Marine Biology (1994) 120: 17–23 © Springer-Verlag 1994

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Nitrate-reductase activity and in vivo nitrate-reduction rate in *Ulva rigida* illuminated by blue light

Received: 10 December 1993 / Accepted: 25 February 1994

Abstract In the marine green alga *Ulva rigida* C. Agardh, nitrate reductase (NR) is synergetically induced by blue light and nitrate. The present study examines the effect of blue light and a large NO_3^- pulse (0.3 m*M*) on relevant variables of NO_3^--assimilation such as NO_3^--uptake, intracellular NO_3^--storage, NR activity, in vivo NO_3^--reduction rate and NO_2^- and NH_4^+-accumulation. Nitrate uptake started immediately upon addition of NO_3^-, suggesting the presence of a constitutive carrier, however in the first 1.5 to 2 h, periods of net NO_3^- efflux were frequent. After this time, NO_3^--uptake and intracellular NO_3^--accumulation proceeded linearly with time, suggesting the existence of a different NO_3^--uptake mechanism, which seems to be inducible. Our results indicate that in vivo NO_3^--reduction is not exclusively dependent on the potential NR activity. In *U. rigida*, during the first 2 h after a NO_3^- pulse (300 μ*M*) there were clear indications that the induction state of the NO_3^--carrier limits the reduction rate of NO_3^-. Once the induction of the NO_3^--transporter had been completed (1.5 to 2 h), the NO_3^--assimilation pathway reached a steady state, NO_3^--uptake rate, NO_3^--reduction rate and NO_2^- and NH_4^+-accumulation being linear with time. Since the reduction of NO_3^- leads mainly to the accumulation of NH_4^+, we conclude that, after the NO_3^--reduction itself, NH_4^+-fixation into carbon skeletons is the limiting step in the assimilation of NO_3^- by *U. rigida* under blue light.

Communicated by J. M. Pérès, Marseille

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Introduction

Blue light acts on a number of physiological processes, triggering different morphogenetic responses in fungi, higher plants and algae, e.g. the development of functionally active chloroplasts, the formation of whorls and caps in the genus *Acetabularia*, and many other processes (Senger 1984; Galland and Senger 1988). As protein synthesis is required for the completion of all photomorphogenic responses, it is of interest to study the assimilation of NO_3^- under blue light. In addition, nitrate reductase (NR), a key enzyme in the inorganic nitrogen metabolism, is inducible by blue light, involving de novo NR synthesis in higher plants (Rao et al. 1982). Additionally, NR from green algae, fungi and higher plants may be activated by blue light in vitro (Aparicio et al. 1976; Roldán and Butler 1980; Aryan et al. 1983; Fritz and Ninnemann 1985). The enzyme-bound FAD (flavin adenine dinucleotide) appears to be the photoreceptor, photoreactivation being caused by the excited triplet flavins (Fritz and Ninnemann 1985; Maldonado and Aparicio 1987). The same mechanism has been suggested to exist in vivo (Maldonado and Aparicio 1987). Despite the economical importance of marine macroalgae both as food for human consumption and as a source of phycocolloids, the information available on the regulation of the key enzymes of inorganic nitrogen metabolism such as nitrate reductase is so far very limited. According to our previous work (Corzo and Niell 1992b), in *Ulva rigida*, blue light-enhancement of NR occurs mainly through the induction of de novo NR synthesis, since the response is inhibited by cycloheximide. Actinomycin D and rifampicin are considerably less effective, suggesting that a mRNA pool coding for NR is already present. The accomplishment of NR-enhancement by blue light is dependent on either newly synthesized carbohydrates, reducing power, or ATP-derived from photosynthesis, since it is inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Corzo and Niell 1992b).

Nitrate assimilation involves the uptake of NO_3^- from the medium, the reduction of NO_3^- to NH_4^+ catalyzed by the

successive operation of nitrate reductase and nitrite reductase (NiR), and NH_4^+ fixation to carbon skeletons catalyzed mainly by the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway. Plants in general can use both NO_3^- and NH_4^+ as alternative nitrogen sources. The uses of both NO_3^- and NH_4^+ saves energy in the form of reducing equivalents; however, neither of them can be stored to a large amount within the cell (Ullrich and Novacky 1990).

The present paper deals with the effect of blue light-enhancement of NR activity in N-starved algae, given a simultaneous pulse of NO_3^-, on a number of relevant variables of inorganic nitrogen metabolism such as NO_3^--uptake, intracellular NO_3^- pool, NO_2^--production, NH_4^+-production, NR activity and NO_3^--reduction rates.

Materials and methods

Plant material

Ulva rigida C. Agardh was collected in 1988 from a rocky shore in the south of Spain (Algeciras, Cadiz). It was maintained in the laboratory at 15 °C in aerated artificial sea water (Kalle 1945 *in* Riley and Skirrow 1975) for different periods of time (up to 9 d), in 3-litre glass containers, the biomass : media ratio being ≃5 g fresh wt l^{-1}. In order to exhaust the NO_3^- internal pool, the alga was kept in white light at high intensity (200 μmol m^{-2} s^{-1}). Previous to the experiment, the alga was transferred to low irradiance (4 μmol m^{-2} s^{-1}) for 12 h. White light was provided with Sylvania F 18 W/GRO.

Experiments

Experiments were performed in a cool chamber at constant temperature (15 °C). Blue-light irradiation (160 μmol m^{-2} s^{-1}) began when pieces of blade (≃0.6 g fresh wt) were transferred to a flask containing 200 ml of artificial sea water + 0.3 m*M* KNO_3. In order to illuminate a large area with the same photon fluence rate, blue light was obtained with Sylvania blue + blue plastic filter and checked with a Spectroradiometer Li-Cor 1800 UW. The maximal transmission wavelength was at 440 nm and the spectral distribution of the photon fluence rate (PFR) was 67.3% from 400 to 500 nm, 26% from 500 to 600 nm and 6.7% from 600 to 800 nm. PFR was measured with a Quantum Radiometer LI-Cor (LI-1000 data logger).

In situ NR assay

Immediately after light treatment, 0.16 g of fresh tissue were introduced into test tubes containing 5 ml of assay medium previously flushed for 2 min with N_2; after introduction of the alga, the test tubes were again flushed with N_2 for a further 2 min. The test tubes were immediately sealed and incubated in the dark for 30 min at 30 °C. At the end of that time, 1 ml of assay medium was sampled and assayed for NO_2^- (Snell and Snell 1949). Two independent in situ NR assays were run for each light treatment. The assay medium consisted of 30 m*M* KNO_3, 0.01 m*M* glucose, 0.1% 1-propanol, 0.5 m*M* Na-EDTA, 0.1 *M* phosphate buffer (pH 8) (Corzo and Niell 1991).

Determination of external and internal nitrate, nitrite and ammonium

At different times, samples of medium (5 ml) were removed and stored frozen until analysis. NO_3^- (Wood et al. 1967), NO_2^- (Shinn 1941) and NH_4^+ (Slawyk and MacIsaac 1972) concentrations were determined by means of a Technicon Autoanalyser. Intracellular NO_3^-, NO_2^- and NH_4^+ concentrations were determined as follows. Samples of fresh tissue (0.1 g fresh wt) were taken and immediately dried overnight (100 °C). After being ground in a mortar, 0.05 g of dry powder were introduced into a test tube containing 5 ml of deionized water. Test tubes were incubated in a shaking water bath

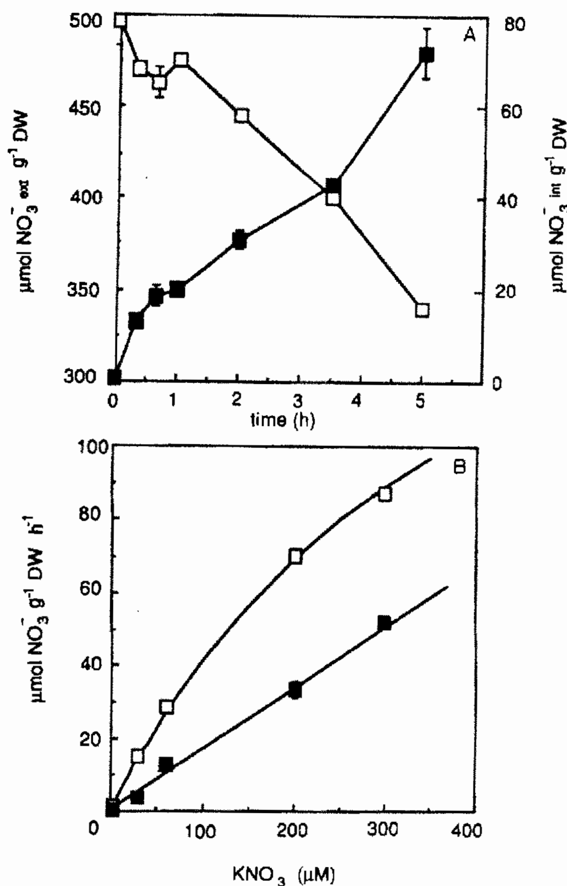

Fig. 1 *Ulva rigida*. **A** Nitrate-depletion from external solution (□) and internal NO_3^- (■) time-courses in blue light (160 μmol m^{-2} s^{-1}); **B** external NO_3^--depletion rate (□) and intracellular NO_3^--replenishment rate (■) as a function of external NO_3^--concentration. *U. rigida* was N-starved for 7 to 9 d. Standard deviation shown as bar if greater than size of symbol (*DW* dry weight)

(30 °C) for 1 h to allow complete dissolution of NO_3^-, NO_2^- and NH_4^+. Finally, the powder was removed by filtration (Whatman GF/C) and NO_3^-, NO_2^- and NH_4^+ concentrations were determined by the analytical methods detailed above.

Results

Nitrate uptake and internal pool of nitrate

After a period of N-starvation, 300 μ*M* KNO_3 was added to the medium and *Ulva rigida* immediately started taking up NO_3^- from the external medium. Over the time interval studied (5 h), the uptake proceeded linearly over time (33.6 μmol NO_3^- g^{-1} dry wt h^{-1}). However, an initial phase (1.5 to 2 h duration) was detected during which a net NO_3^--efflux occurred in different experiments (Fig. 1 A). The existence of a lag period for NO_3^--uptake following NO_3^--starvation has been reported for a number of species, and has been interpreted as the time required to induce the NO_3^--transporter (Ullrich and Novacky 1981; Deane-Drummond 1984). *U. rigida* did not show any lag period,

suggesting that a constitutive system for nitrate uptake exists in N-deprived cells.

The internal NO_3^- pool ($[NO_3^-]_{int}$) in *Ulva rigida* was exhausted by keeping the alga in a medium without any available N source at high light-intensity (200 μmol m^{-2} s^{-1}). After this treatment, the $[NO_3^-]_{int}$ level was very low (0.8±0.15 μmol NO_3^- g^{-1} dry wt). The $[NO_3^-]_{int}$ increased (12.6 μmol NO_3^- g^{-1} dry wt h^{-1}) after NO_3^- addition (300 μ*M*), correspondingly to NO_3^- depletion from the external medium (Fig. 1 A). Intracellular concentrations may be expressed in terms of ml of cell water by dividing by 3.5 (this factor was calculated by assuming that cell water has a density of 1.0 and that cell water is equal to the fresh weight minus the dry weight). According to Grandsted and Huffaker (1982), 58% of the intracellular NO_3^- is located within the vacuole, hence cytosolic concentrations are lower.

Blue-light induction of NR activity has been shown to be linearly dependent on the initial NO_3^- pulse (Corzo and Niell 1992b). It has been argued that NO_3^- has a double role: (1) as an inductor of NR only minor amounts of NO_3^- are probably required; (2) however, if it is to constitute a N-source as well, then larger amounts will be necessary. Two points are particularly important if the main role of NO_3^- is that of a N-source: (i) NO_3^- entry into the cell, and (ii) the fate of NO_3^- within the cell (is it stored in vacuoles or reduced rapidly?) Nitrate consumption rates (calculated for 2 h) were, as predicted, concentration-dependent in a hyperbolic manner. The apparent kinetic parameters were $K_{0.5}$=0.4 m*M*, V_{max}=208 μmol NO_3^- g^{-1} dry wt h^{-1} (Fig. 1 B). In contrast, the rate of $[NO_3^-]_{int}$-replenishment under blue light was linearly dependent on external NO_3^--concentration over the range studied (Fig. 1 B). *Ulva rigida* is capable of storing NO_3^- in large concentrations. After 2 h in blue light, with an initial NO_3^- concentration in the external medium of 300 μ*M*, the $[NO_3^-]_{int}$ pool increased from 1.4 to 105 μmol NO_3^- g^{-1} dry wt. No saturation was observed, although the external concentrations were at least an order of magnitude higher than those frequently measured in coastal marine environments.

Nitrate reductase activity and nitrate-reduction rate

Blue-light enhanced NR activity in *Ulva rigida* mainly through induction of de novo synthesis of NR-protein (Corzo and Niell 1992b). Parallel to the NO_3^- depletion from the external medium and the increase of $[NO_3^-]_{int}$ pool after an NO_3^- pulse (see previous subsection), blue light induced NR activity. In the dark control, the increase in NR activity was only 12% of that achieved under blue light. The increase in NR activity saturates with time (Fig. 2). The time at which the half-maximal activation level is reached is dependent on the N-status of the alga, being longer in N-starved algae (Corzo and Niell 1992b). Since presumably only the NO_3^- incorporated in the cells actually induces NR, it is of interest to investigate the relationship between NR activity and the amount of NO_3^- incorporated into the cell. NR activity showed a hyperbolic relationship with the total amount of NO_3^- incorporated into

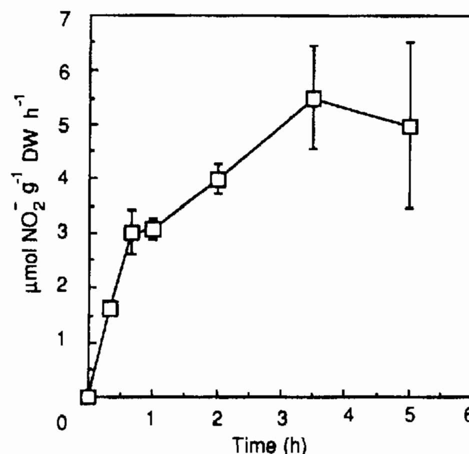

Fig. 2 *Ulva rigida*. Time-course of nitrate reductase (NR)-enhancement by blue light (160 μmol m^{-2} s^{-1}) after pulse of 300 μ*M* KNO_3. *U. rigida* was N-starved for 9 d. Standard deviation shown as bar if greater than size of symbol

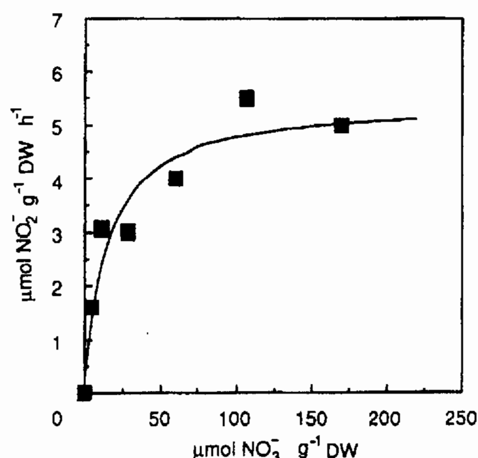

Fig. 3 *Ulva rigida*. NR activity as a function of nitrate content of algal tissue. Data fitted to Michaelis-Menten equation ($K_{0.5}$=14.56 μmol NO_3^- g^{-1} dry wt, V_{max}=5.55 μmol NO_3^- g^{-1} dry wt h^{-1})

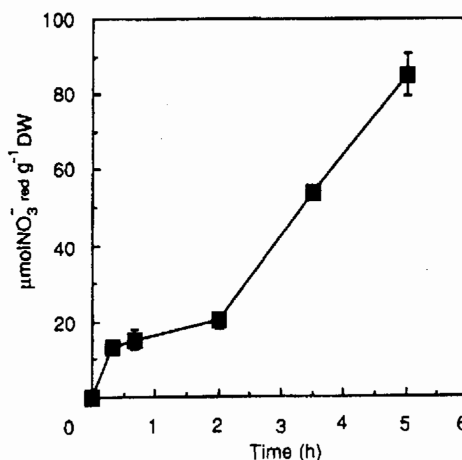

Fig. 4 *Ulva rigida*. Nitrate-reduction rate in vivo calculated as difference between incorporated NO_3^- and internal NO_3^-. Standard deviation shown as bar if greater than size of symbol

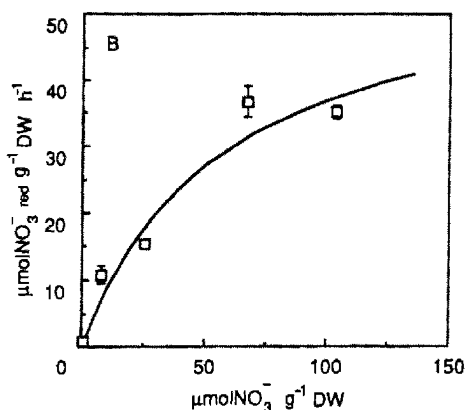

Fig. 5 *Ulva rigida.* Dependence of in vivo NO_3^--reduction rate on internal NO_3^--concentration in blue light. Standard deviation shown as bar if greater than size of symbol

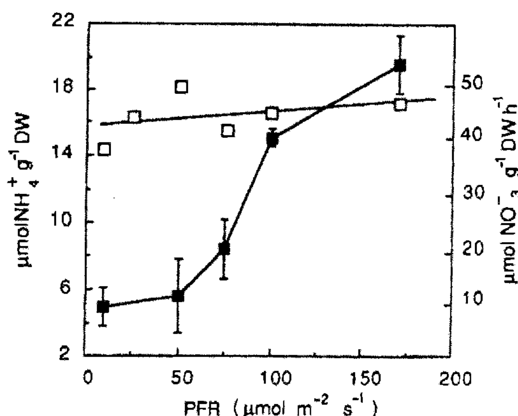

Fig. 7 *Ulva rigida.* NO_3^--uptake rate (□) and total NH_4^+-production (■) as a function of photon fluence rate (*PFR*) in blue light. Standard deviation shown as bar if greater than size of symbol

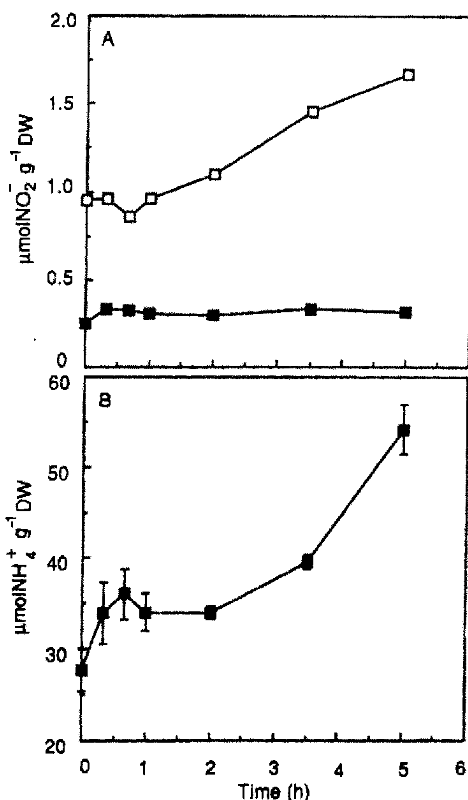

Fig. 6 *Ulva rigida.* Time-courses of **A** external NO_2^- (□) and internal NO_2^- (■) and **B** internal NH_4^+. Experiments performed in blue light (160 μmol m^{-2} s^{-1}). Standard deviation shown as bar if greater than size of symbol

the cell ($K_{0.5}$ = 14.56 μmol NO_3^- g^{-1} dry wt, V_{max} = 5.55 μmol NO_3^- g^{-1} dry wt h^{-1}) (Fig. 3). Obviously, a similar relationship was found between NR-enhancement by blue light and $[NO_3^-]_{int}$. In this case, the $K_{0.5}$ was larger (22.75 μmol NO_3^- g^{-1} dry wt).

As pointed out previously for higher plant systems, NR activity (determined either in vitro, or by the in situ method as in the present study) does not always accurately represent the real rate of NO_3^--reduction. The in vivo NO_3^--reduction rate can be calculated as the difference between the total amount of NO_3^- taken up by the alga and the intracellular NO_3^--concentration after a given period of time. The time-course of NO_3^--reduction suggests the existence of two phases (Fig. 4). An initial phase which reached an almost stable level after 40 min, and a second phase which began after 2 h and was characterized by a linear increase up to 80 μmol NO_3^- reduced g^{-1} dry wt after 5 h. The NO_3^--reduction rate in N-starved *Ulva rigida* was hyperbolic, with both the external NO_3^--concentration ($K_{0.5}$ = 158.96 μ*M* KNO_3, V_{max} = 57.47 μmol NO_3^- reduced g^{-1} dry wt h^{-1}) (result not shown) and $[NO_3^-]_{int}$ ($K_{0.5}$ = 57.7 μmol NO_3^- g^{-1} dry wt, V_{max} = 58.1 μmol NO_3^- reduced g^{-1} dry wt h^{-1}; Fig. 5). The amount of NO_3^- reduced represents 53±9% (n = 10) of the incorporated NO_3^-.

Since it has been claimed that in vivo NO_3^--reduction may be limited by factors (i.e., NO_3^--availability and NADH) other than active NR levels, it was of interest to study the relationships between NR activity and NO_3^--reduction. In *Ula rigida*, the ratio NO_3^--reduction rate: NR activity varied greatly between different experiments (11±7, n = 10). Variations in this ratio were also observed for the same experiment both as a function of different external NO_3^- concentration and with time course (results not shown). Therefore, as previously shown for higher plants, the in vivo rate of NO_3^--reduction cannot be directly assessed for *U. rigida* through the determination of NR activity. Various authors have suggested that the in vivo NO_3^--reduction rate may be limited by the NO_3^--uptake rate (Eisele and Ullrich 1977, Morgan et al. 1985). Our results support this idea, since although 40% of maximum NR activity had been reached after 20 min, only 15.7% of maximum NO_3^--reduction had been achieved at this time.

Nitrite and ammonium production

Under blue light, *Ulva rigida* is not able to assimilate int its carbon skeleton all the NO_3^- incorporated into the cell

and reduced by NR after a large pulse of NO_3^- (300 µ*M*). The excess NO_2^- produced is released to the medium rather than being accumulated in the cell (Fig. 6A), thus maintaining the internal NO_2^--concentration constant and avoiding toxicity effects (Vennesland and Guerrero 1979). However, excess reduced NO_3^- is mainly accumulated within the cell as NH_4^+ (Fig. 6B). The release of NH^+_4 was undetectable (Fig. 6B) or very low (3.5 µmol g^{-1} dry wt after 2 h) in some other experiments (results not shown).

In *Ulva rigida* under blue light, NR activity increased with increasing PFR (Corzo and Niell 1992b). Either a stimulation of the NO_3^--uptake system by the PFR in blue light (Calero et al. 1980), or an increase of reducing power could be responsible for such dependence. We tested the first possibility. NO_3^--uptake rate was practically independent of PFR in blue light (Fig. 7). Similar results were obtained previously using a lower NO_3^--concentration (<10 µ*M*; Corzo and Niell 1992a). However, in the same experiment, the ammonium production rate (intracellular plus released) was dependent on PFR under blue light (Fig. 7), demonstrating further the enhancement of NR activity by PFR in blue light. Therefore under blue light, NH^+_4-fixation in carbon skeletons appears to constitute the limiting step in NO_3^--assimilation.

Discussion

In *Ulva rigida*, blue-light stimulation of NR activity occurs mainly through induction of de novo enzyme synthesis (Corzo and Niell 1992b). Any de novo synthesis of protein requires nitrogen. *U. rigida*, like other marine macroalgae, may store large amounts of NO_3^- (Fig. 1A); therefore, its intracellular NO_3^- pool may constitute a source of nitrogen. In this study we examined the situation in which, since *U. rigida* was N-starved, the only source of N was NO_3^- in the external medium; in such case, NO_3^--uptake plays a major role.

For higher plants, evidence suggests that NO_3^- is transported into the cell by means of a secondary coupling with ATP hydrolysis (Ullrich and Novacky 1981, 1990; McClure et al. 1990a, b). The NO_3^--transporter is considered to be a membrane protein inducible by NO_3^- (Agüera et al. 1990). Induction of the NO_3^--carrier by NO_3^- can be prevented by RNA and protein-synthesis inhibitors; thus, a de novo synthesis of the NO_3^--transporter has been suggested (MacKown and McClure 1988; Hole et al. 1990). After a period of NO_3^--starvation, two different types of responses have been observed: (1) a diminution of the NO_3^--uptake rate to very low values (MacKown and McClure 1988; Agüera et al. 1990), and (2) an increase in the plant's capacity to incorporate NO_3^- (Ullrich and Novacky 1981); this latter response is typical for microalgae. The response of the green macroalgae *Ulva rigida* resembles that of the microalgae. After a high concentration pulse of NO_3^- (0.3 m*M*) to N-starved *U. rigida*, NO_3^- depletion in the external medium occurs immediately at a high rate. An increase of 12 µmol g^{-1} dry wt was detected in $[NO_3^-]_{int}$ after 20 min (Fig. 1A). However, this high rate of NO^-_3-uptake led to a subsequent net NO_3^- efflux later and a short phase of $[NO^-_3]_{int}$ saturation (Fig. 1A). A net NO_3^- efflux is frequent in many species including *U. rigida* (Corzo and Niell 1992a). Various explanations have been offered for this experimental observation (Deane-Drummond 1984). After 1.5 or 2 h, depending on the experiment, NO_3^- uptake proceeds linearly, and is paralleled by an $[NO^-_3]_{int}$ increase. Nitrate, besides being an inductor of its own transporter, is also an inductor of nitrate reductase. There is no information available on the way in which both processes are related in marine macroalgae. Blue-light-enhancement of NR is linearly dependent on external nitrate concentration, but it saturated with respect to the amount of incorporated NO_3^-, the $K_{0.5}$ being 14.56 µmol NO_3^- g^{-1} dry wt.

After a period of N-starvation, NR activity decreased to 4–16% of the inducible activity. This remaining NR activity may be considered as constitutive. Similar levels of constitutive NR have been reported by other investigators (Tischner et al. 1989; Corzo et al. 1991) for NH^+_4-grown microalgae. The existence of a constitutive NR permits the immediate reduction of NO_3^- in N-starved algae after a NO_3^--pulse (Fig. 4), providing NO_3^- can reach the reduction site, which is located intracellularly. While NR activity reached 54% of its maximal activation level in 1 h, NO_3^--reduction was only 16% of maximum values. It seems that during the first 2 h, NO_3^--reduction is limited by NO_3^--uptake; this would provide a simple explanation for the initial plateau observed in the NO_3^--reduction time-course (Fig. 4). This initial limitation in NO_3^--reduction is also visible in the NO_2^- and NH_4^+ accumulation time-courses (Fig. 6).

After 2 h under blue light, the inorganic nitrogen assimilation pathway reached a steady state characterized by constant rates of NO_3^--uptake, NO_3^--reduction and NO_2^- and NH_4^+-production (Figs. 1, 4, 6). In this steady state, the rates of NO_2^--reduction and NH_4^+-fixation may be calculated as the difference between NO_3^--reduction rate and total NO_2^--pool increase (internal + external), and the difference between NO_2^--reduction rate and the total NH_4^+-pool increase.

The internal NO_2^--pool was kept constant (Fig. 6A), the excess of NO_2^- being released to the external medium. Cells maintain a low level of $[NO_2^-]_{int}$ in order to avoid its toxic effects (Vennesland and Guerrero 1979). Therefore, the NO_2^--reduction rate (20.79 µmol NO_2^- reduced g^{-1} dry wt h^{-1}) can be calculated as the difference between the NO_3^--reduction rate and the NO_2^--release rate. In contrast, ammonium accumulates intracellularly rather than in the external medium. Hence, the NH_4^+-fixation rate (14.09 µmol NH_4^+ fixed g^{-1} dry wt h^{-1}) was calculated as the NO^-_2-reduction rate minus the $[NH^+_4]_{int}$ increase rate. According to this calculation, only 41.9% of NO_3^- is incorporated into primary amines (Fig. 8). Nitrate can be stored intracellularly in large amounts, probably within the vacuole (Granstedt and Huffaker 1982). The NO_3^--accumulation rate represents 37.6% of the NO_3^--uptake rate in a steady state. The accumulation of a metabolic intermediary is the result of an imbalance between its rates of synthesis and transfor-

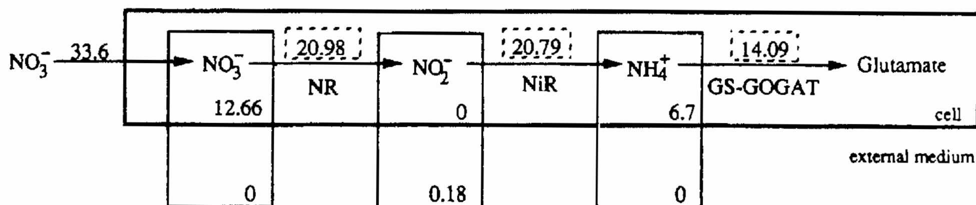

Fig. 8 *Ulva rigida*. Directly measured and calculated (in dashed boxes) rates for processes involved in assimilation of NO_3^- into glutamate. (*NR* nitrate reductase; *NiR* nitrite reductase; *GS-GOGAT* glutamine synthetase–glutamate synthase pathway) NO_3^-, NO_2^- and NH_4^+ pools are divided into two compartments – extracellular (external medium) and intracellular (cell). Rate of increase is shown in bottom right-hand corner of each compartment

mation, synthesis rates being necessarily higher than those of transformation. The larger the accumulation rate of a product, then the more limiting the subsequent enzymatic reaction. Bearing this in mind, NO_3^--reduction seems to be the limiting step in the assimilation of NO_3^- in N-starved *Ulva rigida* under blue light. The total removal of inorganic nitrogen as NO_3^-, NO_2^- and NH_4^+ from the main assimilatory pathway due to storage in internal compartments or release to the external medium was 19.54 μmol N g^{-1} dry wt h^{-1}. Of this, NO_3^- storage was 64.8%, the $[NH_4^+]_{int}$ increase 34.2%, and the NO_2^- release rate 0.9%. Therefore, in the NO_3^--assimilation pathway under blue light, reduction of NO_3^- is the limiting step, followed by NH_4^+-fixation. NR activity has been long considered the limiting step in the assimilation of nitrate. Our results indicate that this is also valid for blue light, even though it has been claimed that blue light may activate NR in vivo (Maldonado and Aparicio 1987). Ammonium fixation is considered to occur in algae and higher plants through the GS-GOGAT pathway. Obviously, the increase of $[NH_4^+]_{int}$ could be a result of catabolic processes; however a high rate of protein and amino acid catabolism after the addition of NO_3^- to N-starved cells appears unlikely. Besides, ammonium accumulation is dependent on the photon fluence rate in blue light (Fig. 7), and this dependence is parallel to that of NR activity (Corzo and Niell 1992b), hence NH_4^+-accumulation would appear mainly to be the result of an imbalance between NO_3^--reduction and NH_4^+-fixation, the GS-GOGAT pathway being unable to cope with the internal availability of NH_4^+. Ammonium is a repressor of NR synthesis; therefore, an inhibitory effect on both NR activity and NO_3^--reduction rate could be expected. However, a different behavior was observed in our study: while the NO_3^--reduction rate was not affected, blue-light-enhancement of NR activity saturated after 3.5 h. Long periods of time in blue light frequently lead to a decrease in the NR activity plateau level in *U. rigida* (results not shown). However, the NO_3^--reduction rate seemed to be unaffected by saturation or even by a drop in the NR activity levels in blue light, which suggests that NR activity is not a limiting factor for the in vivo reduction of NO_3^-. If the potential NR activity does not limit NO_3^--reduction in blue light, the question arises – what is limiting the reduction of NO_3^-? The diminished availability of reducing power has been claimed to limit NO_3^--reduction under certain circumstances (Wallace 1987).

Acknowledgements The research was supported by Grant PB91-0962 from the CICYT (Spain). A. C. holds a fellowship from the Ministerio de Educación y Ciencia (Spain).

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