{35}S] methionine. *T. byssoidea* harvested in the mid exponential growth phase and the dried crusts soon after wetting in BG11 medium were used for experiments. Heat treatment involved exposure to 50 °C (1 h). UV-C radiation stress was applied for 1 h at the fluence rate of $9 \text{ J m}^{-2} \text{ s}^{-1}$ at a distance of 8 cm (15 W Philips T-UV lamp). Ionizing radiation stress of 100 krad from cobalt-60 source (*Gamma radiation cell 220*, AECL, Ottawa, Canada) was also applied. One cm^3 concentrated culture suspensions and the treated crusts were labelled

with $50 \text{ mm}^3 \text{ Bq } [^{35}\text{S}] \text{ methionine}$ (specific activity 1000 - 3000 $\text{Bq mol}^{-1} \text{ cm}^{-3}$) for the last 5 min of exposure to each stress followed by rapid centrifugation and washing of the cells in BG11 medium. The cells were then lysed by boiling for 5 min in a cracking buffer containing 125 mM Tris-HCl (pH 6.8), 5 % (m/v) 2-mercapto-ethanol, 2 % (m/v) SDS, 20 % glycerol, 20 mM sodium azide, 1 mM phenyl methyl sulphonyl fluoride, 20 mM EGTA, and 0.002 % (m/v) bromophenol blue. The cell extracts were centrifuged at 12 000 g for 10 min to remove the insoluble cell debris as a pellet. Incorporation of the label into proteins was estimated by TCA precipitation of 1 mm^3 of the above cell lysate, on a filter disc, which was washed in ethanol, air dried and counted in a liquid scintillation spectrometer (Packard Tri-Carb-3255, New York, USA). For proper comparison, equal amounts of radioactivity (based on TCA-precipitable counts) were loaded on to gels.

Proteins were electrophoresed on 5 to 14 % SDS-polyacrylamide linear gradient slab gels (0.75 mm thickness) in a vertical system (Hoefer Scientific Instruments, San Francisco, USA) overlaid with a stacking gel. The gradient gel was prepared by mixing 9 cm^3 each of freshly prepared solutions A and B through a gradient mixture. Solution A consisted of 0.375 M Tris/HCl (pH 8.8) and 20 % sucrose, 15 % acrylamide, 0.35 % bisacrylamide, 0.1 % SDS, and 0.035 % ammonium peroxydisulphate (APS). Solution B contained 0.375 M Tris/HCl (pH 8.8) and 5 %, acrylamide, 0.135 % bisacrylamide, 0.1 % SDS, and 0.035 % APS. TEMED ($\text{N,N',N',N'-tetramethylethylenediamine}$) was added, to both solutions A and B, at $0.54 \text{ mm}^3 \text{ cm}^{-3}$ just prior to gradient formation. The stacking gel contained 0.125 M Tris/HCl (pH 6.8) and 3.75 % acrylamide, 0.135 % bisacrylamide, 0.1 % SDS, 0.075 % APS, and $0.75 \text{ mm}^3 \text{ cm}^{-3}$ TEMED. Electrophoresis buffer contained 20 mM Tris-glycine (pH 8.3) and SDS (0.1 %, m/v). Molecular mass calibration kits (Sigma Chemical Co., St. Louis, USA) were used.

Electrophoresis was carried out at 40 V overnight (16 h) followed by 200 V for 1.5 h. Alternatively, day time runs at 100 V for 1 h followed by 200 V for 3 h were employed. The gel was stained with Coomassie Brilliant Blue R-250 to visualise the molecular mass markers and destained. The gel was then vacuum dried at 80 °C, for 1 h and exposed to X-ray film (INDU, Mumbai, India) for autoradiography. Typically 24 h exposure was given for 80 000 TCA precipitate counts per lane.

Proteins in which changes are usually induced by stresses (Table 1), belong to low molecular mass stress proteins, LMMs (10 to 25 kDa), wsp-proteins (39 kDa), the 42-group proteins (43 and 49 kDa), high molecular mass stress proteins, HMMs (100 to 130 kDa), chaperonins (57 kDa), stress-70 (62 and 69 kDa), and stress-90 group (84 kDa). Upon exposure of the cyanobacterial crusts to UV-C radiation, none of the proteins were repressed, two HMMs were induced, and several LMMs were

Table 1. Modification of protein synthesis in the epilithic cyanobacterial crust and the corresponding organism *Tolypothrix byssoidea* from culture exposed to γ -radiation, UV-C radiation, and heat stress.

Modification	Stress	Proteins [kDa] crust from exposed rocks	Proteins [kDa] <i>Tolypothrix</i> from culture
Repression	γ - radiation	41, 62, 69, 160	28, 37, 43, 49, 90, 105, 110
	UV-C radiation	-	28, 37, 40, 49, 52.5, 105
	Heat (50 °C)	49, 62, 69, 96, 140, 160	28, 37, 40
Induction	γ - radiation	-	57, 96, 140
	UV-C radiation	110, 130	57, 84, 130
	Heat (50 °C)	-	96, 130
Overproduction	γ -radiation	-	58
	UV-C radiation	10.5, 19, 25, 33, 41, 57, 84	43
	Heat (50 °C)	-	49, 58

overproduced. However, when the crusts were irradiated with γ -radiation or exposed to heat (50 °C, 1 h), 4 or 6 polypeptides were repressed and none of the proteins were either induced or overproduced. However, in the control where the crust collected from nature was used, several LMMs proteins (10.5, 13, 25 kDa), wsp 39 kDa, additional proteins of the 42 kDa group (43, 49 kDa), a chaperonin (57 kDa), and stress-90 group (84 kDa) were prominent (Table 1) which possibly is the reason for the adaptation of the cyanobacterium there-in to the extreme temperature and desiccation of the natural habitat. LMM stress proteins in the crust and those over-produced upon exposure to UV are of special importance unlike stress-90 and chaperonin are not synthesized under normal conditions. Their synthesis is induced under adverse environmental conditions and had long been implicated in thermotolerance (Landry *et al.* 1982, Lindquist 1986).

The protein profile of the cyanobacterial crust and the corresponding organism in laboratory culture showed a close similarity. However, several proteins were synthesized only in the cells inhabiting the crusts (Table 1). This may be because, the cells in the crust live in a different environment than those in the culture; in the crust several stresses might have altered the protein synthesis of the organism. Further, the crust might not contain only *T. byssoidea* cells and proteins of few other microbes in the

crust living in dormant state might have been expressed as visualized in the autoradiographs.

When the *T. byssoidea* cells grown in laboratory culture were exposed to similar UV-C dose, six proteins were repressed, three were induced, and one was overproduced (Table 1). Similarly, on exposing *T. byssoidea* cells to γ -radiation, 7 proteins were repressed; 3 proteins induced, and one overproduced. But when the organism from culture was exposed to heat (50 °C, 1 h), only three proteins of LMMs were repressed, two induced, and two overproduced. Thus unlike the modification pattern of protein synthesis in the cyanobacterium inhabiting the crust, the organism grown in culture could tolerate heat by synthesizing two HMMs, though three proteins under LMM group were repressed. Several proteins of diverse groups were also repressed in the organism upon exposure to UV-C and ionizing radiation stress, however, the adverse effect of repression of protein was counteracted by induction of three new polypeptides under chaperonin and HMMs group and over producing one under 42 kDa and chaperonin groups, respectively.

The fact that certain proteins are repressed or induced in response to such divergent stresses indicates that these proteins are important in the maintenance of vital cellular functions of the organism subjected to the stresses of the extreme environment on the exposed rock surface.

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