Plant Physiol. (1983) 71, 286–290
0032-0889/83/71/0286/05/$00.50/0

In Vivo Blue-Light Activation of *Chlamydomonas reinhardii* Nitrate Reductase[1]

Received for publication June 16, 1982 and in revised form October 19, 1982

MARÍA PILAR AZUARA AND PEDRO J. APARICIO
Instituto de Biología Celular, Consejo Superior de Investigaciones Científicas, Velázequez, 144 Madrid-6, Spain

ABSTRACT

Chlamydomonas reinhardii **cells, growing photoautotrophically under air, excreted to the culture medium much higher amounts of NO_2^- and NH_4^+ under blue than under red light. Under similar conditions, but with NO_3^- as the only nitrogen source, the cells consumed NO_2^- and excreted NH_4^+ at similar rates under blue and red light. In the presence of NO_3^- and air with 2% CO_2 (v/v), no excretion of NO_2^- and NH_4^+ occurred and, moreover, if the bubbling air of the cells that were currently excreting NO_2^- and NH_4^+ was enriched with 2% CO_2 (v/v), the previously excreted reduced nitrogen ions were rapidly reassimilated. The levels of total nitrate reductase and active nitrate reductase increased several times in the blue-light-irradiated cells growing on NO_3^- under air. When tungstate replaced molybdate in the medium (conditions that do not allow the formation of functional nitrate reductase), blue light activated most of the preformed inactive enzyme of the cells. Furthermore, nitrate reductase extracted from the cells in its inactive form was readily activated *in vitro* by blue light. It appears that under high irradiance (90 w m^{-2}) and low CO_2 tensions, cells growing on NO_3^- or NO_2^- may not have sufficient carbon skeletons to incorporate all the photogenerated NH_4^+. Because these cells should have high levels of reducing power, they might use NO_3^- or, in its absence, NO_2^- as terminal electron acceptors. The excretion of the products of NO_2^- and NH_4^+ to the medium may provide a mechanism to control reductant level in the cells. Blue light is suggested as an important regulatory factor of this photorespiratory consumption of NO_3^- and possibly of the whole nitrogen metabolism in green algae.**

Nitrate assimilation in green algae and higher plants is a basic metabolic process because it uses more than 20% of the reducing power generated by their photosynthetic apparatus (11). Among the different steps involved in this metabolic pathway, reduction of NO_3^- to NO_2^- catalyzed by nitrate reductase has become particularly relevant due to its regulatory features on nitrogen metabolism (5, 12, 36).

NADH-nitrate reductase from green algae is a multimeric enzyme of high mol wt with several electron transport components such as flavin adenine dinucleotide, protoheme b_{557}, and molybdenum (8). Recently, it has been reported that molybdenum is held in a special cofactor that contains an unidentified pterin (13).

Depending on the growth conditions, nitrate reductase can be extracted from alga cells in two interconvertible forms: active and inactive. In cells optimally grown on NO_3^- under air enriched in CO_2, nitrate reductase is usually found in its active form. However, after mid-term (1–2 h) NH_4^+ addition to such cells, most of nitrate reductase appears in its inactive form (11, 15, 16).

In vitro nitrate reductase can be inactivated by incubation with NAD(P)H or $S_2O_4^{2-}$ and more readily if CN^- or C_2H_2 are also present (14, 18). Both the *in vivo* inactive form and the *in vitro* CN^--inactivated spinach enzyme can be rapidly reactivated by oxidation with ferricyanide. *In vitro* blue-light irradiation of CN^-- or C_2H_2-inactivated nitrate reductase, as well as the *in vivo* inactive enzyme from *Chlorella fusca*, promotes their full activation (1, 18a). Likewise, CN^--inactivated nitrate reductase from *Neurospora* is also fully reactivated by blue light (23). The photoreactivation of spinach nitrate reductase is greatly accelerated by exogenous flavins, especially under anaerobic conditions. Therefore, excited flavins, but not some highly reactive oxygen species, are directly involved in the photoreactivation. Accordingly, it has been proposed that excited flavins that are strong oxidants (38) reactivate nitrate reductase by oxidizing some of its electron transport component(s), probably molybdenum. Alternatively, the excited flavins could act by removing protein-bound CN^- in a photoaddition reaction that generates cyanoflavins as proposed for the CN^--inactivated enzyme (18a).

In *C. fusca*, the assimilation of NO_3^- under nonsaturating irradiation was higher under blue than under red light (7). However, no direct evidence was reported assigning to blue light an *in vivo* regulatory role of nitrate reductase activity and therefore of nitrogen metabolism. The results presented in this article show that inactive form of nitrate reductase extracted from *Chlamydomonas reinhardii* can be reactivated by blue light and that cells, growing on NO_3^- under air without supplementary CO_2, release NO_2^- and NH_4^+ to the culture medium at much higher rates under blue than under red light. Furthermore, under these growth conditions, irradiation of the cells with blue light increases the enzyme level and maintains most of the intracellular nitrate reductase in active form.

MATERIALS AND METHODS

Chlamydomonas reinhardii 11-32b from the Alga Collection of Göttingen University was grown autotrophically in a mineral medium with NO_3^- as the only nitrogen source (31). The medium was adjusted to pH 7.1 with K-phosphate and gassed with air (containing approximately 0.03% CO_2). Cultures (800 ml) were illuminated with both 160-w model SBR-F, PAR 38, (Eye, Japan) and 100-w tungsten lamps under alternating light-dark periods of 7 and 5 h, respectively. Daily, in the middle of the light period, 300 ml of the growing culture were removed and the remaining culture was refilled up to the initial volume with fresh nutrient solution. The cells were collected by centrifugation and washed three times with the same nutrient solution except that the pH was 7.8, and in some cases NO_3^- was omitted. The resuspended cells were diluted with the pertinent nutrient solution to obtain a final Chl content of 35 $\mu g\ ml^{-1}$. Chl concentration did not change

[1] Supported in part by grants to P.J.A. and R. Malkin from the USA-Spain Joint Committee of Cooperative Research III P-7730394/7, and to P.J.A. from the Comisión Asesora de Investigación científica y Técnica 1222. This investigation was carried out under Consejo Superior de Investigaciones Científicas Programme

appreciably during the subsequent experimental period. From the resulting cell suspensions, 12 to 15 ml were held with magnetic stirring in glass cylindrical tubes (22 mm inner diameter, 68 mm high) placed in small transparent metacrylate baths at 26°C. Where indicated, air with 2% CO_2 (v/v) was bubbled through the cell suspensions with hypodermic needles, making stirring unnecessary. Two slide projectors fitted with DT 450- and 680-nm wide-band Balzers filters were used to illuminate the cell suspensions. Light intensity at the sample location was measured with a Yellow Springs Radiometer and adjusted for both color lights to 90 w m^{-1} using neutral density filters.

To estimate the levels of nitrate reductase activity, 4 ml of cell suspension were supplemented with 20 μM flavin adenine dinucleotide, 2 mM cystein, and 5 mM EDTA and sonicated in translucent polyethylene tubes at 4°C either under background white light at the beginning of the experiment or under background blue or red light according to the particular experiment. The illuminating conditions were obtained with a 100-w tungsten lamp fitted with commercial metacrylate dip blue plate or Kodak safelight red filter No. 1A. Two 45-s periods of sonication with 1-min interval were carried out with a MSE sonifier at 10 kc and 6 μamps to disrupt the cells. Subsequently, parts of the broken cell suspensions were incubated for 2 to 5 min with 0.4 mM ferricyanide. Aliquots (0.5 ml) from both ferricyanide-treated and untreated broken cell suspensions were used both to estimate NADH-nitrate reductase activity and to determine indigenous NO_2^- content. To stop the enzymic reaction and eliminate NADH interference with the diazotization reaction, zinc acetate and phenazine methosulfate were added to the reaction mixture after 20 min incubation at room temperature (25).

For the experiments on *in vitro* photoactivation of nitrate reductase, the cells were grown under the above conditions, although with continuous illumination. Cells (1.5 g) were harvested by centrifugation, washed three times with 50 mM K-phosphate (pH 7.5), and broken by sonication as described above. Cell fragments were removed by centrifugation (28,000*g*, 15 min), and the supernatant was used for the photoactivation studies. The cell-free extract (2.0 ml) was supplemented with 10 μM flavin adenine dinucleotide and illuminated at 4°C in glass tubes under the same conditions described above, but with 180 w m^{-2} for both blue and red lights. Aliquots (0.2 ml) were taken out to estimate NADH-nitrate reductase activity.

Chl was determined according to Arnon (3). Nitrite was measured by the diazotization method of Snell and Snell (27) and NH_4^+ by the phenolhypochlorite method of Solorzano (28). NADH-nitrate reductase activity was assayed as described elsewhere (18). One unit of nitrate reductase corresponds to 1.0 μmol of NO_2^- formed/min. Protein was estimated according to Lowry *et al.* (17) using BSA as standard.

RESULTS

Figure 1 shows that *Chlamydomonas* cells placed in a medium containing both NO_3^- and NO_2^- and gassed with air supplemented with 2% CO_2 assimilated NO_2^- at similar rates under blue or red light of the same intensity. However, when the cells were deprived of supplementary CO_2, NO_2^- uptake eventually stopped and subsequently a net release of NO_2^- began. The rate of NO_2^- excretion increased during light exposure and finally reached much higher values under blue than under red light. Figure 2 shows that, under air, cells growing on NO_3^- not only released NO_2^- but also NH_4^+, blue light being again much more effective than red light. However, the release of NH_4^+ was slightly delayed when compared with that of NO_2^-. These results indicate that, under air, blue light may promote NO_3^- entrance and/or reduction at such high rates that a major portion of NO_2^- and NH_4^+ formed in the cells is released to the culture medium. Strotmann

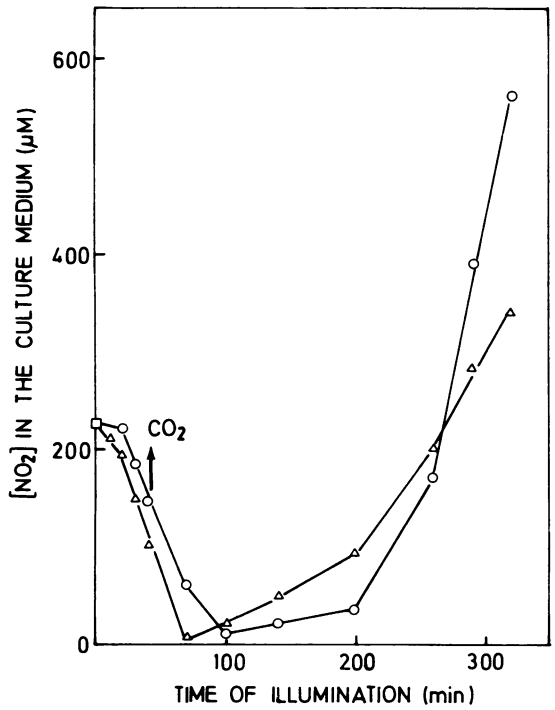

FIG. 1. Effect of CO_2 and light quality on NO_2^- utilization and NO_3^--dependent NO_2^- excretion. Cells were suspended in a medium containing 10 mM NO_3^- and 0.25 mM NO_2^-. Initially, the cell suspensions were gassed with air containing 2% CO_2 (v/v). Where indicated by the arrow, the cell suspensions were gassed with air containing approximately 0.03% CO_2. Blue (○) and red (△) light illumination.

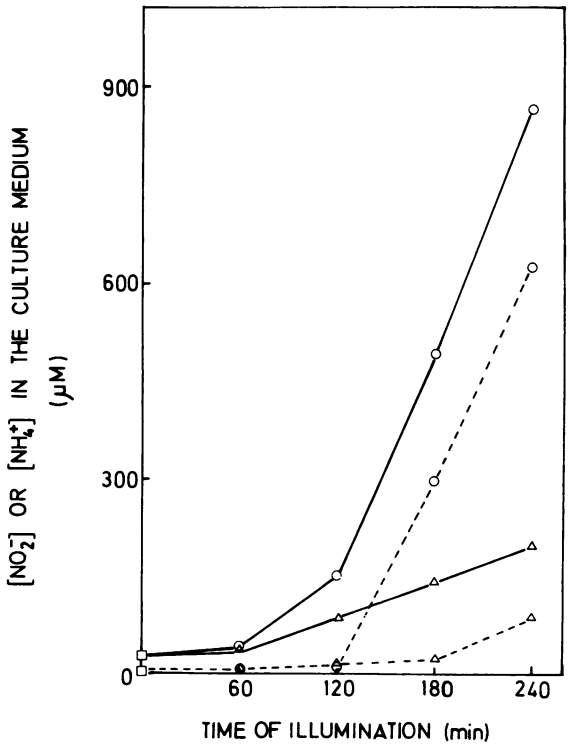

FIG. 2. Blue-light-promoted NO_2^- and NH_4^+ excretion by cells suspended in a nutrient solution with 10 mM NO_3^- under air. NO_2^- excretion (——), NH_4^+ excretion (– – –), blue (○), and red (△) light.

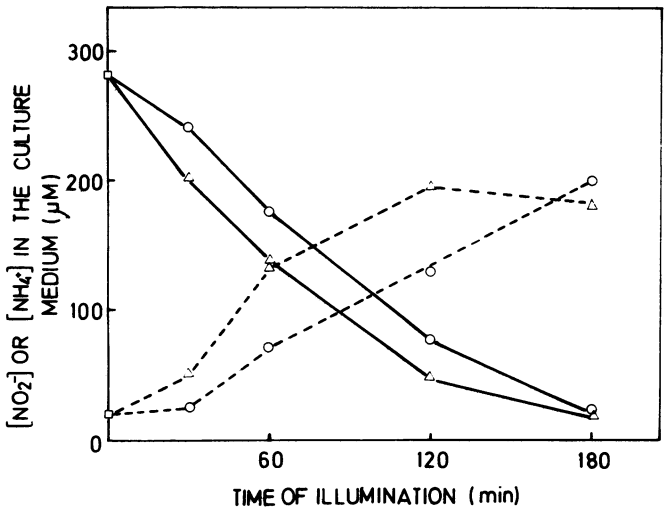

FIG. 3. NO_2^- consumption and NH_4^+ release under blue and red light in cells suspended in a nutrient solution with 0.3 mM NO_2^- under air. NO_2^- consumption (——), NH_4^+ release (- - -), blue (○), and red (△) light.

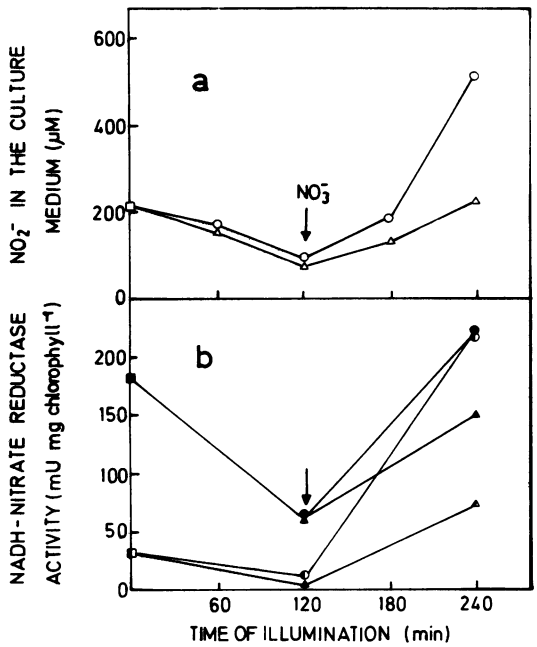

FIG. 4. a, Effect of light quality on NO_2^- consumption and NO_3^--dependent NO_2^- excretion by cells growing under air. At zero time, cells were suspended in a medium containing 0.2 mM NO_2^-. When indicated by the arrow, 10 mM NO_3^- was added to the cultures. Blue (○) and red (△) light. b, Activity levels of NADH-nitrate reductase in the corresponding broken cell suspensions. Activity of the active form (◐, ▲); total activity, after ferricyanide incubation (●, ▲); blue light (◐, ●); red light (▲, ▲).

and Ried (29) have observed NO_2^- excretion from *Chlorella* when NO_3^- was the only nitrogen source in the medium.

To investigate if light quality also modulates NO_2^- consumption, cell suspensions with NO_2^- as the only nitrogen source were illuminated with blue or red light under air. NO_2^- uptake was mostly independent of light quality and proceeded until completion accompanied by a parallel NH_4^+ excretion (Fig. 3).

Under low CO_2 tensions, excretion of both NO_2^- (Figs. 1 and 2) and NH_4^+ (Figs. 2 and 3) is probably determined by the low levels of available carbon skeletons for further assimilation, since, if air was subsequently supplemented with 2% CO_2, eventually all excreted combined nitrogen was rapidly taken up (data not shown).

To study the internal levels of active and inactive nitrate reductase in relation to both light quality and NO_2^- excretion, the cells were disrupted at different stages and conditions of growth. When 10 mM NO_3^- was added to cells suspended in a NO_2^- medium and bubbled with air, the cells became exporters instead of importers of NO_2^- (Fig. 4). The rates of NO_2^- excretion increased during light exposure reaching again much higher values under blue than under red light. Figure 4b shows the NADH-nitrate reductase activity of the corresponding cells at different times. At the beginning, nitrate reductase was found predominantly in its inactive form, in part probably due to the obligate dark intervals necessary to prepare the cells for the experiment. At the end of the NO_2^- consumption period, either under blue or red light, the levels of both the ferricyanide-reactivated enzyme and the standing active enzyme appreciably decreased. However, 2 h after NO_3^- addition, cells illuminated with blue light showed a higher level of nitrate reductase and a fully active enzyme. In the extracts of the cells illuminated with red light, the levels of both the active form and the ferricyanide-reactivated enzyme were much lower. It is worth noting that, on a Chl basis, the different rates of NO_2^- excretion and the levels of the active form of the extracted nitrate reductase show a reasonable relationship (157 nmol NO_2^- excreted $min^{-1} \cdot mg^{-1}$ Chl and 228 enzymic munits $\cdot$ mg^{-1} Chl for the cells illuminated with blue light; and 46 and 78, respectively, for the red-light cells), taking into account that part of the NO_2^- formed undergoes further reduction to NH_4^+.

From the preceding results, we concluded that, in the presence of NO_3^-, blue light reactivates the inactive enzyme and also promotes the *de novo* synthesis and/or assembly of some preexisting enzyme precursors (12, 32, 33, 36). To further establish that, among other possible effects, blue light indeed activates *in vivo* nitrate reductase, the cells were placed in a medium where tungstate replaced molybdate. In green algae, when suitable concentrations of tungsten are present in the culture medium, this metal becomes incorporated in *de novo* synthesized nitrate reductase, presumably replacing the constitutive molybdenum, and generates an enzyme that is not able to reduce NO_3^- (22, 35). However, tungsten cannot displace molybdenum from the native nitrate reductase (20, 21). When tungstate was added to a molybdenum-free cell suspension, again high NO_2^- excretion took place under blue light (Fig. 5a). The values of the ferricyanide-reactivated enzyme remained almost unchanged in the blue-light cells and decreased only moderately in the red-light cells, while the level of active nitrate reductase increased more than four times in the blue-light cell extracts and only a slight increase was detected in the red-light cell extracts (Fig. 5b). These results seem to corroborate that blue light promotes a definite activation of the *in situ* inactive enzyme.

When an extract of *Chlamydomonas* cells containing *in vivo* inactivated nitrate reductase was exposed to blue light, the enzyme was reactivated more than three times in 25 min. In contrast, there was little effect on enzymic activity when the extract was kept in the dark or illuminated with red light (Fig. 6). The observed initial lag on photoreactivation might be due to the presence in the cell-free extract of some internal reductants that, like ascorbate or reduced pyridine nucleotides, may quench excited flavins before they become completely photooxidized (24). As described elsewhere for the *Chlorella* nitrate reductase (2), these data show that blue light is capable of activating the inactive form of the *Chlamydomonas* nitrate reductase.

DISCUSSION

Under the actual prevailing photorespiratory conditions of plant growth, *i.e.* low CO_2 air and high light intensity, *C. reinhardii*

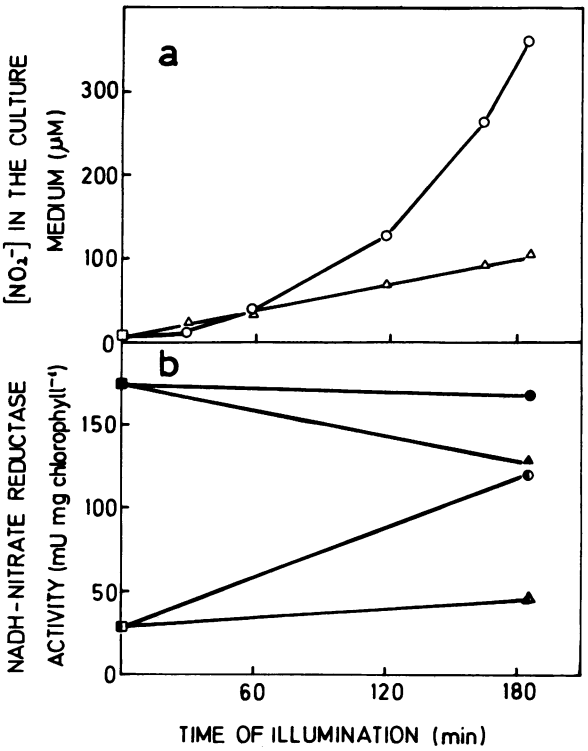

FIG. 5. a, Photochromic effect on NO_2^- excretion in cells growing under air with tungstate replacing molybdate. The cells were suspended at zero time in a medium containing 10 mM NO_3^- and 200 μm tungstate. Blue (○) and red (△) light. b, Activity levels of NADH-nitrate reductase in the corresponding cells suspensions. Activity of the active form (◐, ◭); total activity, after ferricyanide incubation (●, ▲); blue light (◐, ●); red light (◭, ▲).

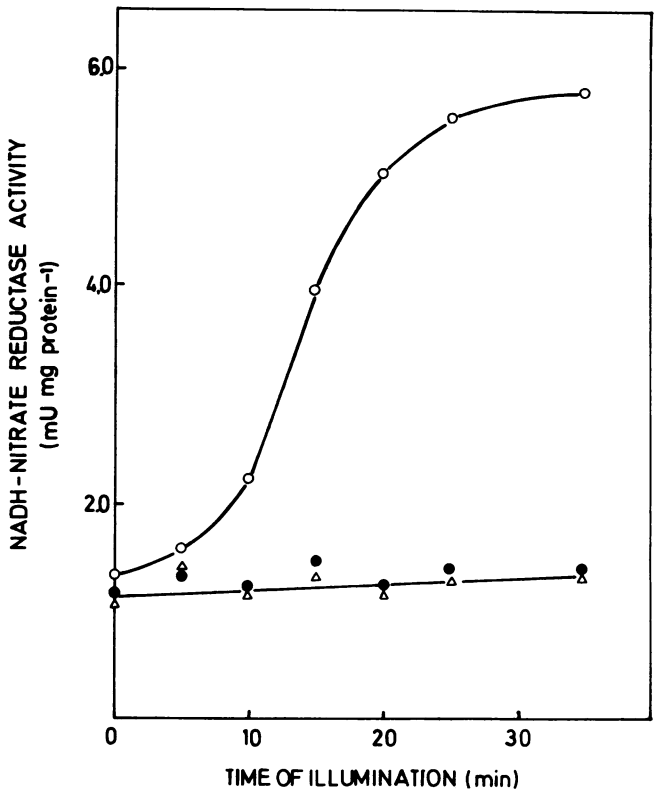

FIG. 6. Activation of nitrate reductase by blue light irradiation of the cell-free extract. Blue light (○); red light (△); dark (●).

nitrogen metabolism appears to be photochromically regulated, inasmuch as cells under these conditions consume NO_3^- and release to the culture medium a major portion of the NO_3^- reduction products, namely NO_2^- and HN_4^+, at much higher rates under blue than under red light. In this regard, preliminary results indicate that *Chlorella pyrenoidosa* also behaves similarly. Blue light *in vivo* may act primarily as a trigger directly activating nitrate reductase and also promoting the biosynthesis and/or the assembly of additional enzyme. The observed *in vitro* blue light activation of different nitrate reductases, including that of *Chlamydomonas*, strongly supports the idea of the *in vivo* regulation of the enzyme activity by the blue component of the light.

More likely, under low CO_2 tensions, the CO_2-fixing capability of the cells is not able to supply sufficient carbon skeletons to incorporate the high amounts of NH_4^+ that can be potentially generated from the excess of internal reducing power caused by high irradiance. Thus, cells that cannot allocate their light-generated reducing power in the assimilation of CO_2 may use the oxidized forms of nitrogen as electron sinks, thus releasing to the culture medium the possibly harmful excess of internal reductants.

Nitrate reductase from photosynthetic eukaryotes is currently considered as an assimilatory enzyme to differentiate it from nitrate reductases of the NO_3^--respiring prokaryotes (11). The experimental evidences described above indicate, however, that nitrate reductase in green algae might have an additional role, reducing NO_3^- in a photorespiratory way that is regulated, among other factors, by blue light. In the long term and with limited amounts of NO_3^- in the culture medium, it can be inferred that, once NO_3^- would become exhausted by cell growth, NO_2^- and NH_4^+ might be subsequently reassimilated as further CO_2 fixation occurs. Hence, photorespiratory NO_3^- reduction does not imply the disappearance of the nitrogen source from the alga environment as it happens to be the case in the NO_3^--respiring prokaryotes that currently evolve dinitrogen (39).

The fact that the cells in the presence of NO_3^- and under low CO_2 tensions excrete not only NH_4^+ but also NO_2^- may imply some unknown regulatory mechanism related to the reduction of NO_2^- to NH_4^+. Preliminary results indicate that in *Chlamydomonas*, as already reported for other photosynthetic eukaryotes (11, 33), nitrite reductase activity is 5 times higher than the values of nitrate reductase from the cells illuminated with blue light and that nitrite reductase exhibited similar activity levels after either short dark periods or under blue or under red light. On the other hand, the results described above show that, in the presence of 2% CO_2 and NO_3^-, NO_2^- is rapidly assimilated, and according to the data from *Chlamydomonas* and other green algae (6, 14, 31), it might be even preferentially utilized to NO_3^-.

Under high CO_2 tensions (2–5% v/v) or in the presence of bicarbonate, the regulation of NO_3^- assimilation in green algae may vary appreciably. Actually, the main difference concerns the strong inhibitory effect exerted by NH_4^+ under high CO_2 tensions or bicarbonate concentrations on the NO_3^- uptake system and on nitrate reductase activity in several green algae including *Chlorella* (6, 11, 27, 30, 36) and *Chlamydomonas* (9, 31). On the contrary, in *Chlamydomonas* kept under air, NO_3^--dependent NH_4^+ and NO_2^- excretion proceeded for long periods of time without any appreciable change in its rate, although NH_4^+ concentration in the medium rose continuously, which indicates that both NO_3^- uptake system and nitrate reductase operated unrestrictively, provided that the cells were illuminated with blue light. Hence, in contrast to recent proposals (9), it seems unlikely that NH_4^+ by itself would block the uptake of NO_3^-, unless this effect also would be reversed by blue light under low CO_2 tensions. Nevertheless, Florencio and Vega (9) have recently shown that darkness inactivated nitrate reductase in *Chlamydomonas* cells kept under high bicarbonate concentrations, and that subsequent illumination

of the cells resulted in the full reactivation of the enzyme in short periods of time (30 min). However, no light quality experiments were conducted by the authors. The actual chemical species that contribute to the *in vivo* inactivation of nitrate reductase is still a matter of controversy (5, 11, 36). Notwithstanding, in all cases tested, *in vitro* blue light irradiation of the enzyme leads to its activation (2, 18a).

The *in vivo* requirement of NO_3^- for the increase in internal levels and activation of nitrate reductase promoted by blue light is in good agreement with the results of Florencio and Vega (9) and others (11, 16). *In vitro*, however, although only spinach nitrate reductase has been studied, photoactivation of CN^--inactivated enzyme was independent of NO_3^-, unless enzyme irradiation was carried out in the presence of NADH, the physiological electron donor of the enzyme. In this case, NO_3^- contributed markedly to the initiation and acceleration of the photoreactivation (23). These experimental conditions might be more close to the physiological ones. On the other hand, NO_3^- by itself does not reactivate *in vitro* nitrate reductase at the same rate that blue light irradiation does (24, 34).

The well-documented fact that plants grown under blue light have relatively a higher protein content, while those under red light are relatively rich in carbohydrates, has contributed greatly to stimulate research on photochromic regulation of carbon metabolism (37; note the papers in Ref. 26). Miyachi and Miyachi, studying *Chlorella* (19), and Gnanam *et al.*, studying higher plants (10), have observed that the presence of NH_4^+ can mimic most of the metabolic effects caused by blue light. According to the present data, their observations could be explained by the blue light effect on the activation, biosynthesis and/or assembly of nitrate reductase, which in turn leads to a definite increase in the availability of NH_4^+ for a number of metabolic purposes.

Acknowledgments—The authors thank Drs. F. F. del Campo, J. M. Ramírez, and G. Giménez-Gallego for their useful discussions and suggestions. We are indebted to Mrs. M. C. Partearroyo and C. F. Cabrera for their secretarial assistance.

LITERATURE CITED

1. Aparicio PJ, JM Maldonado 1979 Regulation of nitrate assimilation in photosynthetic organisms. *In* EJ Hewitt, CV Cutting, eds, Nitrogen Assimilation of Plants, Academic Press, London, pp 207–215
2. Aparicio PJ, JM Roldan, F Calero 1976 Blue light photoreactivation of nitrate reductase from green algae and higher plants. Biochem Biophys Res Commun 70: 1071–1077
3. Arnon DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1–15
4. Barea JL, F Sosa, J Cardenas 1976 Cyanide inactivation of *Chlamydomonas reinhardii* nitrate reductase under reducing conditions. Z Pflanzenphysiol 79: 237–245
5. Beevers L, RH Hageman 1980 Nitrate and nitrite reduction. *In* BJ Miflin, ed, The Biochemistry of Plants, Vol 5. Academic Press, New York, pp 115–168
6. Calero F 1978 Estudios a nivel celular de la asimilación del nitrato en *Chlorella fusca* papel regulador del amoniaco y de la luz azul. PhD thesis. University of Sevilla, Sevilla, Spain
7. Calero F, WR Ullrich, PJ Aparicio 1980 Regulation by monochromatic light of nitrate uptake in *Chlorella fusca*. In H Senger, ed, The Blue Light Syndrome. Springer-Verlag, Berlin, pp 411–421
8. De La Rosa MA, JM Vega, WG Zumft 1981 Composition and structure of assimilatory nitrate reductase from *Aknistrodesmus braunii*. J Biol Chem 256: 5814–5819
9. Florencio FJ, JM Vega 1982 Regulation of the assimilation of nitrate in *Chlamydomonas reinhardii*. Phytochemistry. 21: 1195–1200
10. Gnanam A, AH Mohamed, R Seetha 1980 Comparative studies on the effect of ammonia and blue light on the regulation of photosynthetic carbon metabolism in higher plants. *In* H Senger, ed, Blue Light Syndrome. Springer-Verlag, Berlin, pp 435–443
11. Guerrero MG, JM Vega, M Losada 1981 The assimilatory nitrate-reducing system and its regulation. Annu Rev Plant Physiol 32: 169–240
12. Hewitt EJ, DP Hucklesby, AF Mann, BA Notton, GJ Rucklidge 1979 Regulation of nitrate assimilation in plants. *In* EJ Hewitt, CV Cutting, eds, Nitrogen Assimilation of Plants. Academic Press, London, pp 255–287
13. Johnson JL, BE Hainline, KV Rajagopalan 1980 Characterization of the molybdenum cofactor of sulfite oxidase, xanthine oxidase, and nitrate reductase. J Biol Chem 255: 1783–1786
14. Knutsen G 1965 Induction of nitrite reductase in synchronized cultures of *Chlorella pyrenoidosa*. Biochim Biophys Acta 103: 495–502
15. Losada M, MG Guerrero 1979 The photosynthetic reduction of nitrate and its regulation. *In* J Barber, ed, Photosynthesis in Relation to Model Systems. Elsevier, Amsterdam, pp 365–408
16. Losada M, J Herrera, JM Maldonado, A Paneque 1973 Mechanism of nitrate reductase reversible inactivation by ammonia in *Chlamydomonas*. Plant Sci Lett 1: 31–37
17. Lowry OH, NJ Rosebrough, AL Farr, RJ Randall 1951 Protein measurement with the Folin phenol reagents. J Biol Chem 193: 265–275
18. Maldonado JM, MA Vargas, SG Mauriño, PJ Aparicio 1981 Inactivation by acetylene of spinach nitrate reductase. Biochim Biophys Acta 661: 112–119
18a. Mavriño SG, MA Vargas, PJ Aparicio, JM Maldonado 1983 Blue light reactivation of spinach nitrate reductase inactivated by acetylene or cyanide. Effects of flavins and oxygen. Physiol Plant. In press.
19. Miyachi S, S Miyachi 1980 Effects of ammonia on carbon metabolism in photosynthesizing *Chlorella vulgaris* 11 h: the replacement of blue light by ammonium ion. *In* H Senger, ed, The Blue Light Syndrome. Springer-Verlag, Berlin, pp 429–434
20. Notton BA, EJ Hewitt 1971 The role of tungsten in the inhibition of nitrate reductase actdivity in spinach (*Spinacia oleracea* L.) leaves. Biochem Biophys Res Commun 44: 702–710
21. Notton BA, EJ Hewitt 1971 Reversible cyanide inhibition of spinach (*Spinacia oleracea L.*) nitrate reductase and nonexchangeability *in vitro* of protein bound molybdenum and tungsten. FEBS Lett 18: 22
22. Paneque A, JM Vega, J Cardenas, J Herrera, PJ Aparicio, M Losada 1972. ^{185}W-Labelled nitrate reductase from *Chlorella*. Plant Cell Physiol 13: 175–178
23. Roldan JM, WL Butler 1980 Photoreactivation of nitrate reductase from *Neurospora crassa*. Photochem Photobiol 32: 375–381
24. Roldan JM, F Calero, PJ Aparicio 1978 Photoreactivation of spinach nitrate reductase: role of flavins. Z Pflanzenphysiol 90: 467–474
25. Scholl RL, JE Harper, RH Hageman 1974 Improvements of the nitrite color development in assays of nitrate reductase by phenazine methosulfate and zinc acetate. Plant Physiol 53: 825–828
26. Senger H (ed) 1980 The Blue Light Syndrome. Springer-Verlag, Berlin.
27. Snell FD, CT Snell 1949 Colorimetric Methods of Analysis. D Van Nostrand Company, Inc, New York, pp 802–807
28. Solorzano L 1969 Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr 14: 799–801.
29. Strotmann H, A Ried 1969 Polarographische Messung der Nitritreduktion von *Chlorella* im monochromatischen Licht. Planta 85: 250–269
30. Syrett PJ, I Morris 1963 The inhibition of nitrate assimilation by ammonium in *Chlorella*. Biochim Biophys Acta 67: 566–575
31. Tacker A, PJ Syrett 1972 The assimilation of nitrate and ammonium by *Chlamydomonas reinhardii*. New Phytol 71: 423–433
32. Tischner R, A Huttermann 1978 Light-mediated acivation of nitrate reductase in synchronous *Chlorella*. Plant Physiol 62: 284–286
33. Tischner R, H Lorenzen 1979 Activation of the nitrate reducing system in synchronous *Chlorella* 211-8k (high temperature strain). Biochem Physiol Pflanzen 174: 99–105.
34. Vega JM, FJ Florencio, MA de la Rosa 1981 Studies on the regulation of the photosynthetic assimilation of nitrate in green algae. *In* G Akoyunoglou, ed, Photosynthesis IV. Regulation of Carbon Metabolism. Balaban International Science Service, Philadelphia, pp 721–730
35. Vega JM, J Herrera, PJ Aparicio, A Paneque, M Losada 1971 Role of molybdenum in nitrate reduction by *Chlorella*. Plant Physiol 48: 294–299
36. Vennesland B 1981 HCN and the control of nitrate reduction. The regulation of the amount of active nitrate reductase present in *Chlorella* cells. *In* H Bothe, A Trebst, eds, Biology of Inorganic Nitrogen and Sulfur. Springer-Verlag, Berlin, pp 233–243
37. Voskresenskaya NP 1979 Effect of light quality on carbon metabolism. In M Gibbs, E Latzko, eds, Encyclopedia of Plant Physiol, New Series, Vol 6. Springer-Verlag, Berlin pp 174–179
38. Yamase T 1981 Production of hydrogen by a photogalvanic cell with flavin mononucleotide-EDTA system. Photochem Photobiol 34: 111–114
39. Zumft WG, J Cardenas 1979 The inorganic biochemistry of nitrogen bioenergetic processes. Naturwissenschaften 66: 81–88