EFFECTS OF NITRATE AVAILABILITY AND IRRADIANCE ON INTERNAL NITROGEN CONSTITUENTS IN CORALLINA ELONGATA (RHODOPHYTA)

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ABSTRACT

Short-term (5-h) phycobiliprotein photoacclimation was a NO$_3^-$ dependent process in the red alga Corallina elongata Ellis et Soland. At low irradiance levels, phycobiliprotein synthesis (both r-phycocerritin and r-phycocyanin) took place when N supply was sufficient but was restricted by N limitation. Exposure to saturating irradiance resulted in pigment degradation under N limitation; however, under N-sufficient conditions a partial r-phycocerythrin synthesis was observed, despite the repressing role of high photon flux densities on phycobiliprotein synthesis. Soluble protein was less affected than phycobiliprotein by N limitation at low photon flux densities indicating that N limitation stimulates the flow of internal N metabolites toward the synthesis of nonpigmented proteins rather than pigment proteins.

The addition of protein synthesis inhibitors revealed that new phycobiliprotein synthesis occurs in response to sufficient N conditions. When protein synthesis was blocked in the chloroplast and cytoplasm simultaneously (addition of chloramphenicol and cycloheximide), both pigmented and nonpigmented protein synthesis was inhibited. However, when protein synthesis was blocked in the chloroplast, only phycobiliprotein synthesis was clearly inhibited, whereas nonpigmented protein was less affected, indicating that phycobiliprotein is the main fraction of protein synthesized in the chloroplast at low photon flux densities when external N is available. This inhibition of phycobiliprotein synthesis was consistent with a maximal increase in metabolites of protein synthesis (internal NH$_4^+$ and amino acids).

Our results suggest that phycobiliproteins may be an important N reservoir to meet internal N demands during N limitation in C. elongata. Moreover, r-phycocerythrin, synthesized at saturating irradiance levels, and the major constituent of the phycobiliprotein pigments, may be more sensitive to changes in N supply than r-phycocyanin. The influence of limited irradiance levels on N assimilation and the effects of repressing protein synthesis on internal N accumulation are also discussed.

Key index words: amino acids; Corallina elongata; inorganic nitrogen; irradiance; phycobiliproteins; protein synthesis; Rhodophyta

Photoacclimation to irradiance level is a well-known physiological process among algae (Falkowsky and LaRoche 1991 and references therein). Phycobiliproteins (PBP's), the accessory pigments in cyanobacteria and red algae (Gantt 1981, Glazer 1985, Zilinskas and Greenwald 1986), are synthesized in response to low irradiance levels (Waaland et al. 1974, Rosenburg and Ramus 1982, Levy and Gantt 1988, Algarra and Niell 1990, Gantt 1990). Additionally, as a major component of soluble cell protein (Bogorad 1975, Cohen-Bazire and Bryant 1983), PBP can be an important source of internal N, acting as a N reserve in cyanobacteria (Allen and Smith 1969, Boussiba and Richmond 1980, Yamakana and Glazer 1980, Wyman et al. 1985, De Loura et al. 1987). In these organisms, the acquisition of insoluble N reserves is an important ecological adaptation in the oligotrophic ocean, which is predominantly N-limited (Carr 1988). Of the two kinds of N reserves, cyanophycin and PBP, the polymer cyanophycin is absent from Synechococcus (Newman et al. 1987), further evidence that PBP is a N reserve.

In red algae, changes in PBP content are evident when external N concentrations change (Lapointe 1981, Bird et al. 1982, Lapointe and Duke 1984, Fredriksen and Rueness 1989, Levy and Gantt 1990, Friedlander et al. 1991). Experiments with N deprivation and N resupply indicate that within the protein pool, PBP is an important N reserve (Bird et al. 1982).

The photoacclimation process, as defined by Levy and Gantt (1988), is assumed to occur on a long-time scale of days or weeks. Together with this mechanism, a short-term acclimation of PBP content to irradiance and light quality takes place within a 5-h time scale (Algarra and Niell 1990, Lópex Figueroa and Niell 1990, Algarra et al. 1991) and may be important as a rapid mechanism of adaptation to changes in environmental conditions. Since N is considered to be the most limiting nutrient for macroalgae (Hanisk 1983), it is not surprising that PBP could act in red algae as a N reservoir in an environment where N is available at intermittent periods (Fujita 1985, Duke et al. 1989).

In this paper we report on the effect of NO$_3^-$ availability on short-term pigment acclimation to irradiance and on the content of internal N compounds in the red alga Corallina elongata.

MATERIALS AND METHODS

Plants of Corallina elongata Ellis et Soland were collected on a rocky shore in the bay of Algeciras (strait of Gibraltar, southern Spain). Specimens belonged to two morphotypes, namely, sun and shade, which were acculturated to different photon flux density (PFD) exposures as natural populations (Algarra and Niell 1987). Thalli were transferred and maintained in the laboratory at 17°C in 4-L air-agitated glass containers with unenriched filtered
seawater and preincubated at different PFDs (sun morphotype at 300 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \); shade morphotype at 30 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) for 24 h before conducting the experiments.

In the second experiment, plants corresponding to the sun morphotype were collected on the coast of Málaga (southern Spain) and maintained as already described. Prior to the experiment, plants were nitrogen starved for 24 h in artificial seawater without N (Woelkerling et al. 1983).

**Experimental design.** In the first set of experiments, the time course (0–5 h) of pigment acclimation to irradiance level was studied in light-crossing experiments. The sun morphotype, preconditioned to a high irradiance level, was exposed to a low PFD (30 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)), and the shade morphotype, preconditioned to a low irradiance level, was exposed to a high PFD (300 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)). The influence of N availability on PBP photosynthesis was studied: one set of light treatments was conducted without NO\(_3^-\) addition; the other set received an initial single addition of 10 \( \mu \text{M} \) NO\(_3^-\). These treatments are referred to as N-limited and N-sufficient (as opposed to N-limited, but it does not mean N-saturated). An initial pulse of NO\(_3^-\) within the environmental range was applied to address the problem of the ecological significance of the PBP as a N reservoir. Experiments were conducted in unenriched filtered seawater (Whatman GF/C) with a low concentration of NO\(_3^-\) (<2 \( \mu \text{M} \)). The effect of repressing protein synthesis was investigated in parallel treatments by adding chloramphenicol and cycloheximide (0.24 and 0.5 \( \mu \text{g} \cdot \text{L}^{-1} \)), chloroplasticid and cytoplasmic inhibitors of protein synthesis at the translational level, respectively. These experiments were conducted with 10 \( \mu \text{M} \) NO\(_3^-\) and at low (sun morphotype) and high (shade morphotype) PFDs (30 and 300 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), respectively).

Incubations were carried out in 0.5-L air-agitated flasks (two replicates per treatment) containing 1 g wet wt. of algae at 17°C. The time course of PBP and soluble protein contents was analyzed. The light source was cool-white fluorescent lamps (Sylvania F18 W/GRO), and PFD was measured with a Quantum Radiometer Li-Cor (Li-1908 Data Logger) with a spherical sensor (Li-Cor 193 SB). Appropriate statistical analyses (two-way ANOVA, Student's t-test; Sokal and Rohlf 1981) were applied to test the significance of the results.

Once the time course of NO\(_3^-\)-dependent pigment acclimation was established, the effects of different irradiance levels, NO\(_3^-\) availability, and inhibitor addition (chloramphenicol) on internal N constituents were investigated in a three-way experimental design.

A previous estimate of the photosynthesis–PFD relationship (Fig. 1) allowed the selection of appropriate PFD treatments: darkness, below the light compensation point, a low PFD, and a high PFD (0, 10, 65, and 1000 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), respectively). One set of treatments was conducted without NO\(_3^-\) supply (N-limited); another set received a single initial addition of 10 \( \mu \text{M} \) NO\(_3^-\) (N-sufficient). The third set of treatments was conducted with chloramphenicol (0.24 mg L\(^{-1}\)) in relation to the other sets of treatments without inhibitors.

The experiment was performed for 5 h in 0.5-L air-agitated flasks (four replicates per treatment) in artificial seawater (Woelkerling et al. 1983) containing 1 g wet wt. of algae at 17°C. Changes in the concentration of internal N constituents were determined after 5 h by analyzing PBP (r-phycoerythrin (RPE) and r-phycocyanin (RPC)), soluble protein, amino acid pool, internal NH\(_4^+\) and total internal N content. Total internal C content was also determined. Since PBP are made up of protein, the amount of nonpigmented soluble protein was estimated as the difference between soluble protein and PBP. Soluble protein associated with phycobilisomes is higher than the sum of RPE and RPC since allophycocyanin was not determined (Beer and Elshel 1985), and colorless polypeptides make up about 15% of phycobilisome proteins (Tandeau de Marsac and Cohen-Bazire 1977, Yamakawa et al. 1978).

The light source was a cool Hg-halogen lamp, and PFD was measured as already described. The influence of the treatments on internal N constituents was tested using a three-way ANOVA with replication (n = 4). A multiple mean comparison was conducted using the least significant difference method (LSD) (Sokal and Rohlf 1981).

Since internal N did not accumulate when chloramphenicol was applied, the influence of inhibitor addition (cycloheximide and chloramphenicol) on NO\(_3^-\) uptake was tested by measuring the time course of NO\(_3^-\) depletion from the culture medium at different initial external NO\(_3^-\) concentrations with or without inhibitors (controls).

**Analytical methods.** Samples were ground in Na\(^+\)-phosphate buffer (0.1 M, pH 6.5) at 4°C (Algarra and Niell 1987). The homogenates were extracted overnight and then centrifuged (Heraeus 17-LS 4°C, 19,000 \( \times \) g for 20 min). PBP and soluble protein contents were determined spectrophotometrically (Beckman DU-7 spectrophotometer) from the supernatant fraction. PBP content was calculated using the chromatic equations of Beer and Elshel (1985); soluble protein was calculated according to Bradford's method (1976).

Protein was precipitated by adding trichloroacetic acid (0.37 M, final concentration) and sodium-deoxycholic acid (3.5 mM, final concentration) to the supernatant fraction (Clayton et al. 1988). After centrifuging (19,000 \( \times \) g for 20 min), internal NH\(_4^+\) and amino acid contents were determined from the supernatant by the method of Slawyk and MacIsaac (1972) and by the ninhydrin-ascorbate method (Pezes and Bartos 1974), respectively. The standards for amino acid analysis were made up of a mixture of amino acids according to the amino acid composition in Corallina spp. (Miyazawa et al. 1982). This standard showed the same extinction coefficient as that of the amino acid alanine.

Parallel samples were dried in an oven at 60°C for 48 h to determine total internal C and N content in a Perkin-Elmer 240-C elemental autoanalyzer. Determination of NO\(_3^-\) concentration in seawater was carried out according to Wood et al. (1967).

All results are expressed on an organic dry weight (ODW) basis, since *C. elongata* has a high percentage of carbonates. Therefore, it shows a high dry wt: wet wt relation (DW:WW = 0.43) compared with other red algae (about 0.2). This relation was corrected by the percentage of plant material remaining after burning the sample at 950°C (45% DW, SD = 1%, n = 70). The corrected organic dry wt: wet wt coefficient (ODW:WW) was 0.24.
RESULTS

Short-term response to irradiance level. In the first set of experiments, the time course of PBP concentrations revealed that the photoacclimation process was controlled by NO₃⁻ supply. The sun morphotype, preconditioned to a high irradiance level (and, hence, with a low initial PBP content), synthesized PBP (both RPE and RPC) at low PFD when N was supplied as 10 µM NO₃⁻ (Fig. 2). However, this synthesis was partially repressed by N limitation. The shade morphotype, preconditioned to a low irradiance level (and, hence, with a high initial PBP content), exhibited a net degradation of PBP (both RPE and RPC) when exposed to a high PFD under N-limited conditions. However, under N-sufficient conditions, even a moderate synthesis of RPE took place (Fig. 2, Table 1).

Soluble protein was not affected by N limitation at low PFD, increasing under N-limited and N-sufficient conditions (Fig. 3A, Table 1). At high PFD under N-sufficient conditions, soluble protein synthesis was much lower than at low PFD, whereas its content decreased when N was limited (Fig. 3A).

When protein synthesis was blocked simultaneously in the chloroplast and cytoplasm, PBP synthesis was repressed at low and high PFDs (Fig. 4). Inhibition showed an oscillating pattern (Algarra et al. 1991). An initial enhancement of PBP content was followed by an inhibition of its synthesis. The synthesis of soluble protein at low PFD, after an initial enhancement, was inhibited 2 h after the ad-
condition of inhibitors (Fig. 3B, left). Synthesis did not increase at high PFD either.

Phycobiliproteins. The second experiment showed the same pattern of N regulation of PBP content.

When N supply was sufficient, PBP content increased at low PFD (10 and 65 μmol·m⁻²·s⁻¹), in contrast to darkness and to extremely high PFD (1000 μmol·m⁻²·s⁻¹), where its content was similar to that of initial values (Fig. 5). However, when N was limited, PBP synthesis was restricted, as in the first experiment.

The results of the three-way ANOVA (P-values; Table 2) showed that RPE content was affected by N availability and PFD more than RPC was. RPC synthesis only took place at extremely low PFD, and PFD per se did not influence RPC content (P > 0.05).

Soluble protein. At low PFD, soluble protein was less affected by N limitation than PBP (Fig. 6, Table 1). This result was consistent with those obtained in the first experiment. Moreover, as in the first experiment, soluble protein content was low when N was sufficient at high PFD (Fig. 6). This could be due to its rapid transformation into structural protein, since these conditions resulted in the highest content of internal N (Fig. 9A).

Response to chloramphenicol addition. When protein synthesis was blocked in the chloroplast, PBP synthesis was also repressed. This inhibition was maximal at low PFD without N limitation (Fig. 7). Of the two pigments, RPE was more inhibited than RPC by chloramphenicol (Fig. 5, Tables 1, 2).

Soluble protein synthesis (including PBP) was also affected by chloramphenicol, but no significant inhibition of the synthesis of nonpigmented soluble protein was observed (P > 0.05) (Table 2), in con-
trast to the inhibition observed when protein synthesis was also blocked in the cytoplasm (first trial, Table 1).

Internal \( \text{NH}_4^+ \) and amino acid content. When protein synthesis was blocked during N assimilation, internal \( \text{NH}_4^+ \) and amino acid pools increased by 137\% and 207\% of the control treatment without inhibitor at 65 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), respectively (Fig. 8). These increases were concomitant with the greatest inhibition of PBP synthesis at low PFD (Fig. 7).

The effect of PFD limitation upon \( \text{N} \) assimilation was evidenced by the accumulation under \( \text{N} \)-sufficient conditions of internal \( \text{NH}_4^+ \) and amino acids when light was photosynthetically limited (10 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), as compared to \( \text{N} \)-limiting treatments and initial conditions. Moreover, in the absence of photosynthetic \( \text{C} \) fixation (darkness), \( \text{N} \) was accumulated only as \( \text{NH}_4^+ \) and not as amino acid skeletons (Fig. 8, Table 3). At higher PFDs (65 and 1000 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), a decrease of these intermediary metabolites occurred as a consequence of sufficient PFD conditions, as compared to the light-limited treatments. All these effects are shown by the significant influence of the interaction of the control variables over amino acid and internal \( \text{NH}_4^+ \) content (Table 2).

Total \( \text{internal C and N content}. \) Total internal \( \text{C} \) content is made up of two different sources, organic and inorganic \( \text{C} \). Thus, this alga has high internal \( \text{C} \) concentrations in relation to other red algae as well as high \( \text{C}:\text{N} \) ratios (Niel 1976). The response of internal \( \text{C} \) content was directly influenced by PFD (Table 2), increasing when PFD was not limited and independently of \( \text{N} \) supply (Fig. 9B).

Internal \( \text{N} \) content was higher under \( \text{N} \)-sufficient conditions compared to \( \text{N} \)-limited treatments. However, when chloramphenicol was added, its content was the same as the initial value either in \( \text{N} \)-sufficient or \( \text{N} \)-limiting conditions, indicating the lack of net accumulation of \( \text{N} \) in the cell (Fig. 9A). Because internal \( \text{N} \) was not accumulated when protein synthesis was blocked, the influence of inhibitors of protein synthesis on \( \text{NO}_3^- \) uptake was studied. Protein synthesis inhibitors clearly suppressed \( \text{NO}_3^- \) uptake (Fig. 10). In contrast to the typical exponential decays of external \( \text{NO}_3^- \) concentrations observed in the control treatments, \( \text{NO}_3^- \) was even excreted to the external medium 2–3 h after inhibitor addition.

**Table 2.** F-values of three-way ANOVA with replication (\( n = 4 \)) of internal \( \text{N} \) variables after exposure to different experimental conditions for 5 h: A) chloramphenicol (CLP); B) irradiance (PFD); C) \( \text{NO}_3^- \) availability. \( A \times B, A \times C, \) and \( B \times C \), first-order interactions. \( A \times B \times C \), second-order interaction. Significance levels are *** = \( P < 0.001 \); ** = \( P < 0.01 \); * = \( P < 0.05 \); ns = no significant difference (\( P > 0.05 \)). Internal variables: r-phycoerythrin (RPE), r-phycoerythrin (RPP), phycobiliprotein (PBP), soluble protein (SP), nonpigmented soluble protein (NPSP), amino acids (aa), internal \( \text{NH}_4^+ \), internal \( \text{N} \), and internal \( \text{C} \).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>RPE</th>
<th>NPCP</th>
<th>PBP</th>
<th>SP</th>
<th>NPSP</th>
<th>aa</th>
<th>( \text{NH}_4^+ )</th>
<th>Internal N</th>
<th>Internal C</th>
</tr>
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<tbody>
<tr>
<td>A) CLP</td>
<td>19.66***</td>
<td>ns</td>
<td>15.94***</td>
<td>5.35*</td>
<td>ns</td>
<td>ns</td>
<td>16.34***</td>
<td>8.47**</td>
<td></td>
</tr>
<tr>
<td>B) PFD</td>
<td>4.95**</td>
<td>ns</td>
<td>4.22**</td>
<td>11.85***</td>
<td>10.77***</td>
<td>9.87***</td>
<td>6.41**</td>
<td>ns</td>
<td>30.81***</td>
</tr>
<tr>
<td>C) ( \text{NO}_3^- )</td>
<td>8.51**</td>
<td>10.85**</td>
<td>11.50***</td>
<td>20.50***</td>
<td>16.25***</td>
<td>4.79*</td>
<td>22.25***</td>
<td>11.33**</td>
<td>ns</td>
</tr>
<tr>
<td>A * B</td>
<td>3.11*</td>
<td>ns</td>
<td>3.24**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>A * C</td>
<td>5.80*</td>
<td>ns</td>
<td>4.79**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>B * C</td>
<td>5.70**</td>
<td>3.29*</td>
<td>6.38**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>4.63**</td>
<td>7.65***</td>
<td>ns</td>
</tr>
<tr>
<td>A * B * C</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>6.74***</td>
<td>ns</td>
<td>ns</td>
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</table>

**Fig. 6.** Response of soluble protein (SP) content (mg g\(^{-1}\)ODW) to PFD, \( \text{NO}_3^- \) availability (white and dark columns represent 0 and 10 \( \mu \text{M} \) \( \text{NO}_3^- \), respectively), and chloramphenicol (CLP) addition. The initial value before the experiment is shown in the right column. Bars denote SD (\( n = 4 \)). LSD\(_{0.05}\) indicates least significant difference among means at \( \alpha = 0.05 \).

**Fig. 7.** Percentage of inhibition of phycobiliprotein (PBP) and nonpigmented soluble protein (NPSP) synthesis, and percentage of inhibition of internal \( \text{N} \) accumulation with chloramphenicol (CLP), at different PFDs in \( \text{NO}_3^- \)-limited (0 \( \mu \text{M} \)) or \( \text{NO}_3^- \)-sufficient (10 \( \mu \text{M} \)) conditions.
This inhibition was parallel in time (2 h) with the onset of the inhibition of protein synthesis. (Fig. 3B).

**DISCUSSION**

The acclimation of PBP content to PFD is modulated by N availability in *C. elongata*. PBP synthesis takes place when N is not limited and therefore may be related to the importance of PBP as a N reservoir in red algae when external N becomes limiting. At low PFD, when N supply was limited, there was only a light-stimulated partial synthesis of PBP. In contrast, when an initial pulse of N was applied, optimal PBP synthesis occurred in response to photosynthetically limited irradiances (Table 1). High PFD acts in the opposite way to N under conditions of sufficient NO$_3^-$ supply. Therefore, PBP (both RPE and RPC) decreased when NO$_3^-$ supply was limited, as expected. Nevertheless, when NO$_3^-$ was sufficient, even a nitrogen-stimulated partial RPE synthesis occurred. RPC synthesis, on the contrary, was not stimulated by NO$_3^-$ addition at high PFD. This observation, in spite of the repressor role of high irradiances on PBP synthesis, is consistent with those reported by Wyman et al. (1985) in *Synechococcus* sp. WH7803. Recently, studies with picosecond time-resolved fluorescence spectroscopy have revealed that a significant fraction of RPE is uncoupled from the photosynthetic reaction centers when exposed to sufficient N and high PFD; hence, this mechanism may be a way of avoiding photodamage of photosynthetic antenna when an excess of pigments is synthesized as a N reserve at these saturating irradiances (Heathcote et al. 1992). RPE is the main pigment that responds to N availability, a fact that may be related to the importance of RPE in terms of N reservoir, since RPE content is several times greater than RPC, as shown by Fredriksen and Rue-ness (1989) in *Gelidium latifolium*. The magnitude of PBP synthesis in response to optimal conditions of light and N supply was higher in the first trial. The plant material used in the first set of experiments, with an initial low content of PBP, seemed to be more starved than that used in the second trial (Table 1).

**Table 3.** Percentage of the initial concentration of internal NH$_4^+$ and amino acids after 5 h in the absence (0 μM) or presence (10 μM) of NO$_3^-$ at different PFDs (μmol·m$^{-2}$·s$^{-1}$). LCP = light compensation point.

<table>
<thead>
<tr>
<th>μmol·m$^{-2}$·s$^{-1}$</th>
<th>Internal NH$_4^+$</th>
<th>Amino acids</th>
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<tr>
<td></td>
<td>−NO$_3^-$ +NO$_3^-$</td>
<td>−NO$_3^-$ +NO$_3^-$</td>
</tr>
<tr>
<td><strong>Darkness</strong></td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td><strong>Below LCP</strong></td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td><strong>Low PFD</strong></td>
<td>65</td>
<td>104</td>
</tr>
<tr>
<td><strong>High PFD</strong></td>
<td>1000</td>
<td>120</td>
</tr>
</tbody>
</table>
Soluble protein was not as affected by NO₃⁻ limitation as PBP. What seems to be clear is that N limitation redirects the flow of internal N metabolites (nitrate, ammonia, amino acids) toward the synthesis of nonpigmented proteins and away from PBP (Table 1), as indicated by the phycobiliprotein: soluble protein ratio of 17% when N was limited compared to 24% when N was sufficient after 5 h at low PFD. The same pattern of variation has been observed in Gracilaria tenuis Lemaireformis exposed at different external NH₄⁺ concentrations for 6 h (Vergara, Bird, and Niell, unpubl.).

In both experiments there was a small accumulation of assimilated N into soluble protein at high PFD. Under these conditions, total internal N increased (Fig. 9A). Since N did not accumulate as NH₄⁺ and amino acids (Fig. 8) or as soluble protein (Fig. 6) and since the ratio of C:N assimilated was low (5.5) and close to the C/N content of the pure protein (about 4), we assume that this N was in the form of structural proteins. Alternatively, a shift from low to high irradiance would result in a distribution of carbon flow away from protein toward carbohydrates and lipids (Post et al. 1985, Sukenik et al. 1990), decreasing the synthesis of protein.

In both trials, the addition of protein synthesis inhibitors showed a clear de novo synthesis of PBP, not only in response to optimal light conditions but also to sufficient N supply. PBP synthesis in response to light quality (green light) is inhibited by chloramphenicol in this species (Algarra et al. 1991). This chloroplastidial inhibitor represses the synthesis of the α and β chains of the phycobiliproteins, whereas linker polypeptides are translated in the cytoplasm (Egelhoff and Grossman 1983, Gantt et al. 1985). When protein synthesis was blocked simultaneously in the chloroplast and cytoplasm, the synthesis of proteins, both pigmented and nonpigmented, was inhibited. However, when only chloramphenicol was applied (second experiment), the synthesis of PBP was primarily inhibited, as compared to the lower degree of inhibition of nonpigmented proteins (Fig. 7, Tables 1, 2). This suggests that assimilated N is present in pigment proteins. The synthesis of other plastidial proteins may be less important at limited irradiances levels when N becomes available. This is consistent, in the context of PBP as a N reserve, with the preferential flow of internal N into nonpigmented proteins observed during N limitation, as already discussed. The inhibition of PBP synthesis was accompanied by the accumulation of previous metabolites of protein synthesis (amino acids and internal NH₄⁺). This is evidence of a genetic control at the chloroplastidial level over PBP synthesis, which occurs in response to limited irradiances when N is available. The lower inhibition of RPC synthesis may be due to the minor importance of this pigment in terms of acting as a N reservoir compared to RPE.

With respect to the response of intermediary metabolites during N assimilation, a light-dependent regulation of N assimilation is expected because the assimilation of external N into protein requires both energy and carbon skeletons (Lara et al. 1987a, Turpin 1991).

In the absence of photosynthetic C fixation (darkness), assimilated N was accumulated as NH₄⁺ but not as amino acids (Table 3). The assimilation of N into amino acids is restricted when photosynthetic carbon fixation is inhibited (Romero et al. 1985, Lara and Romero 1986). Although respiratory pathways are able to supply reducing power to maintain N assimilation (Larsson et al. 1985, Weger and Turpin 1989), and could explain NO₃⁻ reduction to NH₄⁺, the assimilation of inorganic N into proteins depends primarily on newly fixed carbon (Syrett 1981, Guerrero and Lara 1987, Lara et al. 1987a, Larsson and Larsson 1987).

Under PFD-limited conditions (below the light compensation point), not only NH₄⁺ but primarily amino acids were accumulated (Table 3), indicating that C demand for N assimilation is less limited than in the absence of photosynthetic C fixation (darkness). During the assimilation of assimilated N into amino acids, the stimulation of respiratory carbon flow by photosynthetic N assimilation (Bassham et al. 1981, Ohmori et al. 1984, Turpin et al. 1988) may have an important role, since carbon requirements for amino acid synthesis may exceed the photosynthetic carbon supply (Elfiri and Turpin 1987). Moreover, nonphotosynthetic carbon fixation (Elfiri and Turpin 1986, Schuller et al. 1990, Vanlerberghe et al. 1990) may be enhanced as an additional source of C to maintain N assimilation even during photosynthesis (Guy et al. 1990, Amory et al. 1991). Van Quy et al. (1991) reported that phosphoanol-pyruvate carboxylase and sucrose phosphate synthase activities are modulated by NO₃⁻ by inducing changes in the phosphorylation state of these enzymes to distribute carbon flow away from sucrose synthase and toward amino acid synthesis during N assimilation in light.

![Fig. 10. Inhibition of NO₃⁻ uptake in C. elongata by the addition of protein synthesis inhibitors (cycloheximide and chloramphenicol, 0.5 and 0.24 mg L⁻¹, respectively). (○) Each line is a control assay at different initial NO₃⁻ concentrations, and (●) treatments with inhibitors. Values are means of two replicates.](image-url)
At higher PFDs, N was not accumulated in these intermediary metabolites, indicating a sufficient provision of reducing power and newly fixed carbon to support N assimilation into proteins. Total internal N, as expected, increased in response to N assimilation, varying the C:N ratio inversely to N supply (Fig. 9C) (Niell 1976, Hanisak 1979, Corzo and Niell 1991). Nevertheless, when chloramphenicol was applied, the accumulation of N into the cell when N was sufficient was clearly inhibited (Fig. 9A). Ulrich et al. (1981) reported the partial inhibition of NO₃⁻ uptake by cycloheximide in Ankistrodesmus braunii. We have obtained a similar result with the application of cycloheximide and chloramphenicol (Fig. 10).

It is unclear how the inhibitors of protein synthesis affect the incorporation and/or assimilation of NO₃⁻. Assimilation of N seems to be controlled at the NO₃⁻ transport level (Lara et al. 1987a, Syrett 1988), and it may be regulated by products of both N and C assimilation, because when NH₄⁺ assimilation is blocked, the inhibition of NO₃⁻ uptake by C limitation is prevented (Flores et al. 1983, Romero et al. 1985, Lara and Romero 1986, Lara et al. 1987b). Thus, a regulatory role of intermediary products of C and N assimilation on NO₃⁻ transport has been suggested (Lara et al. 1987a, Larsson and Larsson 1987). In C. elongata, NO₃⁻ uptake was inhibited when protein synthesis was blocked, and the concomitant increase of intermediary C and N metabolites, such as amino acids (Fig. 8B), could act in a similar way in this proposed mechanism of the regulation of NO₃⁻ transport.

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