Induction of polyunsaturated fatty-acid synthesis enhances tolerance of a cyanobacterium, *Cylindrospermopsis raciborskii*, to low-temperature photoinhibition

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Acyl-lipid desaturation introduces double bonds (unsaturated bonds) at specifically defined positions of fatty acids that are esterified to the glycerol backbone of membrane glycerolipids. Desaturation pattern of the glycerolipids of *Cylindrospermopsis raciborskii* (C. raciborskii), a filamentous cyanobacterial strain, was determined in cells grown at 35°C and 25°C. The lowering of the growth temperature from 35°C to 25°C resulted in a considerable accumulation of polyunsaturated octadecanoic fatty acids in all lipid classes. Lipid unsaturation of *C. raciborskii* was also compared to *Synechocystis* PCC6803. In *C. raciborskii*, a shift in growth temperature induced a much more pronounced alteration in the desaturation pattern of all lipid classes than in *Synechocystis* PCC6803. The tolerance to low-temperature photoinhibition of the *C. raciborskii* cells grown at 25°C and 35°C was also compared to the tolerance of *Synechocystis* cells grown at the same temperatures. Lower growth temperature increased the tolerance of *C. raciborskii* cells but not that of *Synechocystis* cells. These results strengthen the importance of polyunsaturated glycerolipids in the tolerance to environmental stresses and may give a physiological explanation for the determinative role of *C. raciborskii* strain in algal blooming in the Lake Balaton (Hungary).

The physical and biochemical properties of glycerolipids depend on the degree of unsaturation of the fatty acids¹. In the membranes, the level of unsaturation is modulated by specific enzyme system², fatty-acid desaturases, which introduce double bonds at defined positions in the alkyl chain of fatty acids³. In cyanobacterial strains acyl-lipid desaturases are present, which act on fatty acids that are esterified to the glycerol backbone of membrane glycerolipids³. Poikilohermic organisms, which are not able to adapt to the changes in environmental conditions in other way, modulate the level of unsaturation of membrane lipids according to environmental temperature⁴. Thus, acyl-lipid desaturases are the most efficient regulators of the extent of lipid unsaturation in the response to changes in ambient temperature.

Cyanobacteria are ideal objects for studies of temperature-induced changes in the membrane since their fatty acid composition is well known³. On lowering the temperature the level of unsaturated fatty acids in several cyanobacterial strains, such as *Anabaena variabilis*, *Synechococcus* PCC7002, *Synechocystis* PCC6714 and *Synechocystis* PCC6803 markedly increases⁵.

The photosynthetic apparatus of photosynthetic organisms is embedded in thylakoid membrane and surrounded by a lipid matrix. The main constituents of the lipid matrix are glycerolipids which provide the necessary structural background for the functioning of protein complexes⁶, the insertion of proteins, and their translocation across the membranes⁷. In cellular physiology, the unsaturation of membrane glycerolipids plays a key role in the tolerance of living organisms to low-temperature stress⁸. In our earlier studies we demonstrated the importance of polyunsaturated glycerolipids in the tolerance of photosynthetic organisms to low-temperature photoinhibition by using transgenic cyanobacterial strains⁹,¹° and transgenic higher plants¹°.

Cyanobacteria are evolutionarily adapted to changes of environmental temperatures and control the physico-chemical properties of their membranes.
Thus, *Synechocystis* PCC6803, a well-characterized system, can be used as reference organism in studies of temperature dependence. *Cylindrospermopsis raciborskii* is a filamentous, toxic blue-green alga inhabiting sub-tropic/tropic water bodies with optimal growth temperature above 25°C (ref. 15). It appeared in the temperate water of Lake Balaton (Hungary) a few years ago, where it became the most dominant strain in the seasonal alga bloom.

As part of our efforts to understand the physiological roles of the unsaturation of membrane glycerolipids, we studied the effects of enhanced temperature of *Cylindrospermopsis raciborskii* in comparison to *Synechocystis* PCC6803 cells.

**Materials and Methods**

**Organisms and culture conditions**

*Cylindrospermopsis raciborskii* was cultivated in modified BG-11 medium containing 10% of the original microelements. *Synechocystis* PCC6803 was grown photoautotrophically in BG-11 medium supplemented with 20 mM HEPES-NaOH (pH 7.5) as described by Wada and Murata. The strains were illuminated with incandescent lamps providing 40 μmol/m²/sec intensity and aerated by sterile air containing 1% CO₂.

**Analysis of fatty acid and chlorophyll**

Lipids were extracted from the cells by the method of Bligh and Dyer. The fatty-acid composition was analyzed as described by Wada and Murata. The lipid extracts were transmethylated in the presence of methanol containing 5% HCl at 80°C for 3 hr. Fatty acid methyl esters were separated by gas chromatography on a FFAP (Supelco, Bellefonte, PA) capillary column (30 m x 0.25 mm i.d.). Chlorophyll concentrations were determined by the method of Arnon et al.

**Measurement of photosynthetic activity**

Photosynthetic oxygen evolving activity was measured in intact cells with Clark-type electrode according to Gombos et al. without artificial electron donor or acceptor. Actinic light was provided by an incandescent lamp in combination with a red optical filter (R-62; Hoya Glass, Tokyo, Japan) and an infrared-absorbing filter (HA-50; Hoya Glass) at an intensity of 600 μmol/m²/sec. Photosystem II activity was measured in the presence of 1.0 mM 1,4-benzoquinone as electron acceptor.

**Photoinhibition measurements**

The optical density of the cells at 750 nm was 0.5. The suspension of the cells was illuminated at various temperatures in a thermostated reaction vessel with white light of various intensities supplied by two incandescent lamps, by aeration with sterile air containing 1% CO₂.

**Results**

**Lipid composition of C. raciborskii**

Four major lipid classes of *C. raciborskii* were identified: monogalactosyl-diacylglycerol (MGDG), the major lipid component, digalactosyldiacylglycerol (DGDG), sulfolipid (SL), and phosphatidylglycerol (PG). The distribution of the individual lipids in the total lipid extract was similar to other cyanobacteria or thylakoids of higher plants (data not shown). The growth temperature had no effect on the distribution of lipid classes.

**Effect of growth temperature on fatty acid composition**

(i) *Synechocystis* PCC6803

The fatty acid composition of total lipid classes is summarized in Table 1 comparing cell cultures grown at 25°C and 35°C. The cells growing at lower temperature contains α-18:3 and 18:4 fatty acids. The synthesis of these fatty acids is induced under the growth temperature of 30°C. This is in good agreement with the results of Wada and Murata.

<table>
<thead>
<tr>
<th>Growth temperature</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>γ-18:3</th>
<th>α-18:3</th>
<th>18:4</th>
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<td>25°C</td>
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<td>35°C</td>
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<td>9</td>
<td>14</td>
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tr, trace amount (< 0.5%). The results are from three independent experiments; standard deviations are within ± 2% of individual values.
The relative percentage of total polyunsaturated fatty acids was 34% and 32% at 25°C and 35°C, respectively. This result shows that the adaptation to lower growth temperature resulted in a relatively small change in the fatty acid composition. The level of mono unsaturated fatty acid derivatives (palmitoleic acid + oleic acid) was not affected by the change in the growth temperature. The proportion of saturated fatty acids (palmitic acid + stearic acid) slightly increased in the cells grown at higher growth temperature.

(ii) *Cylindrospermopsis raciborskii*

In *C. raciborskii* similarly to the fatty-acid composition of *Synechocystis* cells the major fatty acid species were, in that order, palmitic, linoleic, α-linolenic, oleic, γ-linolenic, stearic and octadecatetraenoic acids (Table 2). According to the mode of fatty-acid desaturation, *C. raciborskii* can be classified as a strain belonging to group 4 (ref. 4). As expected simultaneously with a significant decrease in the saturated-to-unsaturated ratio (sat%/unsat%) of fatty acids, on lowering the temperature the double bond index increased. The level of polyunsaturated fatty acids increased from 34% to 53% in cells grown at 35°C and 25°C, respectively. The slight variation between cultures has to be noted. With decreasing growth temperature the level of linoleic acid decreased from 19% to 2%, while the levels of α-linolenic acid and octadecatetraenoic acid increased from 11% to 49%. This adaptive alteration of fatty acid composition was remarkably higher in *C. raciborskii* cells than in *Synechocystis PCC6803* cells.

![Table 2](image)

<table>
<thead>
<tr>
<th>Growth temperature</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>γ-18:3</th>
<th>α-18:3</th>
<th>18:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>39</td>
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<td>3</td>
<td>2</td>
<td>23</td>
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<tr>
<td>35°C</td>
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<td>7</td>
<td>3</td>
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<td>4</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

The results are from three independent experiments; standard deviations are within ±2% of individual values.

![Fig. 1](image)

**Fig. 1**—Photoinhibition of *C. raciborskii* cells at 20°C (A) and 30°C (B) in white light of 1.0 mmol/m²/sec intensity. The photosynthetic activity was measured at 30°C by monitoring oxygen evolution. The absolute oxygen evolving activities, corresponding to 100%, for *C. raciborskii* cells grown at 25°C (●) and 35°C (○) were 390±10 and 369±10 μmol O₂/mg chlorophyll/hr, respectively. The values are averages from three independent experiments.
Photoinhibition of photosynthesis

The inhibition of the activity of photosystem II of C. raciborskii cells was measured following a long term incubation of the cells grown at 25°C or 35°C at a light intensity of 1.0 mmol/m²/sec (Fig. 1). The measurements were carried out by monitoring photosystem II mediated electron transport from H₂O to 1,4-benzoquinone. Photoinhibition occurred in both cultures grown at 25°C and 35°C and at both temperatures of photoinhibitory treatment, at 20°C and 30°C. However, cells grown at 25°C were more tolerant to high light exposure than the cells grown at 35°C. The difference was more pronounced when measured at 20°C (Fig. 1A) than at 30°C (Fig. 1B).

Both types of cells were more sensitive to light at 20°C than at 30°C. The photosynthetic activity was not altered when the cells were kept in the dark for 2 hr (data not shown). For comparison, the inhibition of photosystem II activity of Synechocystis cells grown at 25°C and 35°C was also measured under similar conditions. These cells also showed higher susceptibility to photoinhibition at lower temperatures, but it was not affected by the growth temperature (data not shown).

Recovery of photosystem II from photoinhibition

Since the kinetics of inhibition of photosynthesis suggested that photoinhibition depended on the growth temperature for C. raciborskii but not for Synechocystis PCC 6803, we compared the recovery kinetics of photosystem II activity after photoinhibition in these two strains. The cells grown at 25°C and 35°C were preincubated in light at 1.5 mmol/m²/sec at 20°C for 40 min, which resulted in ~75% photoinhibition. Then the cells were incubated in low light at 30°C or 20°C. At 30°C, cells of C. raciborskii and Synechocystis PCC6803 grown at 35°C regained 100% of the original activity in 50 and 60 min, respectively (Fig. 2A). At 30°C, the restoration of photosystem II in cells of C. raciborskii and Synechocystis grown at 25°C was complete after incubation for 40 and 60 min, respectively (Fig. 2B). In contrast, the recovery at 20°C was incomplete both for C. raciborskii and Synechocystis cells (Fig. 3A).

A striking difference was observed between the recovery capabilities of C. raciborskii and Synechocystis cells that were grown at 25°C (Fig. 3B). In C. raciborskii, the recovery of photosystem II cells was complete in 2 hr. In contrast, during the

![Fig. 2—Recovery of photosynthetic activity after photoinhibition in Synechocystis PCC 6803 (□) and C. raciborskii (○) cells grown at 35°C (A), and in Synechocystis PCC 6803 (■) and C. raciborskii cells (●) grown at 25°C (B). The recovery was followed at 30°C at light intensity of 40 μmol/m²/sec. For the inactivation of photosynthetic activities to 20% of the original activities the cells were preincubated at 20°C for 40 min at 2.0 μmol/m²/sec light intensity. The recovery from photoinhibition was monitored by oxygen evolving activity of the cells measured with 1 mM 1,4-benzoquinone as the electron acceptor. The absolute oxygen evolving activities, corresponding to 100%, for Synechocystis cells grown at 25°C and 35°C were 480±50 and 440±15 μmol O₂/mg chlorophyll/hr, respectively, and for C. raciborskii cells grown at 25°C and 35°C were 390±10 and 360±10 μmol O₂/mg chlorophyll/hr, respectively. The values are averages from three independent experiments.](image-url)
Fig. 3—Recovery of photosynthetic activity after photoinhibition in Synechocystis PCC6803 (□, ■) and C. raciborskii (○, ●) cells grown at 35°C (A) and 25°C (B), respectively. Recovery was obtained at 20°C in the presence of white light at 40 μmol/m²/sec intensity; other conditions, as in Fig. 2.

same time, only 40% of the original activity of photosystem II of Synechocystis cells was recovered. (The light intensity for recovery treatment was 40 μmol/m²/sec.) In the presence of chloramphenicol, an inhibitor of protein translation, the restoration of photosynthetic activity was completely blocked (data not shown) either at 20°C or at 30°C in both types of cells, indicating that recovery processes depended on protein synthesis. On the other hand, in C. raciborskii the recovery also clearly depends on the temperature of growth, which determines the level of unsaturation of glycerolipids in photosynthetic membranes: the synthesis of polyunsaturated fatty acids in these cells at low temperatures clearly brings about a high recovery of photosystem II activity after photoinhibition.

Discussion

The primary means of thermal adaptation is the alteration of lipid composition of membranes; cyanobacteria can be divided into several groups depending on the desaturase activity at various chain positions. According to unsaturation pattern of glycerolipids⁵ there are four groups of cyanobacteria and in group 4 the fatty acid composition is particularly rich in octadecatetraenoic acid at the expense of linoleic acid. Several data suggest that octadecatetraenoic acid is formed by sequential desaturation of stearic acid in position sn-1 of galactolipids (MGDG) to oleic, linoleic, linolenic and octadecatetraenoic acid requiring Δ-9, -12, -15, and finally Δ6 desaturases in that order.⁶ Synechocystis PCC6803 (Table 1) and C. raciborskii (Table 2) are typical members of group 4 containing octadecatetraenoic acid as the most highly unsaturated fatty acid.⁷

The fatty acid compositions of C. raciborskii and Synechocystis cells grown at optimal condition (35°C) and at suboptimal temperature (25°C) were analyzed. In the cells grown at lower temperature the most unsaturated fatty acids (18:3, 18:4) accumulated with a concomitant decrement of linoleic acid (Tables 1 and 2). The induction of these polyunsaturated fatty acid molecular species requires at least 12 hr assuming the activation of Δ15 desaturase (data not presented). In another cyanobacterial strain, Anabaena variabilis, a detectable accumulation of α-linolenic acid took place in 4-5 hr after lowering the temperature from 38° to 22°C (ref. 22). In the case of Synechocystis PCC6803 Δ15 and Δ6 desaturase is induced within 1 hr upon lowering the temperature from 35° to 25°C (ref. 23). These data indicate vast differences between different blue green algae.

In the case of Synechocystis PCC6803 the level of α-linolenic acid and octadecatetraenoic acid
increased parallel with lowering the temperature from 42°C to 22°C (ref. 9). In contrast, the transformation of Anacystis nidulans R2-SPc, sensitive to chilling temperatures, with the gene encoding Δ12 desaturase resulted in an enhancement of linoleic acid and an increased tolerance to low temperature stresses3. In C. raciborskii cells, the levels of α-linolenic acid and octadecatetraenoic acid increased, instead of linoleic acid, when the cells were exposed to suboptimal growth temperatures. Some data show that in higher plants, α-linolenic acid is involved in the cold tolerance24. St. John et al.25 demonstrated that the inhibition of the formation of linolenic acid led to increased chilling sensitivity of several cereals. In addition, Sommerville and Browse26 suggested that an increase in the level octadecatetraenoic acid in higher plants correlated with chilling tolerance. Kodama et al.27 found that trienoic fatty acids were accumulated in tobacco following Δ15 desaturase induced transformation. In C. raciborskii cells grown at lower temperature, although the levels of octadecapolyenoic acids were increased, there was no indication of increased chilling tolerance in the darkness.

The change in the ratio of unsaturated to saturated fatty acids can result in certain modification in membrane structure, which can explain the alteration of physiological properties of the photosynthetic organisms3. In the glycerolipids of Synechocystis cells the unsat/sat ratio showed very minor change when the cells were transferred from 35°C to 25°C (Table 3) and the change in double bond index (DBI) was negligible as well. In contrast, for C. raciborskii the growth temperature dramatically affected the unsat/sat ratio and the DBI of glycerolipids (Table 3). These findings suggest that while the unsaturation level of glycerolipids from Synechocystis is unchanged C. raciborskii cells show a more flexible adaptation to the temperature of environment on the level of lipid unsaturation.

In our earlier studies we investigated the effect of polyunsaturated glycerolipids on the susceptibility of photosynthetic organisms to low-temperature photoinhibition using transgenic cyanobacterial strains8,10,28. In the present study we investigated C. raciborskii, a cyanobacterial strain which was found to be extremely stress resistant in natural environmental conditions. Therefore, we compared their susceptibility to photoinhibition at low temperature with that of the well-characterized Synechocystis cells; and also compared the susceptibility of cells grown at different temperatures.

Results of these experiments (Figs. 2 and 3) revealed that the extent of the photoinhibition was higher in the C. raciborskii cells grown at higher temperature than in the cells grown at lower temperature possessing considerably higher amount of polyunsaturated glycerolipid components. These findings indicated a good correlation between the amounts of polyunsaturated lipid molecules and the ability of cells to protect photosystem II against low-temperature photoinhibition.

Recent studies on the photoinhibition and recovery29 have demonstrated that these processes are related to the degradation of the D1 protein and reconstitution of the de novo synthesis of D1 protein of photosystem II. The D1 protein is embedded in the membrane. Thus, the incorporation of precursor of D1 to the photosystem II complex, and the processing of the precursor of the D1 protein to the mature D1 protein depend on the membrane environment. Previously it was suggested that the processing of pre-D1 is related to the unsaturation level of glycerolipids in the membrane30,31. Experiments to determine how this process is modulated by the level of unsaturation of membrane lipids in C. raciborskii cells is in progress.

In summary, we investigated the physiological consequences of the altered growth temperatures in the adaptation to high light at low temperature. In the case of Synechocystis PCC6803 the growth temperature did not affect the tolerance to low-temperature photoinhibition. We could reach alteration of the tolerance to low-temperature photoinhibition by the complete elimination of polyunsaturated glycerolipids32. In C. raciborskii, a decrease in the growth temperature enhanced the

<table>
<thead>
<tr>
<th>Strains</th>
<th>Growth temp.</th>
<th>unsat/sat(%)</th>
<th>DBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synechocystis</td>
<td>25°C</td>
<td>0.89 ± 0.05</td>
<td>1.09 ± 0.05</td>
</tr>
<tr>
<td>PCC6803</td>
<td>35°C</td>
<td>0.79 ± 0.04</td>
<td>0.94 ± 0.05</td>
</tr>
<tr>
<td>C. raciborskii</td>
<td>25°C</td>
<td>1.50 ± 0.07</td>
<td>1.90 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>35°C</td>
<td>1.08 ± 0.05</td>
<td>1.03 ± 0.05</td>
</tr>
</tbody>
</table>

unsat/sat(%), represents the ratio between unsaturated and saturated fatty acids

DBI, double bond index was calculated from the percentage of unsaturated fatty acids and multiplied with the number of double bonds
tolerance to low-temperature photoinhibition. The results of fatty acid analyses revealed that the variations in the level of polyunsaturated glycerolipids of the thylakoid membranes as a function of growth temperature were much higher in C. raciborskii than Synechocystis PCC6803. These data show that in natural conditions the photosynthetic organisms can manipulate their tolerance to stress conditions by adjusting the lipid unsaturation; as demonstrated in this work, this influences significantly the degree of protection against low temperature photoinhibition. In particular, our data point to the importance of polyunsaturated glycerolipids in the recovery process after photoinhibition of photosynthesis\(^\text{13}\).

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References
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