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ORIGINAL RESEARCH ARTICLE

Changes in hysteretic kinetics and properties of key regulatory enzymes of wild type & mutant strains of *Synechococcus cedrorum* during pesticide (endosulfan) stress combat

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Abstract: The effects of the hexachlorinated pesticide "endosulfan" on the regulatory kinetics of glucose -6-phosphate dehydrogenase (G6PDH EC 1.1.1.49) from wild type (WT) and pesticide resistant mutant type (End^R) strains of the cyanobacterium *Synechococcus cedrorum* were analysed. G6PDH from both strains was a "hysteretic" enzyme which could exist either in the aggregated "hyperactive" form which had an initial burst of activity or in the dissociated "hypoactive" form. The Km values for substrate G6P were 0.83 and 0.96 µM in WT and End^R strains, respectively. Hyperactivity following growth in endosulfan increased in the End^R strain whereas it decreased in the WT strain. These changes were accompanied by synthesis of a new class of membrane localized proteins with molecular weights of about 70-80 kDa The Km of nitrate reductase of wild type and mutant strains were observed to be 15.3 and 29.4 µM, respectively. This was concomitant with phycobilisome disintegration in the wild type only, diagnostic of nitrogen deficiency. The results taken together point to a coordination of carbon and nitrogen metabolism during endosulfan stress combat emphasizing the role of global regulatory networks of the metabolome.

Key words: hysteretic; endosulfan; *Synechococcus cedrorum*

Introduction

Endosulfan is an organochlorine acaricide that has been shown to be hazardous to some non-target organisms.1-3 Cyanobacteria, by virtue of their cosmopolitan habitat4, encounter a wide range of chemical toxicants. In this study, a fresh water, unicellular, non-N2-fixing cyanobacterium Synechococcus cedrorum has been used as a model.

Cyanobacterial glucose-6-phosphate dehydrogenase (G6PDH) is a typical enzyme of the hysteretic type. Hysteretic enzymes undergo relatively slow reversible transitions between different states of aggregation, each having a different degree of catalytic activity. In Anabaena sp. three principal forms of G6PDH with approximate molecular weights of 120, 000 (M1), 240, 000 (M2) and 345, 000 (M3) have been reported5. The equilibrium between these different forms is shifted in favour of the more active oligomeric forms at high enzyme concentrations, high levels of G6P, and a slightly acidic pH (6.5). Dilution in the absence of substrates, NADP+, RuDP and alkaline pH, shift the equilibrium in favour of the M1, form with marked dimunition in catalytic activity. G6PDH from several cyanobacterial sources is reported to be inhibited by products of photosynthesis like NADPH and ATP5-7, by light and/or by dithiothrietol, a reducing agent6-8, by reduced thioredoxin8, 9 and by virus infection.10, 11These effectors have been reported to affect activity of G6PDH from several other cyanobacterial sources also.12-16 Such regulation of G6PDH by metabolites has also been reported from a variety of unrelated organisms, e.g. inhibition by NADPH in fungi, sweet potato tuber, bacteria, pea chloroplasts [17, 18, 19, 20]. Being subject to the control of a number of metabolic regulators, the stress response would also be mediated via regulation of this key enzyme; possibly by way of inter-conversions between its different forms.

Further, in order to overcome the detrimental effect of pesticides, N2-fixing cyanobacteria have been reported to replete their nitrogen resources by increasing their N2 -fixing capacity21-22 or depend on reserve nitrogen found within the cell. While cvanophycin is the nitrogen reserve found in all cyanobacteria23, evidence is now rising that phycobiliproteins, viz. phvcocvanin (PC), allophycocvanin (AP) and phycoerythrin (PE) which are aggregated together as phycobilisomes in an arrangement favourable for efficient solar energy transfer, also serve as nitrogen reserves24-28.

The catalytic action of assimilatory nitrate and nitrite reductases helps in the incorporation of nitrate which is the most common form of naturally available nitrogen for most photosynthetic organisms. 29Nitrate is reduced to nitrite in a 2-electron reduction catalysed by nitrate reductase (NR) and the resulting nitrate to ammonium in a 6-electron reaction catalysed by nitrite reductase (NiR)30.

The present work analyses (a) the nature of G6PDH from wild-type (WT) and endosulfan resistant mutant (EndR) strains of the cyanobacterium Synechococcus cedrorum (b) the change in hysteretic properties of G6PDH from both of the above strains in response to pesticide stress. (c) the effect of endosulfan on nitrate & nitrite reductases and phycobilisome integrity during pesticide stress.

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Materials and Methods

Organism and growth conditions. Synechococcus cedrorum obtained from U.S. Environmental Protection Agency Cincinna-ti, Ohio (U.S.A.), was grown in Hughes medium modified by Allen 31 buffered to pH 7.5 with 20 mM HEPES-NaOH buffer, the cultures were incubated at 28±2°C and illuminated with cool white fluorescent light having a photon fluence rate of 50 µmol m-2 sec-1 at the surface of the vessels.

Mutant isolation. WT cells were mutagenised with 100 mg ml-1 N-methyl-N ' -nitro-N-nitrosoguanidine (MNNG) in 50 mM citrate buffer pH 6.0 for 15 min in light under shaking conditions at room temperature 32, Survivors were plated on plates containing increasing concentrations of endosulfan.

After 30 days, mutant colonies appeared on the plate containing maximum pesticide dose (ll.0 mg1-1). One such colony was picked and inoculated in the same concentration of endosulfan. The mutant was designated EndR.

Extraction of G6PDH. All operations were carried out at 0-4°C. 1 g wet weight of packed fresh cells from exponentially growing cultures of WT and EndR strains were suspended in 3-fold volume of 50 mM Tris-maleate buffer, pH 6.5 containing 10 mM MgCl2, 0.1% (v/v) 2-mercaptoethanol and 10 mM G6P5. The suspension was sonicated using ultrasonicator (Soniprep, MSE Scientific Instruments, Crawley, England) at 15 microns amplitude, for 15 min. The suspension was centrifuged at 10, 000 × g for 10 min and the supernatant was used as enzyme stock solution. 34

Assay of G6PDH activity. G6PDH activity was assayed by measuring the rate of NADP reduction by following the change in absorbance every 30 sec. at 340 nm, at 25°C using "Kinetics Soft PacTM module" of UV-visible spectrophotometer (DU-64, Beckman). The reaction was initiated by the addition of enzyme stock solution to the reaction mixture which contained in a final volume of 1 ml: 5 mM G6P, 0.5 mM NADP+ and 10 mM MgCl2 in 50 mM Tris-maleate buffer pH 7.5 5.

Measurement of lag time, l (tau). Aliquots of the enzyme stock solution were added in 0.95 ml 50 mM Tris-maleate buffer, pH 7.5, containing 10 mM MgCl2. These were incubated in ice for 10 min and the reaction was started by the addition of 0.05 ml solution of G6P and NADP having a final concentration of 5 and 0.5 mM, respectively. The course of NADP reduction was measured until reaction rates became constant. The lag time was determined graphically from the intercept of the extrapolation of the steady state rate with the time axis 5.

Pesticide treatment. Exponentially growing cells of WT and EndR strains were grown in several parallel replicates. On the second day of growth, the commercial formulation of endosulfan 35% EC was added at the rate of 1.1 mg, 2.2 mg, 4.4 mg and 11.0mg active ingredient per litre of culture. The pesticide stock solutions prepared in double distilled water were filter sterilized (0.45 µm millipore) before being added to the cultures. One flask each of WT and EndR without addition of endosulfan served as control. After 72 h growth in endosulfan, G6PDH was extracted from cells and activity assayed as described above.

Determination of reaction kinetics of G6PDH with respect to substrate (G6P). G6PDH activity was assayed with varying G6P concentrations and saturating NADP+ (0.5 mM) concentration. Kinetic constants, Km and Vmax were determined by double - reciprocal plots 35.

All assays were performed with equal aliquots of enzyme stock solution so that the final concentration of enzyme in 1 ml remained constant (30 pg). Care was also taken to see that the enzyme stock solution always contained a protein content of 300pg / ml. Protein contents in the extracts were measured as given 36.

Isolation of membrane proteins for analysis by SDS-PAGE. Cells of WT and EndR strains were treated for 72 h with 4.4 and 11.0 mgl-1 endosulfan, respectively, since these were the concentrations at which there was maximum inhibition of growth. These and their respective controls without endosulfan treatment were harvested and transferred with one washing to 10 mM Tris-HCl buffer, pH 7.5 containing 2% (v/v) 2-mercaptoethanol and 1 mM phenylmethylsulfonylfluoride (PMSF). This was sonicated at an amplitude of 15 microns for 20 min at 4°C (Soniprep, MSE) and centrifuged at 10, 000 x g for 10 min at 4°C to collect membrane fractions in the pellet. These were taken up in cracking buffer made in 10 mM Tris-HCl buffer, pH 8.0 containing 1 mM EDTA, 2.5% SDS, 5% 2-mercaptoethanol, 10% glycerol, 1 mM PMSF and 0.01% tracking dye, bromophenol blue. Before loading, samples were heated for 3 min at 100°C. 20-30 µg protein from all samples was loaded. SDS-PAGE was performed according to Laemmli37 on 10-20% step-gradient slab gels. Gels were run overnight at 4°C under a constant current of 10 mA. Peptides were visualised by staining with Coomassie Brilliant Blue R-250.

Assay of nitrate reductase (NR) and nitrite reductase (NiR) in situ in WT and EndR strains Activity levels of NR 38 and NiR 39 were determined in cells permeabilised with 1% lysozyme 40, using dithionite-reduced methylviologen as reductant. One unit (U) of enzyme activity corresponded to 1 uM of nitrite formed (NR) or nitrite disappeared (NiR) per minute. Nitrite was determined colorimetrically 41 Kinetic constants Km and Vmax were determined from double reciprocal plots35

NR and NiR activities of WT and EndR strains in situ, after treatment with endosulfan Exponentially growing cells of WT and EndR strains of cell density whose A660 = 0.7 to 0.75, were treated with 4.4 and 11 mgl-1 endosulfan, respectively, since these were the concentrations at which there was maximum inhibition of growth in both the strains. One flask each of WT and EndR unsupplemented with endosulfan served as control. After 72h of incubation in endosulfan, cells were harvested, washed, permeabilised and assayed for NR and NiR activities with 20µM KNO3and 100µM NaNO2, respectively.

Measurement of Hill activity and chlorophyll a fluorescence
Hill activity was measured by following reduction of dichlorophenolindophenol (DCPIP) 42, in permeabilised cells of WT and EndR treated for 72h with 4.4 mg 1-1 and 11 mg 1 -1 endosulfan44.

Chlorophyll a fluorescence was measured using a photofluorimeter (Systronics India Ltd.) fitted with a tungsten halogen lamp (SOW) and two monochromatic corning filters for excitation (435 nm) and emission. Endosulfan treated (72h) and untreated cultures of WT and EndR were normalised to equal concentrations of Chl a (approximately 2µg ml-1 by dilution with medium. Cells were dark adapted for 30 min before assay 45. Fluorescence was recorded as per cent fluorescence relative to excitation energy.

Extraction of phycobiliproteins from cells broken by ultrasonication (15microns amplitude, for 20 min at 4°C) phycobiliproteins were extracted from endosulfan-treated (72h) cells of WT and EndR strains and their untreated controls, as described by Tandeau de Marsac and Houmard45. Quantitation of phycobiliproteins, phycocyanin (PC), allophycocyanin (AP) and phycoerythrin (PE) was made from absorbance values at 620 nm, 650 nm and 565 nm using the equations of Bryant et.al. 46

Isolation of phycobilisomes for electrophoretic analysis
Phycobilisomes were extracted at room temperature as per procedure of Grossman and Brand 47 and untreated WT and EndR strains. The phycobilisomes were taken up in cracking buffer made in 10 nm Tris-HCl buffer, pH 8.0 containing 1 mM EDTA, 2.5% SDS, 5% 2-mercaptoethanol, 10% glycerol. 1 mM phenylmethylsulfonylfluoride (PMSF) and 0.01% tracking dye, bromophenol blue. Before loading, samples were heated for 3 min at 100°C. 20-30 µg protein from all samples was loaded. Standard protein mixture was also run simultaneously with the samples. Electrophoresis was performed according to Laemmli37 at 4°C under a constant current of 10 mA on 10-18% step-gradient slab gels (gel) size (0.15 x 15 x 15 cm).

Results

Effect of dilution on the kinetics of NADP reduction by G6PDH. When aliquots of the enzyme stock solution were added to reaction mixture containing substrates, there was an initial burst of activity, followed by a lower constant rate. The activity curve followed a hyperbolic pattern hence enzyme in this state was called "hyperactive" enzyme (Fig. 1).

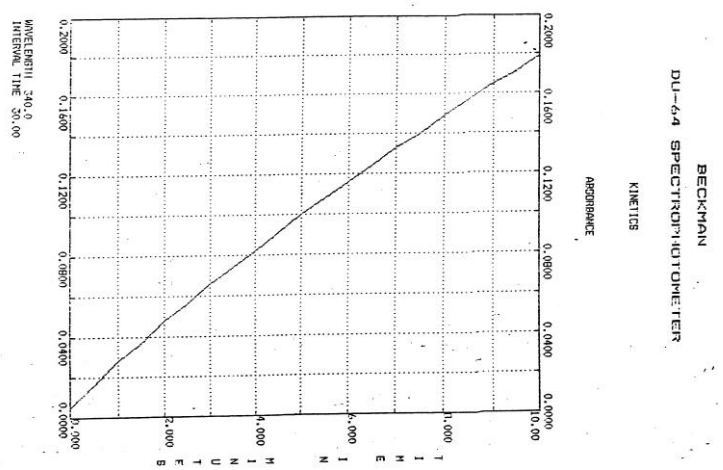

Fig. 1: Direct recording of activity of G6PDH in WT strain of Synechococcus cedrorum after 0 min of dilution in absence of substrates.

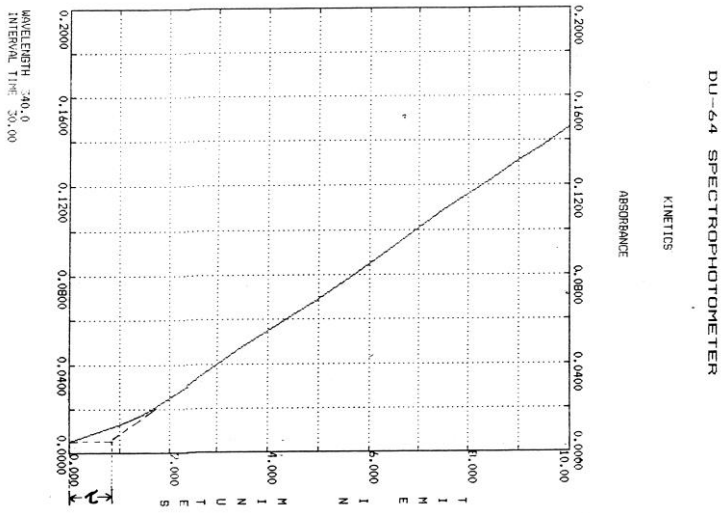

Fig. 2: Direct recording of activity of G6PDH in WT strain of Synechococcus cedrorum after 10 min of dilution in absence of substrates.

When the same volume of enzyme stock solution was diluted in the absence of substrates for 10 min and the reaction started thereafter by addition of substrates, there was an initial lag (Fig. 2). The activity followed a hypobolic curve. Hence the enzyme in this state was called "hypoactive" enzyme.

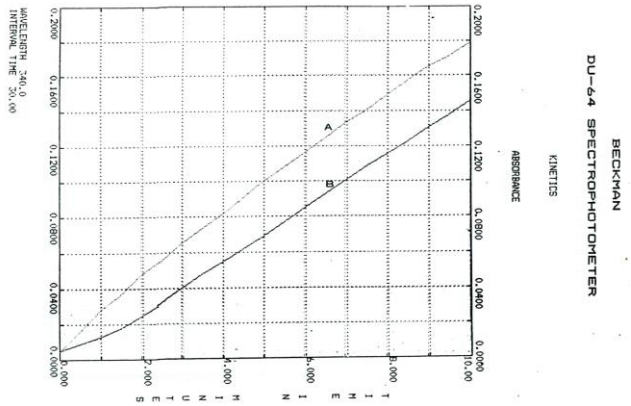

Fig.3: Superimposition of direct recordings of hyperactivity (A) and hypoactivity (B) curves of WT strain showing the same constant steady state velocity

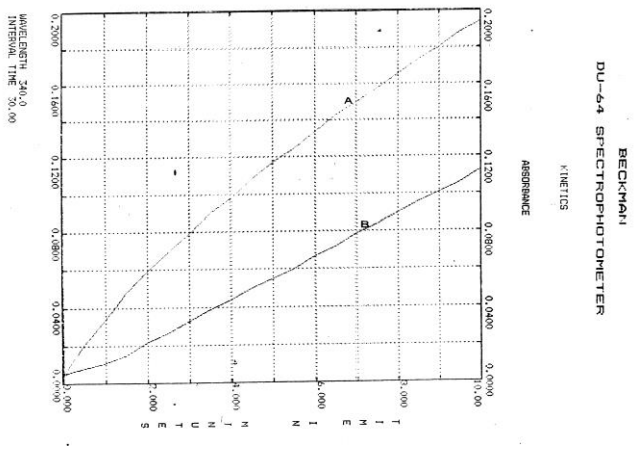

Fig.4: Superimposition of direct recordings of hyperactivity (A) and hypoactivity (B) of G6PDH of EndR strain.

The two forms viz. hyperactive and hypoactive reached the same constant rate after 5 min (Fig. 3). G6DPH in the End R strain also showed the same dilution effect (Fig. 4).

Effect of varying concentrations of endosulfan in vivo on G6PDH activity. The WT and EndR mutant strain showed marked differences in the activities of G6PDH extracted from cultures growing for 72 h in the presence of endosulfan. The initial velocity (vo) of hyperactive G6PDH decreased with increasing concentration of endosulfan as compared to the control culture devoid of endosulfan. At 11.0 mg l-1 endosulfan, no activity was observed. In contrast, the vo of hyperactive G6PDH of End R strain increased with increasing doses of endosulfan upto 4.4 mgl-1 and decreased only at a considerably high dose of endosulfan (11.0 mgl-1) as compared to control (Fig 5 &6).

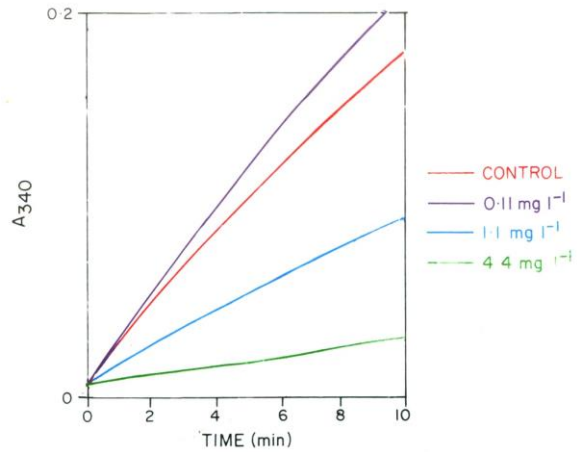

Fig.5: Hyperactivity of G6PDH from WT strain following growth for 72 h in varying concentration of endosulfan.

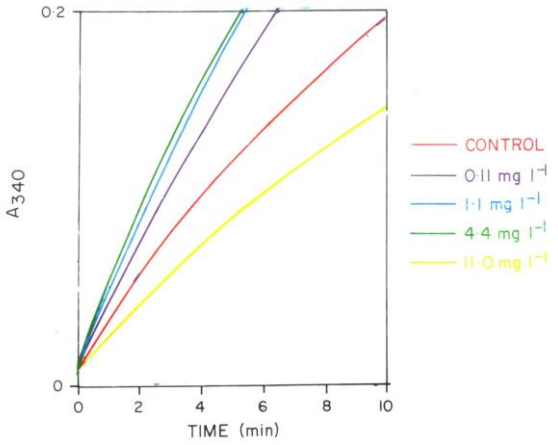

Fig.6: Hyperactivity of G6PDH from EndR strain following growth for 72 h in varying concentration of endosulfan.

Dilution of the same aliquots of enzyme stock solution resulted in an increase in lag period (Tau) as compared to the control (Table 1).

Table 1: Effect of endosulfan on time-lag in hypoactivity of G6PDH of WT and EndR strains of Synechococcus cedrorum

Endosulfan(mgl^{-1})	*I* (tau in sec)	
	WT	End^{R}
0.0	30.0	25.7
0.11	26.0	17.1
1.1	34.0	15.9
4.4	180.0	15.9
11.0	NA	19.9

The cells of WT and EndR strains were incubated for 72h in varying concentration of endosulfan under standard culture conditions.
NA = No activity
(l) = Time-lag

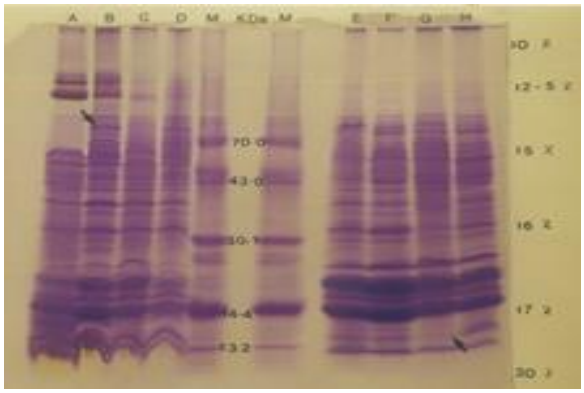

Figure 7: SDS-PAGE of membrance proteins (A-D) and cytoplasmic proteins (E-H) from WT and EndR strains of Synechococcus cedrorum
Lanes A & E – WT untreated, Lanes B & F – EndR untreated, Lanes C & G – WT strains treated with 4.4. mg l-1 endosulfan for 72h, Lanes D & H – EndR strain treated with 11.0 mg l-1 endosulfan for 72h, Lane M – Standard markers (MW in Kda)

SDS-PAGE profile of membrane proteins. Electrophoretic resolution of membrane proteins distinctly showed that new popypeptides (70-80 kDa) were synthesized in the

endosulfan resistant EndR strain even when grown in absence of endosulfan (Fig.7 lane B). These polypeptides were altogether absent in membrane proteins of WT strain (Fig.6 lane A). 72 h of incubation of the cells in endosulfan induced the synthesis of these polypeptides in the WT strain as evidenced by a few faint bands at the same position (Fig. 7 lane C).

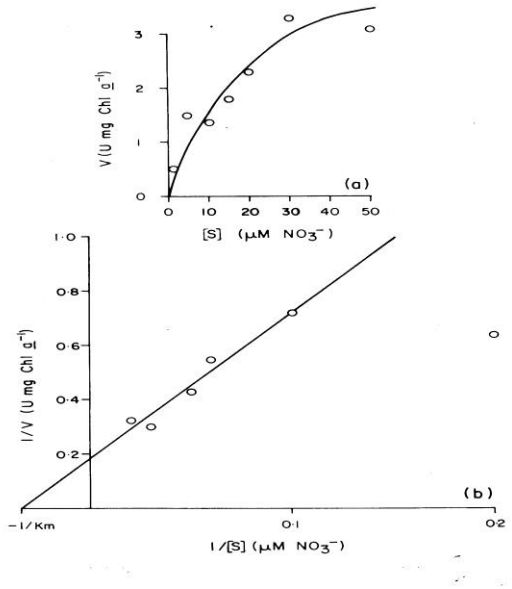

Fig.8(a): Michaelis-Menten and (b) Lineweaver-Burk plots of nitrate reductase activity in WT
(IU = mM NO2- formed min-1

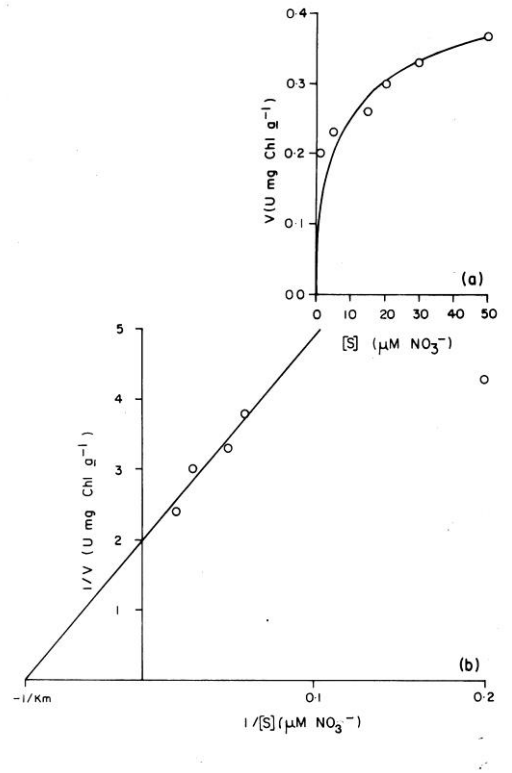

Fig.9(a): Michaelis-Menten and (b) Lineweaver-Burk plots of nitrate reductase activity in EndR (IU = mM NO2- formed min-1)

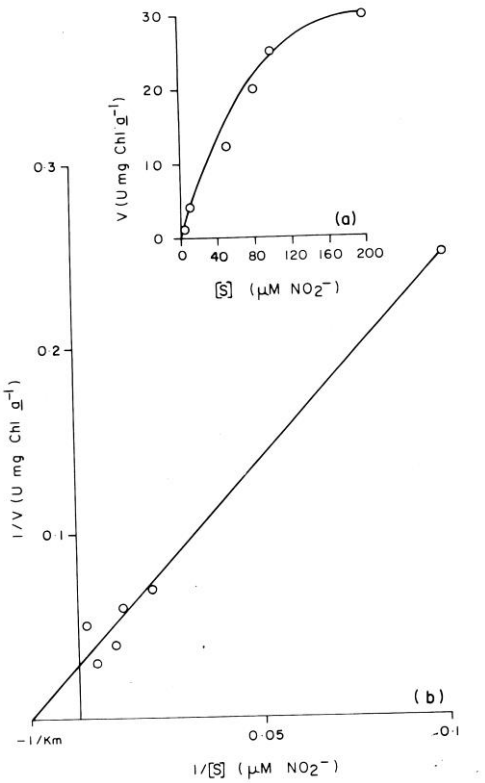

Fig.10: NiR activity as a function of nitrite concentration in WT and EndR strains (a) Michaelis-Menten and (b) Lineweaver-Burk plots. (IU = mM NO2- disappeared min-1).

Dependence of nitrate reductase (NR) and nitrite reductase (NiR) activities on substrate concentrations in WT and End R strains

Both NR and NiR activities in WT and End R strains followed Michaelis-Menten saturation kinetics with respect to their substrates (Figures 8, 9 &10, respectively)). The rates of maximum velocity (Table 2) achieved by the NR in EndR strain (0.5 U mg Chl a-1 min-1) was an order of magnitude less than that of WT strain (5.5 U mg Chl a -1min-1). However, NR from the EndR strain had a two-fold greater affinity for its substrate (Km =15.3 μm) as compared to the NR from the WT strain (Km = 29.4μm) (Fig. 8 & 9).

The NiR from both WT and EndR strains was identical with Km of 76.6 μM and Vmax of 0.03 Umg Chl a-1 min-1 (Figure 10).

Effect of endosulfan on nitrate and nitrite reductases of WT and EndR strainsNR and NiR activities following endosulfan treatment decreased in the WT strain but increased in the EndR strain (Table 2). NR activity in untreated EndR strain (0.66 UmgChl a-1 min-1) was around ten fold less than NR activity in the untreated WT (5.5 U mg Chla -1min-1).

Table 2: Effect of endosulfan on various activities in WT and EndR strains of Synechococcus cedrorum

Activity	WT		End^R	
	control	treated	Control	Treated
NR(U mg chla^{-1})	5.50	4.70	0.66	1.32
NiR(u mg chla^{-1})	30.80	19.90	22.70	60.70
Hill activity (mM DCPIP reduced mg Chl *a*min^{-1})	53.00	21.60	63.00	57.00
Photosynthetic O_2 evolution (µM mg Chl a^{-1}min^{-1})	2.60	2.00	4.10	3.00
O_2consumption (µM mg Chl a^{-1}min^{-1})	7.36	4.60	8.50	10.00
Relative fluorosence (%)	5.00	9.00	5.00	4.5

The cells of WT and EndR strains were incubated for 72h with endosulfan (4.4 mg 1-1) for WT and 11.0 mg 1-1 for EndR strain) under standard culture conditions.

The results are the mean of three independent experiments.
Effect of endosulfan on Hill activity and Chl a fluorescence
Table 2 shows that after 72h of incubation in endosulfan, there was a four-fold increase in the Hill reaction activity of WT strain (as depicted by DCP1P reduction rates). The activity in the EndR strain was not much affected by endosulfan treatment and was almost similar to the Hill reaction activity of WT control culture.

A similar pattern of response was observed by relative fluorescence emission of Chl a. The treated WT strain showed an almost 2-fold increase as compared to its untreated control. In EndR strain, a slight decrease in relative fluorescence in the endosulfan treated culture was observed.

Changes in phycobiliproteins following endosulfan treatment in WT and EndR strains Colorimetric analysis of extracted phycobiliproteins showed that endosulfan treatment caused considerable loss of PC and AP and the complete loss of PE in WT strain (Table 3).

Table 3: Change in phycobiliproteins in response to endosulfan treatment in WT and EndR strains of Synechococcus cedrorum

Activity	WT		End^R	
	Control	Treated	Control	Treated
PC	33.00	0.40	23.00	2.00
AP	47.00	1.40	40.00	12.00
PE	2.00	0.00	1.00	5.00
PC/AP	0.70	0.28	0.57	0.16

The cells of WT and EndR strains were incubated for 72h with endosulfan (4.4 mg 1-1) for WT and 11.0 mg 1-1 for EndR strain) under standard culture conditions. The results are the mean of three independent experiments.
PC = Phycocyanin AP = Allophycocyanin PE = Phycoerythrin

Similar results were also observed in EndR strain. However, the PE content showed a five-fold increase. The fall in PC/AP ratio of WT and EndR strains after incubation in endosulfan, as compared to the untreated control cultures, was indicative of ultrastructural changes in the phycobilisome assembly. The difference in PC/AP ratio between the treated and untreated WT strain was 0.22 while it was 0.41 in the EndR strain. These correspond to the loss of 1.3 and 2.6 PC hexamers in WT and EndR strains respectively 48

Effect of endosulfan treatment on phycobilisomes of WT and EndR strains Analysis by sodium dodecyl su]fate polyacrylamide gel electrophoresis (SDS-PAGE) (Figure7) revealed the loss of structural integrity of phycobilisomes of the WT strains due to incubation in endosulfan for 72h. As such they could not be isolated (and hence did not appear in the gel electrophoretic run) under conditions identical to those in which phycobilisomes from the pesticide-treated and untreated EndR mutant were isolated. The process of isolation yielded energetically intact phycobilisomes from the other cultures, as observed from absorbance spectra of isolated phycobilisomes. (results not shown).

Discussion

Like the enzyme from other cyanobacterial sources 5, 51-53, G6PDH from WT and EndR strains of Synechococcus cedrorum is a hysteretic enzyme which exists in a hyperactive associated form or hypoactive dissociated form. The hypoactive form shows an initial lag in its activity after addition of substrates. The lower catalytic activity of the hypoactive form causes a greater lag. The duration of lag depends on the state of molecular aggregation at the time of substrate addition5, 20a characteristic feature of hysteretic enzymes. Thus, as an outcome of hysteresis, G6PDH activity essentially depends on the relative abundance of the hyperactive oligomeric form and hypoactive monomeric forms at the time of substrate addition and activity measurement. The present study shows that G6PDH of EndR strain has a greater concentration of hyperactive oligomer than the WT strain after growth for 72h in endosulfan accounting for (a) higher burst in activity and (b) shorter lag periods in the EndR strain at all doses of the pesticide. A similar shift in equilibrium towards hyperactive form has also been observed in Anacystis nidulans upon cyanophage infection10, In fact, G6PDH activity increases whenever cyanobacteria are exposed to energy requiring conditions, for example, to maintain metabolism at high temperatures in order to exist as thermophiles56, or when solar energy is not utilized, by blocking PS II using DCMU in Aphanocapsa sp.57, under heterotrophic growth regimes in several cyanobacteria 18, 58, 59 and in dark in pea leaf chloroplasts60, A high rate of G6PDH activity also exists in heterocysts 58, 59 and in germinating akinetes 57 (as evidenced from increased respiratory oxygen uptake and decreased carbon content) where the energy intensive processes of N2- fixation and germling formation take place, respectively. Combating adverse conditions results in increase in activity of oxidative pentose phosphate pathway, particularly of G6PDH activity in several other organisms; in sweet potato tuber upon infection with black-rot fungus Ceratocystis fimbriata 60 and aerobic incubation of sliced sweet potato tissue61.

This stress response is absent in the WT strain in which the equilibrium does not shift to the oligomeric forms, but rather in the opposite direction resulting in the decrease in activity upon endosulfan addition during growth.

The End R strain may also be enabled to counteract the effects of Endosulfan by the ability to inherently synthesise a new class of membrane- localized proteins even in the absence of the pesticide. The resultant demand for reduced nitrogen is met with a doubly efficient NR of EndRas

apparent from Km value of NR. This creates a nitrogen deficiency due to increased rates of nutrient uptake from the growth medium which effectively makes the cell environment nitrogen deficient65. Increased rates of nutrient uptake have been reported in cells grown in media minus nitrogen source65, 66. Protein synthesis is inherently upscaled in EndR strain even in the absence of endosulfan in order to synthesise a new class of membrane-localised (70-80 kDa) proteins, as observed in SDS-PAGE profile of membrane proteins following 72h treatment with endosulfan. In the WT, these proteins are synthesised only in the presence of endosulfan at a much lower level. This is because the WT is limited by the inefficiency of its NR (Km =2.9μM) as compared to the almost doubly efficient NR in EndR (Km = 15. 3μM) strain. The lower Vmax values (0.5 U mg Chl a), is attributed to the greater degree of nitrogen deficiency that results from a more efficient NR activity. Nitrogen deficiency is reported to cause a drastic decrease in NR activity of illuminated Anacystis nidulans cells, lacking any nitrogenous nutrient in the media 68.

The less efficient NR of WT strain compels the breakdown of phycobilisomes as a source of reduced nitrogen. This is not observed in the EndR strain whose phycobilisomes are intact (Figure 7). Phycobilisome breakdown mediated by membrane-bound proteases under nitrogen starvation has been reported in Synechococcus 6301 25. Phycobiliprotein loss due to pesticide stress, as observed in this study, has also been reported to occur under nitrogen deprived conditions25, 64. The fall in PC/AP ratio due to endosulfan treatment has also been observed to occur due to high light stress in *Agmenellum quadruplicatum* PR-6 (Synechococcus.sp. PCC7002)47. Among the phycobiliproteins, PE shows the most dramatic changes. While it is completely lost in the WT strain after endosulfan treatment, it increases five-fold in the EndR strain following endosulfan treatment. This suggests that the EndR strain, under a stress condition develops the capability to store the nutrient which is likely to be limiting, i.e., nitrogen. Here, nitrogen is stored as PE. These results are consistent with the elucidated role of surplus PE as nitrogen reserve with the concomitant decoupling of energy transfer to prevent photooxidative damage to the photosynthetic reaction centre in Synechococcus sp. WH 780363. A similar decoupling of energy transfer by PE could have occurred in the present case since increase in PE was not accompanied by increase in DCPIP reduction rates. The disarray of phycobilisomes in the treated WT causes inefficient energy transfer so that an increased proportion of excitation energy is lost as fluorescence.

These results taken together show that the mutant EndR strain achieves resistance to 2.5-fold higher doses of endosulfan by regulation of the hysteretic behaviour of G6PDH, mediated via protein factors. Both the inherent abundance of hyperactive form and the ability to shift equilibrium towards the hyperactive form meets the increased energy demand during the energy intensive stress conditions of pesticide stress combat in the mutant EndR strain..These integrated metabolic changes enabled by alterations in its blueprint-DNA as evident from de novo protein synthesis.These point to a coordination of carbon and nitrogen metabolism during endosulfan stress combat emphasizing the role of global regulatory networks of the metabolome.

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