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Nitrogen Metabolism of the Marine Microalga *Chlorella autotrophica*[1]

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ABSTRACT

The levels of glutamine synthetase (GS) and glutamate dehydrogenase (GDH) in *Chlorella autotrophica* (clone 580) are strongly regulated by the nitrogen source and salt concentration of the medium. GS is present at high levels in NO_3^--grown cells, and at maximum levels in nitrogen-starved cells. However, the levels of GS in these cells are somewhat decreased by increasing salinity. Cells growing on NH_4^+ have high NADPH-GDH activity, the levels of which increase with increasing NH_4^+ supply, while GS decreases to a very low level under these conditions. Salinity intensifies the induction of NADPH-GDH activity in NH_4^+-grown cells. The levels of NADH-GDH are low in this alga, but present under all growth conditions. Methionine sulfoximine (MSX) has little effect on growth and nitrogen assimilation of the alga in the presence of NH_4^+.

Numerous studies have dealt with the uptake and utilization by marine microalgae of various nitrogen sources found in seawater (5, 8, 14, 17, 26). However, only a few of these have focused on metabolic pathways of nitrogen assimilation in these organisms (3, 7, 26). Furthermore, there appears to be a need to examine nitrogen assimilation in microalgae in relation to metabolic adaptation to salinity fluctuations. This consideration is particularly important for euryhaline microalgae which are subject to rapid changes in salinity in their natural environments (2). Metabolic activities such as photosynthesis have been shown to react to changes in external salt concentrations (4, 10, 13), but little is known about the effect of external salinity on nitrogen assimilation in microalgae.

Eukaryotic green plants have the enzymic potential for the assimilation of NH_4^+ by the reductive amination of 2-oxoglutarate either by GDH[2] or by the combined action of GS and GOGAT (19, 24, 26). In higher plants, the GS-GOGAT cycle appears to be the primary pathway of NH_4^+ assimilation, and GDH plays only a minor role (19, 24). Also, for some microalgae such as *Platymonas striata* (7) and *Chlamydomonas reinhardii* (6), only the activities of GS and GOGAT were found to be sufficient to carry out the required rate of nitrogen assimilation. However, many microalgae contain high levels of GDH activities (9, 20, 22–24, 27) indicating a potential role for this enzyme in NH_4^+ assimilation. The only clear evidence to date of the participation of GDH in NH_4^+ assimilation by green algae has been provided by Everest and Syrett (9) for NO_3^--grown *Stichococcus bacillaris* which has an inducible NADPH-GDH with a low K_m for NH_4^+ (K_m^{app}, 1 mM). Several strains of *Chlorella* are also found to contain an inducible NADPH-GDH; however, in this case the enzyme is synthesized in NH_4^+-grown cells (9, 20, 22, 23, 27). Shatilov and Kretovich (22) have proposed that this GDH might have a role in NH_4^+ assimilation of *Chlorella* under conditions of high NH_4^+ availability. Recently, Everest and Syrett (9) have questioned this suggestion by demonstrating that in *C. vulgaris* NADPH-GDH from NH_4^+-grown cells have an unfavorable K_m^{app} for NH_4^+ of about 50 mM. Everest and Syrett (9) have also shown that *C. vulgaris* cannot utilize NH_4^+ in the presence of MSX. However, the lack of NH_4^+ assimilation by MSX-treated cells of *C. vulgaris* shown in their study does not necessarily exclude a role for NADPH-GDH in NH_4^+ assimilation of this alga, as the experimental conditions used were such that the activity of NADPH-GDH disappeared rapidly in both cells incubated with or without MSX.

In this report, we present a study of nitrogen assimilation by the euryhaline green microalga *Chlorella autotrophica*, which can grow in salinities several times greater than that of seawater with either NO_3^- of NH_4^+ as a nitrogen source, and which accumulates very large quantities of the imino acid proline at high salinities (2).

MATERIALS AND METHODS

Algal Culture. *Chlorella autotrophica* Shihira and Krauss (clone 580 obtained from Dr. R. R. L. Guillard, Woods Hole Oceanographic Institute, Woods Hole, MA, Culture Collection) was grown axenically in ASW with nutrient enrichments as described previously (2, 13). The nitrogen source was substituted with varying concentrations of sodium nitrate and ammonium chloride. In the time course studies, cells were switched from one nitrogen source to another by 10 min centrifugation in sterilized centrifuge bottles, removal of supernatant and resuspension of cells under axenic conditions. In growth experiments with MSX, filtered sterilized solutions of MSX were added to the autoclaved growth media.

[^{14}C]Methylamine Uptake. Aliquots of 100 ml NH_4^+-grown cultures (2–4 × 10^6 cells ml^{-1}) were washed in inorganic N-free isoosmotic ASW media, centrifuged, and resuspended in 10 ml. Two-ml fractions were incubated with 400 μmol [^{14}C]methylamine (2.5 μCi) at 20°C and 600 ft-c light for 15 min, and the uptake of [^{14}C]methylamine was determined by the method of Wheeler (29).

Preparation of Cell-Free Extracts. Algal cells grown on a 12:12 LD cycle (13) were harvested by centrifugation 3 to 4 h after the start of the light period. In time course studies following changing of nitrogen source, cells at zero time were harvested 3 h after the start of the light period, and then kept under constant light for subsequent samplings. The cell density of each sample was determined separately. The cells were washed in inorganic N-

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[2] Abbreviations: GDH, glutamate dehydrogenase; GS, glutamine synthetase; GOGAT, glutamate synthase; ASW, artificial seawater; MSX, L-methionine DL-sulfoximine.

free isoosmotic ASW media, suspended in 1.5 to 3 ml of buffer (pH 7.8) which contained 50 mM imidazole, 1 mM DTT, 1 mM reduced glutathione, 1 mM mercaptoethanol, 0.5 mM EDTA, and 10% ethylene glycol (v/v). Cells were disrupted by passage through a French pressure cell at 200 MPa and 0°C. The resulting crude preparation was assayed directly for GS activities. For GDH assays, 0.5 ml crude extract was mixed with 0.5 ml of 50% $(NH_4)_2SO_4$ solution prepared in EDTA-free buffer (pH 7.8). After 1 h incubation at 0°C, the preparation was centrifuged for 10 min at 7000*g* and 0°C, and the supernatant was collected. For routine GDH determinations, 100 μl of the resulting 25% $(NH_4)_2SO_4$-soluble fraction was assayed for enzyme activities. For kinetic studies, the preparation was desalted on a 0.5- × 3-cm G-25 Sephadex column equilibrated with EDTA-free buffer (pH 7.8).

Enzyme Assays. For all enzyme assays, the reaction mixtures were incubated with cell-free extracts at 25°C.

GS. The transferase activity of GS was determined by the Mn^{2+}-dependent transferase assay (21, 24). The reaction mixture contained in a volume of 1 ml: 100 μmol Tris, 0.3 μmol ADP, 2.5 μmol $MnCl_2$, 35 μmol hydroxylamine, 70 μmol L-glutamine, 33 μmol sodium arsenate, and 100 μl cell-free extract. Prior to the addition of the cell-free extract, the pH of the reaction mixture was adjusted with acetic acid to 6.4. The synthetase activity of GS was determined by the assay procedure in which hydroxylamine replaces ammonia (1, 24). The reaction mixture contained in a volume of 1 ml: 50 μmol imidazole, 85 μmol L-glutamate, 20 μmol ATP, 12 μmol hydroxylamine, 45 μmol $MgSO_4$, and 200 μl cell-free extract. The pH of the reaction mixture was adjusted to 7.6 with KOH prior to the addition of cell-free extract. Both transferase and synthetase reactions were terminated by the addition of ferric chloride reagent, and the resulting Fe-hydroxamate was measured colorimetrically (21).

NAD(P)H-GDH. Reductive amination activities were determined in partly purified cell-free extracts by following the 2-oxoglutarate-dependent oxidation of NAD(P)H. The assay mixture was prepared to contain in a volume of 2.9 ml: 75 μmol glycine, 300 μmol ammonium acetate, 25 μmol 2-oxoglutarate, and 0.25 μmol NAD(P)H. The pH of the reaction mixture was adjusted with Tris to 7.8. The reaction was initiated by the addition of 100 μl enzyme extract.

Protein and Total Nitrogen Determinations. For protein determination, cells washed in inorganic N-free isoosmotic ASW media and suspended in 1 ml of NaOH were heated at 80°C for 1 h, and then left overnight at room temperature. The resulting extract was clarified by centrifugation and protein determined by a modification of the Lowry method as described by Hartree (11). For total nitrogen determination, cells were washed in N-free isoosmotic ASW media, dried at 80°C, and digested in 2 ml concentrated H_2SO_4 in the presence of 25 mg Se. The digest was neutralized with KOH and ammonia was determined by the method described by Weatherburn (28).

RESULTS

C. autotrophica was grown for a period of up to 3 weeks over the salinity range of 10 to 300% ASW with 0.2, 2, and 10 mM of either NH_4Cl or $NaNO_3$ as the nitrogen source. Results obtained from these studies were consistent with our previous observation (2) in that cells growing exponentially exhibited maximum division rates at a salinity range of 10 to 50% ASW and the culture's final cell yield was also optimal over this salinity range. Furthermore, growth of the alga was also influenced by the medium nitrogen concentration; the division rates and cell yield showed a significant increase when the media nitrogen concentration was increased above 0.2 mM. At 300% ASW, the alga was unable to grow on 0.2 mM of either NH_4Cl or $NaNO_3$. Because of these differences in the alga's capacity to grow at various salt and nitrogen concentrations, the relative physiological age of the culture after different periods of growth was taken into account when planning further studies. Typically, the alga was grown from an initial cell density of 0.5×10^6 cells ml^{-1} to mid exponential growth phase to yield between 2×10^6 and 4×10^6 cells ml^{-1}.

Figure 1 shows the protein and nitrogen content of *C. autotrophica* grown at various salinities and concentrations of $NaNO_3$ or NH_4Cl. At each salinity, increasing the concentration of nitrogen from 0.2 to 2 mM with either nitrogen source brought about an increase in cell protein and nitrogen contents. NH_4^+-grown cells exhibited a further increase in cell protein and nitrogen content when the NH_4^+ concentration was raised from 2 to 10 mM. This effect was particularly marked at salinities from 10 to 50% ASW, and consequently over this salinity range the protein and nitrogen content of the cell grown on 10 mM NH_4^+ was twice as high as that of the cells grown on 2 mM NH_4^+. Figure 1 also shows the effect of salinity on the protein and nitrogen content of the alga. Cell protein content decreased with increasing salinity, and the effect was most pronounced in cells grown on 10 mM NH_4^+. Cell nitrogen content also decreased with increasing salinity for cultures with low concentration (0.2 mM) of either nitrogen source, or with high levels (10 mM) of NH_4^+.

As *Chlorella autotrophica* (clone 580) only excretes small amounts of nitrogenous compounds (12), the rates of nitrogen uptake required at mid-exponential growth phase can be calculated from the cell nitrogen content (Fig. 1) and division rates. Table I shows these calculated rates for cells grown at various salinities and with different concentrations of the two nitrogen sources. As a consequence of inhibition in growth rates at high salinities, the estimated required rates of nitrogen uptake are decreased significantly. It can also be seen from Table I that the cell's ability to acquire nitrogen from the media is markedly improved by increasing the concentration of either nitrogen source.

The uptake of [^{14}C]methylamine, an analog of ammonia, was measured for cells pregrown with 2 mM NH_4^+ over the salinity range of 10 to 200% ASW. The rates of methylamine uptake at 10, 50, 100, and 200% ASW were 60, 49, 44, and 37 attomol $cell^{-1}$ min^{-1}, respectively, being 25 to 70% higher than the rates of NH_4^+ uptake calculated for the alga grown under these conditions (Table I).

The activities of both GS and GDH were measured in the aminating direction in cell-free extracts of *C. autotrophica.* Both GS and GDH were unstable in either imidazole or Tris-HCl buffer (pH 7.8) at 0°C. GS was stabilized by the addition of compounds with thiol groups to these buffers, whereas both NADPH and NADH-GDH activities were stable when ethylene glycol and $(NH_4)_2SO_4$ were also present.

Some characteristics of NADPH-GDH were determined in desalted partly purified cell-free preparations of *C. autotrophica.* The pH optimum for aminating reaction of NADPH-GDH was found to be pH 7.8. The apparent K_m (K_m^{app}) for NH_4^+ of this GDH isoenzyme was about 20 mM.

Because of the stability of GDH activities in the presence of $(NH_4)_2SO_4$, the levels of these activities were measured in preparations purified by $(NH_4)_2SO_4$ fractionation without any desalting treatments. About 25 nmol EDTA contained in 100 μl of these enzyme preparations (see "Materials and Methods") which was added to the 3-ml assay system was confirmed to be noninhibitory in a study in which these preparations were compared for their GDH activities with those containing no EDTA (results not shown). The addition of 2 mM Ca^{2+} both in the presence and absence of 25 nmol EDTA in the assay system had no effect on the levels of GDH activities.

The levels of NADPH-GDH activities in *C. autotrophica* were

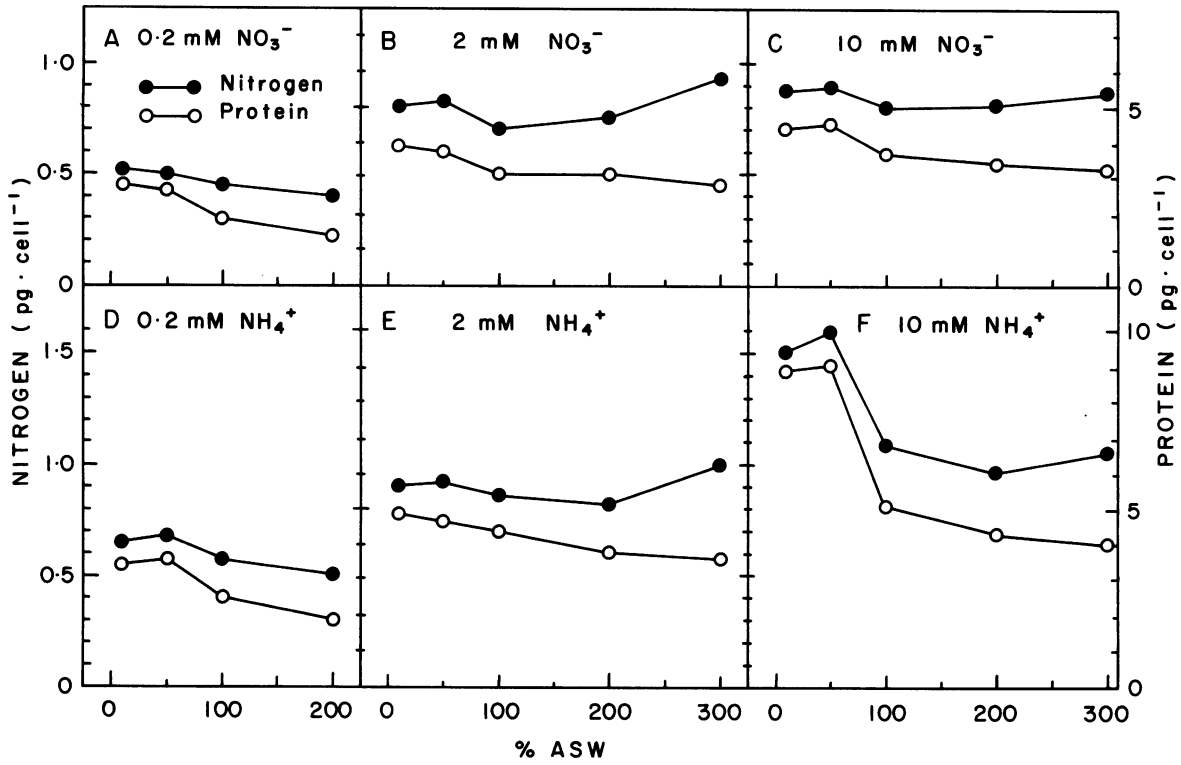

FIG. 1. The effect of nitrogen source and salinity on cell protein and nitrogen content in *C. autotrophica.*

Table I. *Estimated Nitrogen Assimilation Rates for Cells Grown at Different Salinities and Different Nitrogen Sources and Concentrations*

Rates based on measured cell nitrogen contents (Fig. 1) and cell division rates.

Salinity	NO_3^--Grown Cells[a]			NH_4^+-Grown Cells[a]		
	0.2 mM	2 mM	10 mM	0.2 mM	2 mM	10 mM
% ASW	*attomol N cell^{-1} min^{-1}*					
10	19	36	39	21	42	53
50	18	34	39	20	40	58
100	12	26	35	12	35	38
200	8	21	21	6	22	20
300	—[b]	16	14	—[b]	20	21

[a] Grown at 0.2, 2, or 10 mM $NaNO_3$ or NH_4Cl.
[b] No growth observed.

Table II. *NADPH-Glutamate Dehydrogenase Activities in Cells Grown at Different Salinities and with Different Nitrogen Sources and Concentrations*

One-L cultures with 2×10^6 to 4×10^6 cells ml^{-1} were harvested and washed with N-free isoosmotic ASW media. Cell-free extracts were prepared in imidazole buffer (pH 7.8) and partially purified by $(NH_4)_2SO_4$ fractionation. The NADPH-GDH activities in 25% $(NH_4)_2SO_4$-soluble fractions were determined in the aminating direction by following the 2-oxoglutarate-dependent oxidation of NADPH.

Salinity	NO_3^--Grown Cells[a]			NH_4^+-Grown Cells[a]		
	0.2 mM	2 mM	10 mM	0.2 mM	2 mM	10 mM
% ASW	*attomol cell^{-1} min^{-1}*					
10	47	31	30	73	220	396
50	53	34	36	68	265	437
100	44	38	37	66	305	592
200	40	36	29	68	617	722
300	—[b]	28	27	—[b]	566	636

[a] Grown at 0.2, 2, or 10 mM of $NaNO_3$ or NH_4Cl.
[b] No growth observed.

dependent upon the source and concentration of nitrogen and salinity of the media. NO_3^--grown cells showed an increase in their NADPH-GDH activities when the NO_3^- concentration in the growth media was decreased from 10 or 2 mM (Table II). Growth of the alga on NH_4^+, on the other hand, resulted in high levels of NADPH-GDH activities. An induction of this activity was noticeable even in cells grown with low concentration (0.2 mM) of NH_4^+ (Table II). The levels of NADPH-GDH activities increased markedly with increasing NH_4^+ concentration, and as a consequence, at each salinity the alga grown with 10 mM NH_4^+ exhibited the highest levels of activities (Table II). Interestingly, increasing the salt concentration of media containing 2 and 10 mM NH_4^+ intensified the induction of NADPH-GDH activities with a maximum being reached at 200% ASW (Table II). Cells grown with 0.2 mM NH_4^+ and those grown with nitrate showed little change in their NADPH-GDH activities with changes in external salt concentration.

The levels of NADH-GDH were in the range of 30 to 70 attomol cell^{-1} min^{-1}, and showed little change in response to either nitrogen source or external salinity (results not shown).

The levels of GS activities were also determined for cells at different salinities and with different nitrogen sources and concentration. Table III shows that the pattern of changes in GS levels was almost a mirror image of that for NADPH-GDH activities (Table II). Low nitrogen concentration of the medium was found to be most favorable for high levels of GS activities in the alga. At each salinity, the alga grown with 0.2 mM of either NO_3^- or NH_4^+ exhibited the highest levels of GS activities. In other studies (data not shown) when these low nitrogen cultures were allowed to grow beyond exponential growth phase, the GS levels were found to increase further as the depletion of nitrogen progressed with cell growth. The maximum levels were found at stationary phase and were about 40 to 70% higher than those

Table III. *Glutamine Synthetase Activities in Cells Grown at Different Salinities and with Different Nitrogen Sources and Concentrations*

One-L cultures with 2×10^6 to 4×10^6 cells ml^{-1} were harvested and washed with N-free isoosmotic ASW media. Glutamine synthetase (GSs) activities were determined in cell-free extracts prepared in imidazole buffer (pH 7.8).

Salinity	NO_3^--Grown Cells[a]			NH_4^+-Grown Cells[a]		
	0.2 mM	2 mM	10 mM	0.2 mM	2 mM	10 mM
% ASW	*attomol cell^{-1} min^{-1}*					
10	326	212	199	293	36	34
50	306	189	202	284	43	36
100	254	161	158	221	72	57
200	188	149	130	169	98	73
300	—[b]	111	105	—[b]	86	90

[a] Grown at 0.2, 2, or 10 mM of $NaNO_3$ or NH_4Cl.
[b] No growth observed.

found at mid-exponential phase. At mid-exponential phase, nitrate-grown cells showed a 20 to 40% decrease in the levels of GS activities when the media nitrate concentration was increased from 0.2 to 2 mM and above. A much more rapid decrease in the levels of GS activities occurred, however, when the alga was grown with increasing concentration of NH_4^+, and as a consequence, cells grown with 10 mM NH_4^+ showed the lowest levels of GS activities at each salinity (Table III). The effect of salinity on GS levels was dependent on the source of nitrogen in the media. Increasing the salt concentration of high NH_4^+ (2 and 10 mM) cultures, diminished the NH_4^+-induced decline in the levels of GS activities, and consequently, GS activities of NH_4^+-grown cells were about 2 to 3 times higher at 200 to 300% ASW than those found at 10% ASW (Table III). Conversely, increasing the salt concentration of cultures containing low NH_4^+ or the three tested concentrations of NO_3^-; *i.e.* the nitrogen sources favoring high GS levels, brought about a decrease in the levels of GS activities. These declines may be associated with the reduction in the rates of nitrogen uptake which these cells show with increasing salinity (Table I). The transferase activity of GS in *C. autotrophica* grown under various nitrogen and salt concentrations was between 5 and 10 times higher than the synthetase activity.

As anticipated, changing the nitrogen source from high NH_4^+ to low NO_3^- and vice versa completely reversed the balance between the levels of GS and GDH in the cells (Fig. 2), and the change was completed within the period required for completion of one cell cycle in the 50% ASW cultures (2). There was a rapid and immediate decline in the NADPH-GDH activities in cells switched from 2 mM NH_4^+ to 0.2 mM NO_3^-, and an almost equally rapid increase in both transferase and synthetase activities. Conversely, cells switched from 0.2 mM NO_3^- to 2 mM NH_4^+ exhibited rapid and immediate decline in synthetase activities which after a lag of about 1 h was accompanied by a similar decline in transferase activity (Fig. 2). The NADPH-GDH activity of cells transferred to 2 mM NH_4^+ showed little change for the first 2 h, and then increased rapidly resulting in fully developed activity at about 24 h. The levels of NADH-GDH activities showed little change in response to changes in the media nitrogen source or concentration (Fig. 2).

An experiment to ascertain growth and nitrogen assimilation in the presence of 5 mM MSX was undertaken (Table IV). In Table IV, it can be seen that the alga can grow in the presence of MSX, and the cell protein and nitrogen contents at various growth periods were identical with or without MSX.

DISCUSSION

Recently, we have shown that in *C. autotrophica*, osmoregulation at high salinities is primarily effected by the synthesis and accumulation of the amino acid proline (2). Our present study shows an important connection between the nitrogen supply and salinity tolerance of this euryhaline microalga. When supplied with only 0.2 mM of either NH_4Cl or $NaNO_3$, the alga was unable to grow at salinities higher than 200% ASW, whereas at higher nitrogen concentrations it can grow at salinities of up to 400%

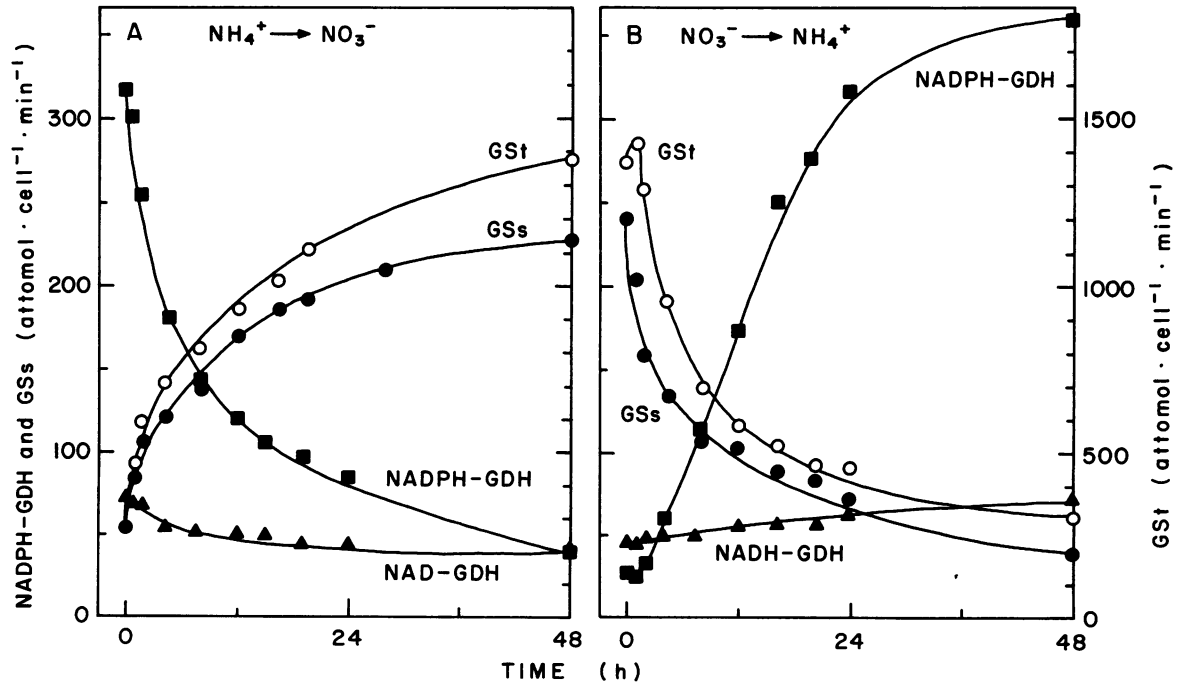

FIG. 2. The effect of changing the nitrogen source on GS and NAD(P)H-GDH activities in *C. autotrophica*. Cells grown at 50% ASW with 2 mM NH_4^+ (A) or 0.2 mM NO_3^- (B) were harvested, washed, and resuspended in 50% ASW containing 0.2 mM NO_3^- (A) or 2 mM NH_4^+ (B) at zero time.

Table IV. *Cell Density, Nitrogen Content, and Protein Content of Cultures Growing in the Presence and Absence of 5 mM MSX*

A sample of 100 to 250 ml from 2-L cultures growing with or without MSX in 50% ASW with 2 mM NH_4^+ was collected at each interval. Cells were washed in nitrogen-free isoosmotic ASW prior to protein and nitrogen determinations.

Day	Cell Density		Cell N		Protein	
	Control	MSX	Control	MSX	Control	MSX
	cells ml^{-1} × 10^{-6}		*fg cell^{-1}*		*pg cell^{-1}*	
0	0.32	0.32	885	885	5.1	5.1
2	1.24	1.20	813	836	4.7	4.6
4	3.14	2.98	875	895	4.9	4.5
7	8.87	8.21	967	982	4.9	4.6

ASW (2). Our data on methylamine uptake as a function of salinity suggest that the requirement of the alga for increased inorganic nitrogen concentration at higher salinities is at least partly attributable to the decline in the cell's transport ability. This effect of salinity on the cell's capacity to take up inorganic nitrogen appears not only to decrease growth, but also to affect cell composition (Fig. 1). At high salinities, the alga incorporates less of its nitrogen into protein. This appears to arise from an increasing diversion of cell nitrogen into free proline (2) with increasing salinity. The free proline content of both NO_3^- and NH_4^+-grown cells at 300% ASW was about 1600 mM (2).

The nitrogen and salinity status of the growth media influences the levels of both GDH and GS activities in *C. autotrophica*. GS was present in this alga under all growth conditions, but its levels were high in NO_3^- compared with NH_4^+-grown cells, and maximum activities were shown by cells subject to nitrogen starvation. Similar changes in GS levels in response to changes in nitrogen source have been recently reported in two freshwater *Chlorella* species, *C. vulgaris* (9) and *C. kessleri* (25). In *C. kessleri* (25), two isoenzymes (GSI and GSII [1]) were isolated and it was shown that the increase in GS activities during nitrogen starvation was largely due to an increase in GSI levels. In our experiments, in which *C. autotrophica* was transferred from NO_3^- to NH_4^+ and vice versa, the changes in GS levels were time-dependent suggesting that the effect of nitrogen source on GS levels may simply be attributable to the regulation of *de novo* synthesis. Another feature of the relationship between GS levels and nitrogen source in this alga was the influence of external salinity. The cell's ability to regulate GS levels in response to changes in nitrogen source and supply declined with increased salinity. It is not clear as to how salinity interferes with the regulation of GS in this alga. However, a somewhat similar influence of growth conditions on GS regulation by nitrogen source has been shown in *C. kessleri* (25), where light was found to diminish the regulation of GS.

As with several other species of *Chlorella* (20, 22, 23, 27), GDH in *C. autotrophica* appears to be present in two forms, a constitutive GDH which has low nucleotide specificity (22, 27), and an inducible form highly specific for NADPH. Cells grown on NO_3^- appear to contain only the constitutive form, whereas the inducible NADPH-GDH is present in NH_4^+-grown cells, and increasing levels of this form were observed with increasing NH_4^+ concentration (Table II). Results obtained from experiments in which the alga was transferred from NO_3^- to NH_4^+ and vice versa agree with the suggestions that the increase in NADPH-GDH activities after the cells transfer to high NH_4^+ is due to *de novo* synthesis of the enzyme (20, 22, 27), and that its decline after removal of NH_4^+ from the media is attributable to its degradation (22) or inactivation (9). The present study reveals that, in this euryhaline alga, salinity acts as an intensifier for the induction of NADPH-GDH in NH_4^+-grown cells. Similar stimulation of GDH activities by inorganic salts have been reported in bacteria (15, 18) and marine invertebrates (15) and, interestingly, most of these organisms have been found to accumulate amino acids including proline at high salinities. This has led Hochachka and Somero (15) to suggest that salt stimulation of GDH initiates a metabolic cascade in which increased availability of glutamate leads to accumulation of amino acids.

The question arises as to the role of two potential pathways in the assimilation of NH_4^+ in *C. autotrophica*. Cells grown on low NH_4^+ or a range of NO_3^- concentrations contained GS activities at levels several times higher than those required by the cells and it appears likely that the GS-GOGAT cycle is the primary pathway for NH_4^+ assimilation under those conditions. Cells grown on high concentrations of NH_4^+ at salinities below 100% ASW, however, contain GS levels (Table III) lower than the estimated rates of NH_4^+ assimilation (Table I). The presence of high NADPH-GDH in these cells therefore suggests that this enzyme may play at least a partial role in NH_4^+ assimilation under these conditions. This situation, however, becomes less certain at salinities above 100% ASW. NH_4^+-grown cells showed an increase in both GS and NADPH-GDH activities with increases in external salt concentrations. Nonetheless, the GS activities in NH_4^+-grown cells at high salinities did not reach a level where an exclusive role in NH_4^+ assimilation could be established unequivocally. Although the activities of GS in NH_4^+-grown cells at high salinities were about 2 to 3 times higher than the required rates estimated in Table I, it must be pointed out that these estimated rates do not take into account the expected reassimilation of NH_4^+ produced by photorespiration (19). Furthermore, the GOGAT activity has been shown to be dependent on Fd in *Chlorella* (16), which suggests that GS-GOGAT cycle in *C. autotrophica* grown on 12:12 LD cycle (see "Materials and Methods") may operate only for half of the growth period. Normally, a fully functional GS is present at a level several times higher than the rate of primary NH_4^+ assimilation in higher plants (19). In NH_4^+-grown cells, the levels of NADPH-GDH activities at various salinities are about 5 to 30 times higher than the required assimilation rates, and it is tempting to assume that in *C. autotrophica* NADPH-GDH provides an alternative pathway for NH_4^+ assimilation under the conditions of high NH_4^+ availability. A similar role for NADPH-GDH in *C. pyrenoidosa* Pringsheim 82 T was proposed by Shatilov and Kretovich (22).

The ability of NH_4^+ cells of *C. autotrophica* to grow and maintain normal protein and nitrogen content in the presence of 5 mM MSX provides the most direct evidence in support of a role for NADPH-GDH in NH_4^+ assimilation of this alga. GS was almost completely inhibited and the levels of NADPH-GDH were normal in MSX-treated cells of this alga (results not shown).

Certainly, as with most green plants, the K_m^{app} of GDH for NH_4^+ has been shown to be high in *Chlorella* (9, 22, 24), and our estimate of about 20 mM NH_4^+ for NADPH-GDH activity in *C. autotrophica* is consistent with these observations. However, these results by themselves do not exclude a role of GDH in NH_4^+ assimilation. First of all, it is not certain that the affinity of GDH for NH_4^+ in cell-free extracts can be related to its function in the highly organized intact metabolic machinery of plant cells. Second, there is evidence that in certain fungi NH_4^+ assimilation takes place via NADPH-GDH with a similarly high K_m^{app} for NH_4^+, as these organisms lack GOGAT activity (19, 24).

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