Iron induced metabolic changes in the diazotrophic cyanobacterium 

*Anabaena* PCC 7120

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Iron induced changes in growth, N₂-fixation, CO₂ fixation and photosynthetic activity were studied in a diazotrophic cyanobacterium *Anabaena* PCC 7120. Iron at 50 µM concentration supported the maximum growth, heterocyst frequency, CO₂ fixation, photosystem I (PS I), photosystem II (PS II) and nitrogenase activities in the organism. Higher concentration of iron inhibited these processes. Chl *a* and PS II activities were more sensitive to iron than the protein and PS I activity.

**Keywords:** *Anabaena*, Cyanobacterium, Iron, Metabolic change

Iron acts as a necessary cofactor of many enzymes and is often crucial for the catabolic activity. The effect of iron stress on metabolism of cyanobacteria reflected loss of biochemical pathway¹, rather than decrease in substrate transport. Low solubility of iron in water limits its availability for biochemical processes like photosynthesis and N₂-fixation. Nitrogenase, the key enzyme responsible for N₂ fixation contains up to 36 iron atoms per enzyme complex². Iron containing electron transport proteins (cytochromes, non-heme iron proteins, and ferredoxin) are also required to supply sufficient reductant for N₂-fixation³. Iron stress also leads to alterations in the photosynthetic apparatus resulting in decreased phycocyanin and chlorophyll content and reduced photosynthetic activity⁴,⁵.

In the present study the effect of exogenous iron on growth, PS I and II activities, ¹⁴CO₂ and N₂-fixation of the diazotrophic cyanobacterium *Anabaena* PCC 7120 has been investigated.

**Materials and Methods**

*Organism and culture conditions—* *Anabaena* PCC 7120 (obtained from National Centre for Conservation and Utilization of Blue Green Algae, IARI, New Delhi) was axenically grown in modified Chu 10 medium⁶ devoid of combined nitrogen source. The medium in the final volume of 1 litre contains (mg): CaCl₂, 2H₂O, 55; MgSO₄, 7H₂O, 25; K₂HPO₄, 10; Na₂CO₃, 20; Na₂SiO₃, 44; Ferric citrate, 3; citric acid, 3; MnCl₂, 1.81; H₂BO₃, 2.86; MoO₃, 0.018; ZnSO₄, 7 H₂O, 0.222; CuSO₄, 5H₂O, 0.079 and CoCl₂, 6H₂O, 0.010. The medium was buffered to pH 7.5 with 4-(2–hydroxy ethyl)–1-piperazine ethane sulphonic acid (HEPES)/NaOH. The cultures were maintained at 25°±1°C and illuminated with daylight fluorescent tubes having the photon fluence rate of 50 µmol m⁻² s⁻¹ at the surface of the vessel with 18/6 hr light/dark cycle. The Chu 10 medium devoid of iron was passed through a chelex 100 (Biorad) column (pH 7.5) and filtered through acid washed polycarbonate filter (pore size 0.4 µm) to make it completely iron free. For experiments, FeCl₃ was added to the basal medium to obtain Fe concentration of 20, 50, 100, 300 and 500 µM in the culture.

Growth was measured in terms of increase in the protein and chl *a* contents. Chl *a* was estimated following Mackinney⁷. Protein was assayed using the method of Lowry *et al*.⁸ with lysozyme (Sigma) as the standard. Heterocyst frequency was calculated as number of heterocysts per hundred vegetative cells. It was determined microscopically taking ten

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independent replicates. Nitrogenase activity was assayed following the acetylene reduction technique. Exponentially growing Anabaena PCC 7120 cells (5 ml) cultured in presence of different concentrations of iron were placed in 15 ml serum vials with 10% acetylene (v/v) and incubated for 1 hr at 25°C under fluorescent light having the photon fluence rate of 50 µmol m⁻² s⁻¹. One ml aliquot of the gas phase was withdrawn from each vial and the amount of C₂H₄ formed was assayed using a Gas Chromatograph (14C-Shimadzu, Japan) fitted with a Flame Ionization Detector (FID) (porapack R column; injector temp: 70°C; detector temp: 90°C; carrier gas N₂ flow: 30 ml min⁻¹). Nitrogenase activity was expressed in terms of nmol C₂H₄ produced mg⁻¹ protein min⁻¹.

CO₂-fixation was assayed by measuring the incorporation of NaH¹⁴CO₃ (final concentration 0.05 µci ml⁻¹) in the cells following the method followed by Pandey et al. The amount of¹⁴C incorporation was quantified using a Liquid Scintillation Counter (Wallac 1409, Finland) and the incorporation was quantified using a Liquid Scintillation Counter (Wallac 1409, Finland) and the incorporation was quantified using a Liquid Scintillation Counter (Wallac 1409, Finland) and the incorporation was quantified using a Liquid Scintillation Counter (Wallac 1409, Finland).

Results and Discussion

Changes in growth in terms of chl a and protein of Anabaena PCC 7120, in presence of different concentrations of iron (20-500 µM) were studied. The cells devoid of iron (iron-starved) contained slightly lower level of protein and chl a as compared to the control culture with 20 µM iron (Table 1). Further increase in the concentration of iron to 50 µM resulted in a maximum increase in protein and chl a content. However, higher concentrations of iron in culture (100-500 µM) resulted in a significant decrease in both the chl a and protein contents; chl a showed higher sensitivity to iron as compared to protein (Table 1). The persistence of significant amounts of protein as well as chl a in the cells cultured without iron may presumably be due to siderophore-mediated intracellular iron build-up inside the cells. These results are in agreement with those reported earlier in Anabaena sp.

Heterocyst frequency also increased with increase in iron concentrations and attained its maximum level (10.5%) at 50 µM iron (Table 1). Higher concentration of iron (100-500µM) resulted in a significant reduction in heterocyst differentiation. Nitrogenase activity also increased with increase in iron concentration up to 50 µM, however, further increase in its concentration (100-500 µM) resulted in a significant inhibition in enzyme activity (Table 1). This shows that similar to growth, heterocyst

Table 1—Iron induced changes in chlorophyll a, protein, heterocyst frequency, ¹⁴CO₂ fixation, nitrogenase, PS I and PS II activity of Anabaena PCC 7120

<table>
<thead>
<tr>
<th>Iron (µM)</th>
<th>Chl a (µg ml⁻¹)</th>
<th>Protein (µg ml⁻¹)</th>
<th>Heterocyst Frequency (%)</th>
<th>Nitrogenase activity (nmol C₂H₄ produced mg⁻¹ protein min⁻¹)</th>
<th>¹⁴CO₂ fixation (CPM × 10⁵ mg⁻¹ protein min⁻¹)</th>
<th>PSI activity (µmol O₂ consumed mg⁻¹ protein hr⁻¹)</th>
<th>PSII activity (µmol O₂ evolved mg⁻¹ protein hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.92</td>
<td>18.05</td>
<td>6.81</td>
<td>3.82</td>
<td>80.5</td>
<td>21.84</td>
<td>15.34</td>
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<td>20</td>
<td>9.64</td>
<td>190.21</td>
<td>7.01</td>
<td>4.82</td>
<td>90</td>
<td>42.8</td>
<td>30.26</td>
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<tr>
<td>50</td>
<td>12.96</td>
<td>240.82</td>
<td>10.5</td>
<td>6.26</td>
<td>116</td>
<td>58.93</td>
<td>42.2</td>
</tr>
<tr>
<td>100</td>
<td>4.62</td>
<td>101.52</td>
<td>5.26</td>
<td>1.56</td>
<td>36</td>
<td>27.18</td>
<td>18.16</td>
</tr>
<tr>
<td>300</td>
<td>2.12</td>
<td>46.23</td>
<td>2.33</td>
<td>0.621</td>
<td>9</td>
<td>16.5</td>
<td>2.13</td>
</tr>
<tr>
<td>500</td>
<td>1.98</td>
<td>0</td>
<td>1.21</td>
<td>0.213</td>
<td>4</td>
<td>10.16</td>
<td>0</td>
</tr>
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</table>

Protein, chl a, heterocyst frequency, nitrogenase, ¹⁴CO₂ fixation, PS I and PS II activity were measured on 6th day of growth. The data in each columns are mean of three independent experiments with four replicates each. The maximum variation from the mean value was <5%.
differentiation and nitrogenase activity were maximum at 50 \mu M iron. About 40% reduction in N\textsubscript{2} fixation rate in the cells devoid of iron may be due to less photosynthetic activity in iron-starved cells rather than the direct removal of iron from the nitrogenase enzyme complex\textsuperscript{15}.

The cells devoid of iron (iron-starved) exhibited appreciable level of \textsuperscript{14}C-incorporation which further increased with increase in iron concentration up to 50 \mu M iron. However, at higher concentrations of iron (100-500 \mu M) inhibition of \textsuperscript{14}C incorporation was significant and about 89% reduction in \textsuperscript{14}C-incorporation was observed at 300 \mu M iron. Similar findings were also reported with other heavy metals in cyanobacteria\textsuperscript{14}.

Iron starved cells of Anabaena PCC 7120 exhibited lower PS II activity as compared to the cells incubated with 20 to 50 \mu M iron. The cells treated with 50 \mu M iron, however, exhibited an enhanced level of PS II activity as compared to the cells treated with 20 \mu M iron. In contrast, the cells incubated with higher iron concentration (100-500 \mu M) showed reduced level of PS II activity. High concentration of iron causing strong inhibition in PS II activity may presumably be due to the extreme sensitivity of chl a pigment under iron stress\textsuperscript{15}. Similarly iron starved cells of Anabaena PCC 7120 exhibited lower level of PS I activity as compared to the cells incubated with 20 \mu M and 50 \mu M iron. Similar to PS II activity, PS I activity also attained its maximum level in the cells incubated with 50 \mu M iron. The reduction in PS I activity at higher iron concentration (100-500 \mu M) suggested that iron like other metals also acts as cytotoxin, inhibiting the various cellular processes\textsuperscript{16}. However, PS I activity never attained its zero level even at the maximum iron concentration (500 \mu M), suggesting that PS I activity is less sensitive to iron as compared to PS II activity in the cyanobacterium Anabaena PCC 7120.

It therefore, seems that siderophore mediated iron build up inside the Anabaena PCC 7120 cells may be involved in affecting all the cellular activities related to iron starvation. Moreover, iron, at higher concentrations possibly act as a cytotoxin, leading to the inhibition of various cellular processes like growth, N\textsubscript{2}-fixation, CO\textsubscript{2} fixation and photosynthetic activities in Anabaena PCC 7120.

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**References**