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THE PHYSIOLOGICAL SIGNIFICANCE OF LIGHT AND DARK NH_4^+ METABOLISM IN *CHLORELLA SOROKINIANA*

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Key Word Index—*Chlorella sorokiniana*; Chlorophyta; algal respiration; NH_4^+ assimilation; nitrogen metabolism.

Abstract—N-limited cells of *Chlorella sorokiniana* Shihira and Krauss 211/8k exhibited rates of respiratory oxygen consumption and photosynthetic oxygen evolution which were 70 and 17%, respectively, of those of N-sufficient cells. N-sufficient cells absorbed NH_4^+ in light at a linear rate, and absorption was 100% inhibited by darkness. N-limited cells, by contrast, absorbed NH_4^+ at almost similar rates in light and darkness. The rate of uptake in darkness decreased after 20 min, and decreased further after 60 min. In the light, by contrast, it decreased to a constant higher value, which appeared to approximate the sum of the rate of the dark absorption plus the rate of the light-dependent NH_4^+ absorption by N-sufficient cells. NH_4^+ in darkness did not affect respiration of N-sufficient cells. However, NH_4^+ supply to N-limited cells in darkness resulted in a sudden increase of respiratory oxygen consumption, suggesting that short-term control mechanisms were triggered; it also resulted in time-dependent increase in photosynthetic activity and chlorophyll content, suggesting long term control of the photosynthetic apparatus. NH_4^+ supply also resulted in a resumption of growth, in light but not in darkness. It is proposed that the use of NH_4^+ in darkness by N-limited cells serves to recover from N-limitation, whereas in light it serves to sustain both recovery and growth. This could also explain why cells which are in an N-sufficient status and need nitrogen for growth only, exhibited exclusively a light-dependent NH_4^+ uptake. © 1997 Elsevier Science Ltd

INTRODUCTION

Unicellular algae, cultured in a medium containing sufficient nitrogen for growth, assimilate NH_4^+ only under light and CO_2 conditions [1, 2], which suggests that they derive the necessary carbon skeletons from recent photosynthate [3–6]. However, exposure of algal cells to N-deprivation brings about an abrupt halt in cell division and reduces carbon flow into nitrogen-containing compounds, so that lipids and carbohydrates are preferentially synthesized [7–9]. When N becomes available, N-limited cells can use such carbon compounds for NH_4^+ assimilation and amino acid synthesis in darkness, and they do so at a rate that is much higher than that of light- and CO_2-dependent NH_4^+ assimilation by N-sufficient cells [1, 2, 10, 11].

The ability of N-starved or N-limited cells to assimilate NH_4^+ under dark conditions indicates that the addition of NH_4^+ results in an increase in dark respiratory oxygen consumption [10–14], and short term control mechanisms triggered by rapid change in ATP and ADP levels occurring during NH_4^+ absorption and assimilation into organic matter have been proposed [12, 15]. An integrative model for the control of carbon partitioning from starch to respiration by adenylate levels during NH_4^+ assimilation by *Selenastrum minutum*, was recently described [16].

In this paper we report studies with N-limited cells of *Chlorella sorokiniana* regarding the effects of light or darkness on recovery from N-limitation upon NH_4^+ supply, and related changes in respiratory and photosynthetic activities. It is shown that NH_4^+ metabolism associated with recovery from N-limitation is distinct from that associated with growth, that the two activities can be summed, and that they are independently governed by different signals arising from the N status of the cell or from cell growth.

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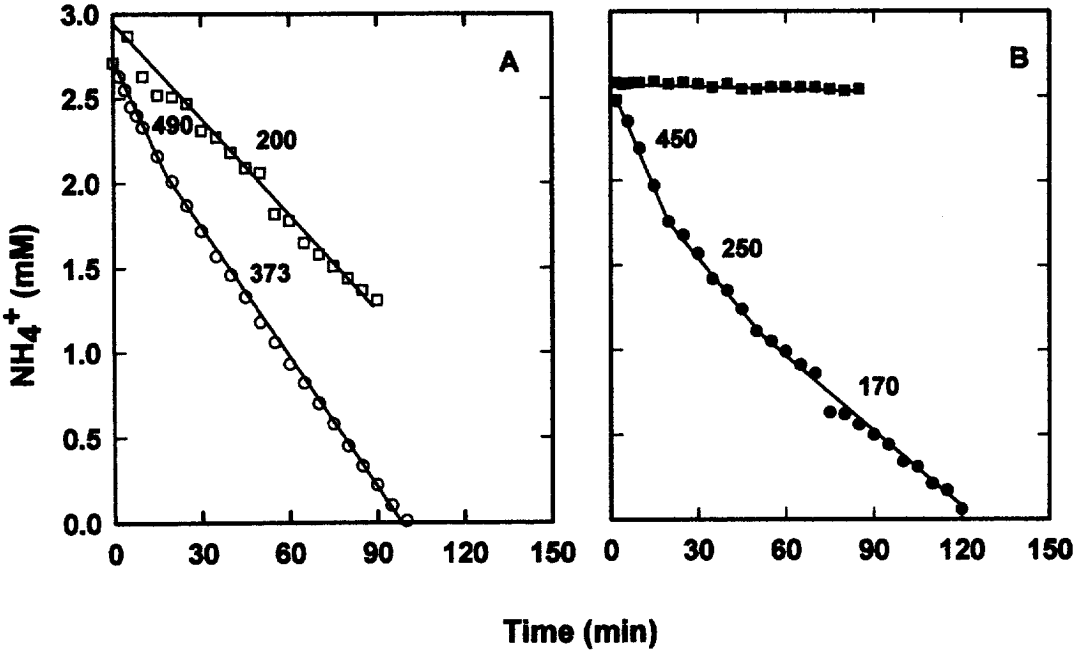

Fig. 1. The effect of darkness on ammonium uptake by N-sufficient and N-limited cells of *Chlorella sorokiniana*. A: (□–□) N-sufficient cells and (○–○) N-limited cells illuminated throughout; B: (■–■) N-sufficient cells and (●–●) N-limited cells in darkness. All cell suspensions were gassed with air containing 2% CO_2, and were kept at 35°. Addition of ammonium at zero time. Numbers above trace lines indicate uptake rates expressed as μmol ammonium absorbed hr^{-1} ml^{-1} pcv.

RESULTS

NH_4^+ uptake by N-sufficient and N-limited cells in light and darkness

N-sufficient cells absorbed NH_4^+ in light at the constant rate of 200 μmol hr^{-1} ml^{-1} packed cell volume (pcv) [Fig. 1(A)]. The net uptake of NH_4^+ was low or insignificant when cells were transferred to darkness [Fig. 1(B)]. By contrast, N-limited cells absorbed NH_4^+ at rates of 490 and 450 μmol hr^{-1} ml^{-1} pcv in light and darkness, respectively [Fig. 1(A and B)]. After a 20 min period the rate of uptake in darkness decreased to 250 μmol hr^{-1} ml^{-1} pcv, and decreased further to 170 μmol hr^{-1} ml^{-1} pcv after 60 min. In the light, by contrast, it decreased to a constant 373 μmol hr^{-1} ml^{-1} pcv, which appears to be the sum of the light-dependent NH_4^+ absorption by N-sufficient cells and the dark absorption by N-limited cells.

Effects of NH_4^+ on respiratory activity

The respiratory oxygen consumption by N-sufficient and N-limited cells of *Chlorella sorokiniana* occurred at the rate of 0.130 ± 0.004 and 0.092 ± 0.003 mmol hr^{-1} ml^{-1} pcv, respectively. Addition of NH_4^+ to N-limited cells of *C. sorokiniana* resulted in a sharp 3-fold increase in respiratory oxygen consumption during *ca* 9 min. Thereafter, the increase declined with time to a constant 2-fold. This latter value was maintained throughout the rest of the incubation period (Fig. 2). Resupply of NH_4^+ to N-sufficient cells of *C. sorokiniana* that had been temporarily transferred to an N-free medium for a few min did not affect respiratory activity of these cells (not shown).

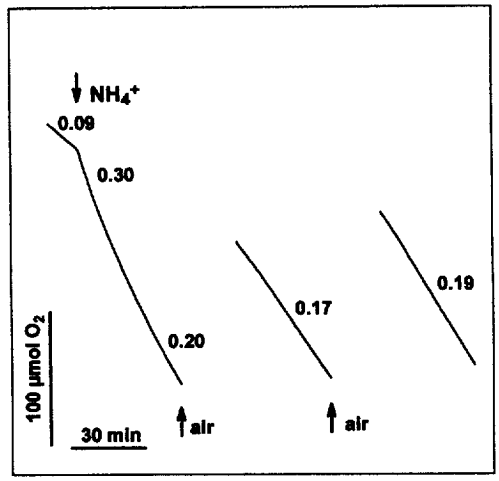

Fig. 2. Effect of ammonium on dark respiratory oxygen consumption by cells of N-limited *Chlorella sorokiniana*. Cell density was 1 μl pcv ml^{-1}. At the time indicated by the arrows, ammonium 2 mM was added. The upward arrow indicates gassing with air to restore oxygen consumed by respiration. Numbers above trace lines indicate uptake rates expressed as mmol oxygen consumed hr^{-1} ml^{-1} pcv. Measurements were made as indicated under Experimental.

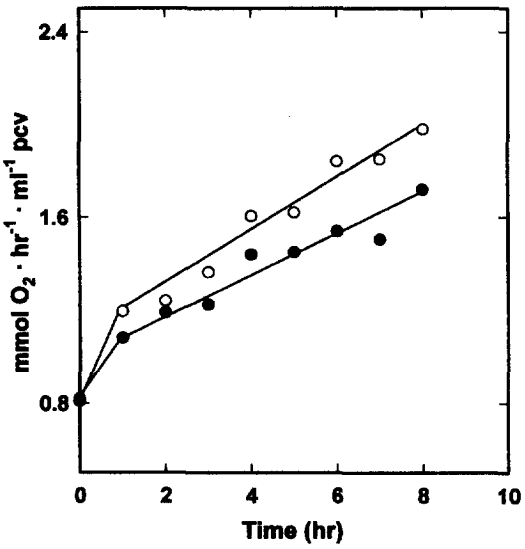

Fig. 3. Time-dependent increase in rates of photosynthetic oxygen evolution upon 2 mM ammonium addition to N-limited cells of *C. sorokiniana* illuminated (○–○) and darkened (●–●). Cells collected from continuous culture were resuspended in two separate water-jacketed cuvettes, one of which was kept in darkness, and then had 2 mM ammonium added. At the indicated times aliquots of cells suspensions were collected and tested for photosynthetic O_2 evolution as indicated under Experimental.

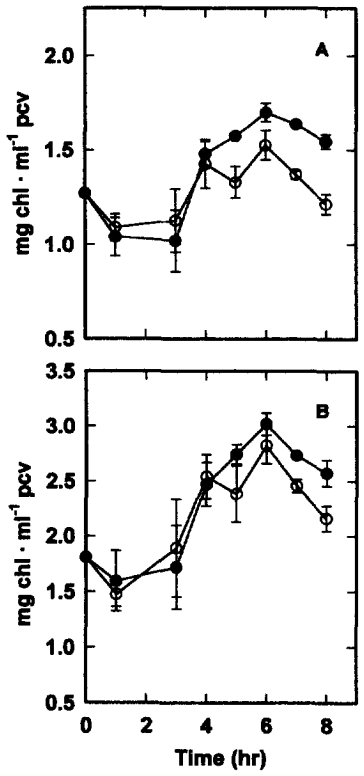

Fig. 4. Time-dependent increase of chlorophyll a (A) and total chlorophyll (B) content upon 2 mM ammonium addition to N-limited cells of *C. sorokiniana* resuspended in light (○–○) or darkness (●–●). Chlorophyll was estimated as reported under Experimental.

Effects of NH_4^+ resupply in light or darkness to N-limited cells on photosynthetic activity, chlorophyll content and cell growth

The gross photosynthetic oxygen evolution by N-sufficient and N-limited cells of *Chlorella sorokiniana* occurred at the rate of 3.260 ± 0.110 and 0.550 ± 0.013 mmol hr^{-1} ml^{-1} pcv, respectively. The addition of NH_4^+ to N-limited cells resuspended both in light or darkness, promoted a time-dependent increase in photosynthetic activity (Fig. 3) and in the chlorophyll content of the cell (Fig. 4). NH_4^+ addition also caused a linear increase in cell biomass, which after 8 hr was 30% higher than the starting point (Fig. 5). This increase occurred strictly in the light and not in darkness.

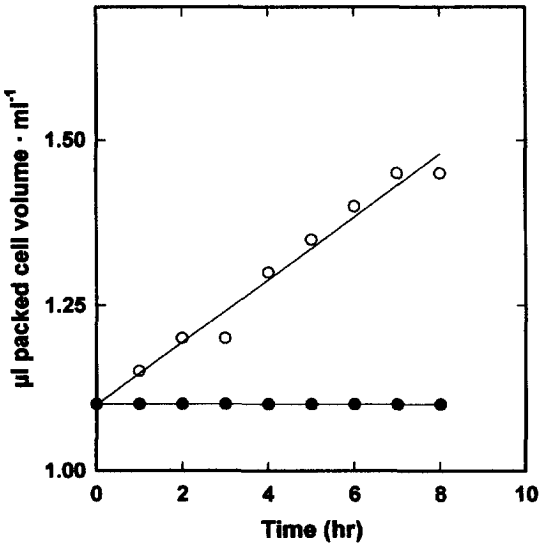

Fig. 5. Variations of packed cell volume, upon NH_4^+ addition, in suspensions of N-limited cells of *Chlorella sorokiniana* kept in light (○–○) and darkness (●–●).

DISCUSSION

NH_4^+, immediately after being added, was absorbed by N-limited *Chlorella sorokiniana* at almost similar rates both in light or darkness, but after 20 min the rate in darkness decreased with respect to that in light. NH_4^+ uptake by N-starved *Lemna gibba* also decreased in darkness more drastically than in light, showing that after the initial phase the uptake rate of NH_4^+ was determined by its assimilation rates [17, 18].

As with several other N-limited algae [14] and the root of barley plants [19], NH_4^+ addition was followed by an immediate enhancement of the dark respiratory oxygen consumption. As shown here with *C. sorokiniana*, enhancement was maximal initially and decreased along with the decline in the rate of NH_4^+ uptake. This indicates that between the two phenomena there was a strict correlation, and that the size of enhancement varied according to the rate of NH_4^+ utilization.

Respiratory activity of N-limited cells, measured under N-free conditions, was as high as 70% that of N-sufficient cells. The immediate enhancement in respiratory oxygen consumption by *C. sorokiniana* caused by NH_4^+ supply, however, suggests that respiratory enzymes were not affected by N-limitation, and that N metabolism influences respiratory activity mainly through short term control mechanisms; this is in agreement with the short term control phenomena at the level of regulatory glycolytic enzymes, triggered by NH_4^+ metabolism through changes in adenylates, described in *Selenastrum minutum* [16]. Photosynthetic activity of N-limited cells of *C. sorokiniana* was only 17% that of N-sufficient cells, and chlorophyll content also was noticeably lower, and both photosynthetic activity and chlorophyll increased with time upon NH_4^+ supply. These findings suggest that the photosynthetic apparatus, unlike the respiratory one, undergoes long-term control phenomena according to the N-status of the cell. In *Isochrysis galbana* also, N-deprivation affected the photosynthetic apparatus, and N-supply increased photosynthetic proteins such as ribulose bisphosphate carboxylase [9]. As shown, in *C. sorokiniana* recovery of gross photosynthesis and chlorophyll content, can take place also in darkness, although in *Euglena* light has been found to represent a necessary stimulus for induction [20].

Similar to N-deficient cells of *Euglena* supplemented with N [21], N-limited *C. sorokiniana* after resupply of NH_4^+ failed to grow in the dark, but the packed cell volume increased in cultures maintained in the light.

It is thus apparent that N-limited cells of *C. sorokiniana*, absorbed NH_4^+ in light at higher rates than did N-sufficient cells because, in addition to N-compounds required to support cell growth stimulated by the concomitant presence of light and NH_4^+, they also synthesize N-compounds necessary for recovery from N-limitation. In this respect it is worth noting that at steady conditions (20 min after NH_4^+ supply), N-limited cells of *C. sorokiniana* absorbed NH_4^+ in light at a rate (373 μmol hr^{-1} ml^{-1} pcv) which was apparently the sum of the rate at which NH_4^+ was absorbed in darkness by N-limited cells (170 μmol hr^{-1} ml^{-1} pcv) plus the rate of the light-dependent NH_4^+ assimilation by N-sufficient cells (200 μmol hr^{-1} ml^{-1} pcv). Different rates in light and dark ammonium assimilation also occurred in cell suspensions of the N-limited unicellular red alga *Cyanidium caldarium* [14]. These observations suggest that the dark NH_4^+ assimilation by N-limited algae is under the control of the N-status of the cells, whereas light-dependent NH_4^+ assimilation is under the control of the N-requirement for growth. It would appear that the two types of NH_4^+ assimilation have a different physiological significant. These results also explain why N-sufficient cells, which have to synthesize only those N-compounds necessary for growth, exhibit, in turn, NH_4^+ assimilation which is strictly dependent on light, and lack dark NH_4^+ assimilation.

EXPERIMENTAL

Organism and culture conditions. Chlorella sorokiniana Shihira and Krauss strain 211/8K (CCAP of Cambridge University) was grown in both a batch culture under conditions of nutrient sufficiency, including NH_4^+, and in a chemostat under conditions of NH_4^+ limitation. The cultures were kept at 35°, and were continuously illuminated (Philips TLD 30 W/55 fluorescent lamps, 160 μmol photons m^{-2} sec^{-1}), and flushed with air containing 2% CO_2 at a flow rate of about 80–100 l hr^{-1}. In the batch culture NH_4^+ 10 mM (supplied as $(NH_4)_2HPO_4$) was used; in the chemostat culture the fresh medium containing NH_4^+ 2 mM was added at a dilution rate of 0.15 day^{-1}. The *Chlorella* batch had a growth rate of 3.3 day^{-1}, and the chemostat had a growth rate of 0.33 day^{-1}. The composition of the basal medium and the growth procedure in batch and in chemostat was as previously reported [22]. The cells for experiments were collected from the batch culture when cell density was the same as that in the chemostat culture.

NH_4^+ uptake. Cells of *Chlorella* were harvested and concd by centrifugation (4000 *g* for 5 min), resuspended in a N-free culture medium and then incubated at 35° using a water-jacketed cuvette in the light or darkness as reported in the text. The cell suspensions were flushed with air containing 2% CO_2.NH_4^+ uptake experiments started with the addition of NH_4^+ as indicated in the text. NH_4^+ utilization by the cells was assayed by measuring its disappearance from the external medium. Samples of the cell suspension were taken at 5 min intervals and centrifuged. The residual NH_4^+ in the clear supernatant was treated with 0.2 M NaOH to obtain NH_3 which was assayed using an NH_3-specific electrode (Orion 95-12, Cambridge, MA, U.S.A.) connected to an ion analyser (Orion model EA 920) adjusted in concn mode.

Respiration and photosynthesis. Rates of O_2 exchange were measured in a water-jacketed vessel equipped with an O_2 electrode (Orion 97-08) connected to an EA 920 ion analyser adjusted in O_2 mode and connected to a data printer. Data were printed at 1 min intervals.

Packed cell volume (pcv). Estimated by centrifuging a known aliquot of cells suspension in a haematocrit tube.

Chlorophyll estimation. Chlorophyll content was estimated according to ref. [23].

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REFERENCES

1. Thacker, A. and Syrett, P. J., *New Phytology*, 1972, **71**, 423.
2. Di Martino Rigano, V., Vona, V., Di Martino, C. and Rigano, C., *Physiologia Plantarum*, 1986, **68**, 34.

3. Kanazawa, T., Kirk, M. R. and Bassham, J. A., *Biochimica et Biophysica Acta*, 1970, **205**, 401.
4. Kanazawa, T., Kanazawa, K., Kirk, M. R. and Bassham, J. A., *Biochimica et Biophysica Acta*, 1972, **256**, 656.
5. Kanazawa, T., Distefano, M. and Bassham, J. A., *Plant Cell Physiology*, 1983, **24**, 979.
6. Turpin, D. H., *Journal of Phycology*, 1991, **27**, 14.
7. Ohmori, M., Wolf, F. R. and Bassham, J. A., *Archives of Microbiology*, 1984, **140**, 101.
8. Hipkin, C. R. and Syrett, P. J., *Planta*, 1987, **133**, 209.
9. Livne, A. and Sukenik, A., *Plant Cell Physiology*, 1992, **33**, 1175.
10. Syrett, P. J., *Annals of Botany*, 1953, **65**, 1.
11. Syrett, P. J., *Annals of Botany*, 1953, **65**, 21.
12. Ohmori, M., Miyachi, S., Okabe, K. and Miyachi, S., *Plant Cell Physiology*, 1984, **25**, 749.
13. Weger, H. G., Birch, D. G., Elrifi, I. R. and Turpin, D. H., *Plant Physiology*, 1988, **86**, 688.
14. Rigano, C., Di Martino Rigano, V., Vona, V., Esposito, S. and Di Martino, C., in *Inorganic Nitrogen in Plants and Micro-organisms*, ed. W. R. Ullrich, C. Rigano, A. Fuggi and P. J. Aparicio. Springer, Berlin, 1990, p. 131.
15. Syrett, P. J., *Archives of Biochemistry and Biophysics*, 1958, **75**, 117.
16. Turpin, D. H., Botha, F. C., Smith, R. G., Feil, R., Horsey, A. K. and Vanlerberghe, G. C. *Plant Physiology*, 1990, **93**, 166.
17. Ullrich, W. R., Larsson, M., Larsson, C. M., Lesch, S. and Novacky, A., *Physiologia Plantarum*, 1984, **61**, 369.
18. Ullrich, W. R., Lesch, S., Jarczyk, L., Herterich, M. and Trogich, G. D., in *Inorganic Nitrogen in Plants and Micro-organisms*, ed. W. R. Ullrich, C. Rigano, A. Fuggi and P. J. Aparicio. Springer, Berlin, 1990, p. 44.
19. Rigano, C., Di Martino Rigano, V., Vona, V., Carfagna, S., Carillo, P. and Esposito, S., *New Phytology*, 1996, **132**, 375.
20. Sukenik, A., Bennett, J., Mortain-Bertrand, A. and Falkowski, P. G., *Plant Physiology*, 1990, **92**, 891.
21. Coleman, L. W., Rosen, B. H. and Schwartzbach, S. D., *Plant Cell Physiology*, 1988, **29**, 423.
22. Di Martino Rigano, V., Vona, V., Fuggi, A., Di Martino, C. and Rigano, C., *Plant Science Letters*, 1983, **28**, 265.
23. William, P. I. and Bloom, P. R., *Plant Physiology*, 1985, **77**, 483.