Localization of Nitrogen-Assimilating Enzymes in the Chloroplast of Chlamydomonas reinhardtii

PETRA FISCHER and UWE KLEIN*

Botanical Institute, University of Bonn, Kirschallee 1, 5300 Bonn 1, Federal Republic of Germany

ABSTRACT

The specific activities of nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase, and glutamate dehydrogenase were determined in intact protoplasts and intact chloroplasts from Chlamydomonas reinhardtii. After correction for contamination, the data were used to calculate the portion of each enzyme in the algal chloroplast. The chloroplast of C. reinhardtii contained all enzyme activities for nitrogen assimilation, except nitrate reductase, which could not be detected in this organelle. Glutamate synthase (NADH- and ferredoxin-dependent) and glutamate dehydrogenase were located exclusively in the chloroplast, while for nitrite reductase and glutamine synthetase an extraplastidic activity of about 20 and 60%, respectively, was measured. Cells grown on ammonium, instead of nitrate as nitrogen source, had a higher total cellular activity of the NADH-dependent glutamate synthase (+95%) and glutamate dehydrogenase (+33%) but less activity of glutamine synthetase (-10%). No activity of nitrate reductase could be detected in ammonium-grown cells. The distribution of nitrogen-assimilating enzymes among the chloroplast and the rest of the cell did not differ significantly between nitrate-grown and ammonium-grown cells. Only the plastidic portion of the glutamine synthetase increased to about 80% in cells grown on ammonium (compared to about 40% in cells grown on nitrate).

More uniform results have been published for the location of other nitrogen-assimilating enzymes. Nitrite reductase and glutamate synthase were found mainly in chloroplasts after cellular fractionation of higher plant cells (5, 13, 30, 41). Two isoforms of glutamine synthetase occur in most plants, one of which is located in the cytoplasm (GS,) and the other in plastids (GS2). The intracellular ratio of cytoplasmic to plastidic enzyme was found to be extremely different among various plants, depending on the species (28).

In unicellular green algae the enzymes of the GS/GOGAT pathway are present, too, and several investigations support their role in algal nitrogen assimilation (38). Like in higher plants, the individual steps of algal nitrate reduction and ammonia assimilation have been characterized, especially in Chlamydomonas (38). However, due to major difficulties in the isolation of organelles from unicellular green algae, a comprehensive study of the compartmentation of nitrogen-assimilating enzymes in algae has not been reported to date. To our knowledge only nitrate reductase and glutamate dehydrogenase have been localized in unicellular green algae. Nitrate reductase was found in the pyrenoid of several green algae by immuno-gold labeling (26). By use of the same method and by cytochemical procedures evidence has been provided for a plastidic location of the ammonium-inducible NADP-specific glutamate dehydrogenase in Chlorella (32). However, no information is available on the location of the other nitrogen-assimilating enzymes in unicellular green algae.

During our studies on carbon partitioning in C. reinhardtii, it became evident that nitrogen assimilation is involved in the regulation of the intracellular flow of carbon (19). In order to fully understand the influence of nitrogen on carbon partitioning in the alga it was necessary to survey the pathways of nitrogen metabolism in Chlamydomonas. A lot of information could be obtained from the literature (38), but the compartmentation of enzymes, which is an important aspect for the regulation of nitrogen assimilation, remained an open question. Therefore, we started an investigation on the distribution of nitrogen-assimilating enzymes in C. reinhardtii. In this communication we report on the portion of these enzymes in the chloroplast of the alga for cells grown on nitrate and on ammonium. The method of enzyme localization we used was already applied for the localization of glycolytic enzymes in Chlamydomonas (18).

MATERIALS AND METHODS

Growth of Algae. Chlamydomonas reinhardtii, strains 11-32/b and 11-32/c, from the “Sammlung für Algenkulturen at Göttingen (SAG),” FRG, was grown autotrophically at 34°C on a mineral medium with nitrate or ammonium as nitrogen source. The ammonium medium contained the following (21): 10 mM (NH₄)₂HPO₄, 2.6 mM Na₂HPO₄, 0.7 mM KH₂PO₄, 1 mM KCl, 0.2 mM MgSO₄, 0.1 mM CaCl₂ (pH 6.8), and 1 mL/L of trace

1 Supported by the Deutsche Forschungsgemeinschaft.
2 Abbreviations: GS, glutamine synthetase; GOGAT, glutamate synthase; MV, methylviologen.
element solution (15). Growth on nitrate was as described in a previous publication (20).

Algae were kept in glass flasks (300 mL) and were continuously bubbled with CO2-enriched air (2 to 5% CO2). They were synchronized by 12 h light/12 h dark cycles (light intensity about 320 W m⁻² from 18 fluorescent lamps, L18W/30 warm white, Osram, München, FRG) and diluted with fresh medium every day at the beginning of the light period to a cell density of 1 to 3 \times 10^6 cells per mL.

Cell number was determined with a hemocytometer. Chl was extracted with 90% (v/v) acetone and measured at 647 and 664 nm using the absorption coefficients given by Jeffrey and Humphrey (16).

**Isolation of Protoplasts and Chloroplasts.** Protoplasts and chloroplasts were isolated from synchronized cells that were harvested after about 9 h in the dark period. The algae had just divided and were in the sporangial stage. We also tried to use synchronized cells taken from the light period of the dark/dark cycle. However, in these preparations the yield of chloroplasts was very low and the organelles were not as intact as those isolated from dark-adapted synchronized cells. We do not know the reason why chloroplasts from light-adapted cells are more fragile than those from dark-adapted cells, but the starch content or the composition of the chloroplast envelope may play a role during the purification procedure. This problem has been discussed in a previous communication (20). The method for cellular fractionation and chloroplast purification was identical with a published procedure (20). Protoplasts were produced by incubation of the cells with a solution of autolysine (0.5 mg mL⁻¹ in 1 mM potassium phosphate, pH 6.0, 0.5 mg mL⁻¹ of BSA, 10 μg mL⁻¹ of cycloheximide). The autolysine was obtained as described earlier (20). Protoplasts could be produced with an integrity of 100% because the autolysine specifically lyses only the algal cell wall (35). Chloroplasts were isolated from protoplasts by lysis with digitonin and purification by differential and Percoll cushion centrifugation (for details see ref. 20). The isolated organelles were about 80 to 90% intact, as judged by the ferri cyanide assay and by microscopic observation (20).

The production of protoplasts with autolysine took about 40 min, and an additional 15 min were needed to purify the chloroplasts. Protoplasts and chloroplasts were finally resuspended in a buffer containing 0.1 mM potassium phosphate, 0.3 mM Na₂EDTA, 10 μM L-cysteine, pH 7.5, and were immediately used for the enzyme assays.

**Enzyme Localization.** The method for the localization of enzymes in chloroplasts of *C. reinhardtii* was described in detail in a previous communication (18). It consists of the measurement of specific enzyme activities (related to Chl) in the protoplast and chloroplast fraction, followed by a correction of the latter data, in order to eliminate any error due to contaminating enzyme activities. The exact amount of contamination was determined for each preparation and the specific enzyme activities were corrected for this contamination with the formula

\[
x = \frac{a - \frac{a}{b}}{1 - \frac{a}{b}}
\]

where \(x\) is the true specific enzyme activity in the chloroplast; \(a\) is the specific enzyme activity measured in the isolated chloroplast suspensions; \(b\) is the specific enzyme activity measured in the isolated protoplast suspension; \(a\) and \(b\) are the specific enzyme activities of an extraplastidic marker enzyme in the isolated chloroplasts and protoplasts, respectively. All enzyme activities must be related to Chl.

The formula is derived from the following two simultaneous equations

\[
b = x + y
\]
\[
a = x + \frac{a}{b} y
\]

where \(x, a, b, \alpha, \) and \(\beta\) are defined as above and \(y\) is the specific enzyme activity outside the chloroplast.

Equation 2 states that the total activity \((b)\) of an enzyme measured in the protoplasm is the sum of the true activity in the chloroplast \((x)\) plus the extraplastidic activity of the enzyme, termed \(y\). Equation 3 says that the enzyme activity \((a)\) measured in the isolated chloroplast is the sum of the true activity in the chloroplast \((x)\) plus a contamination by the extraplastidic activity of the enzyme \((\alpha/\beta)y\). The term \(\alpha/\beta\), which is the ratio of the activities of an extraplastidic marker enzyme measured in the isolated chloroplasts \((\alpha)\) and protoplasts \((\beta)\), determines the size of the term \((\alpha/\beta)y\) and hence the amount of contamination. Values for \(\alpha/\beta\) can range from zero (if \(\alpha\) is zero, that is no contamination of the isolated chloroplasts) to 1 (if \(\alpha\) is as high as \(\beta\), that is 100% contamination).

If \(a, b, \alpha, \) and \(\beta\) are measured accurately, Equation 1 can correct high amounts of contamination so that an enzyme can be exactly localized even when the chloroplast preparation is not very pure. The corrected values can be directly compared and were used in our study to calculate the percentage of each enzyme in the algal chloroplast.

Since previous investigations showed that mitochondria are the major contaminants of the chloroplast fraction (18, 20), Cyt c oxidase was generally used as a marker to estimate the contamination of the isolated chloroplasts. In the experiments described in this report the average contamination was about 7% on a Chl basis (mean of 16 preparations). The individual contamination of a preparation was never higher than 10%. Other marker enzymes tested, like catalase (marker for microbodies) or phosphoeneolpyruvate carboxylase (marker for the cytoplasm), were found to contaminate the chloroplasts to the same (catalase) or to a lower extent (PEP carboxylase).

**Enzyme Assays.** All enzyme activities were measured with 0.05% (v/v) Triton X-100 in the reaction mixture in order to solubilize protoplasts and chloroplasts.

NADH-nitrate reductase was assayed by measuring the formation of nitrite from added nitrate and NADH in an incubation mixture containing in 2 mL: 60 μmol potassium phosphate (pH 7.5), 10 μmol KNO₃, 0.85 μmol NADH₂, and 0.4 mL sample with about 0.5 mg Chl ml⁻¹. Before addition of the other reagents, the sample was incubated for 2 min with 6 mM ferricyanide in the presence of 0.05% (v/v) Triton X-100 to activate the enzyme. After incubation for 30 min at 30°C the reaction was stopped by boiling (1 min), and the mixture was cleared by centrifugation (27,000g). For the determination of nitrite the supernatant was mixed with 1 mL each of 1% (w/v) sulfanilamide in 2 N HCl and 0.2% (w/v) N-(1-naphthyl)ethylenediamine. The absorption of the resulting violet color was measured at 540 nm against a blank (= sample in which the reaction was stopped by boiling immediately after addition of all reagents).

The activity of nitrite reductase was determined by measuring the disappearance of nitrite from a reaction mixture containing in 2 mL: 45 μmol sodium borate (pH 8.6), 2 μmol NaN₃, 10 μmol methylviologen (MV), 0.7 mL distilled water, and 0.3 mL sample with about 0.5 mg Chl ml⁻¹. The reaction was started by addition of 43 μmol of dithionite (dissolved in 0.29 m NaHCO₃) as electron donor. After incubation for 1 h at 30°C the reaction was stopped by oxidation of dithionite and methylviologen (tubes were vigorously shaken in air until the blue color disappeared) and by boiling. After centrifugation (27,000g), 0.1 mL of the supernatant was diluted to 2 mL with distilled water, and nitrite in this sample was determined as in the nitrate reductase assay.

The activity of glutamine synthetase was assayed by measuring
The ATP-dependent formation of the γ-glutamyl-hydroxamate from L-glutamate and hydroxylamine as published by Rhodes et al. (33).

NADH-GOGAT and ferredoxin-GOGAT were assayed according to Márquez et al. (27) and Galván et al. (9), respectively. Most assays of ferredoxin-GOGAT were run with methylviologen plus dithionite as electron donor, but the results were also checked with reduced ferredoxin from spinach. With this electron donor about 35% of the methylviologen activity was measured.

The activity of glutamate dehydrogenase was determined as described by Cullimore and Sims (3). The enzyme is equally active with NAD and NADP as cosubstrate. Cyt c oxidase was measured by the method of Wharton and Tzagoloff (43).

Chemicals. Enzymes and substrates were purchased from Boehringer Mannheim GmbH and Sigma. Polyethylene glycol was obtained from Baker. All other chemicals were of reagent grade.

RESULTS

Enzymes Assayed. All enzymes known today to participate in nitrogen assimilation in C. reinhardtii (38) were measured. Besides nitrate and nitrite reductase these included glutamine synthetase and two glutamate synthases, an NADH- and a ferredoxin-dependent enzyme, previously found in the alga (4, 9, 27). Glutamate dehydrogenase was also assayed, although it is generally accepted now that this enzyme does not contribute to the assimilation of nitrogen in green cells of higher plants and algae. Rather it seems to be involved in turnover processes of intracellular nitrogen-containing compounds (3). The enzyme was included in this investigation because its substrates (2-oxoglutarate; NADH, and glutamate) are also substrates of the GS/GOGAT cycle. Sharing of substrates among glutamate dehydrogenase and the GS/GOGAT cycle may be important for the flow of nitrogen under certain physiological conditions.

Enzyme activities, given in this report, represent dark values, except for nitrate reductase, which did not show any activity at the time of sampling (9 h in the dark). To overcome this problem in the assay of nitrate reductase, the enzyme had to be activated (Fig. 1). For activation, the algae were illuminated under growth conditions for 30 min prior to protoplast and organelle isolation. After this period, the nitrate reductase activity was about 40% of that measured at maximum in protoplasts isolated from a synchronized culture during the light period. Thirty minutes of illumination were chosen for the activation of the nitrate reductase in all preparations because this period of time sufficed to get a measurable enzyme activity while the decrease in the yield of intact chloroplasts was still tolerable. Extending the activation period was unfavorable due to increasing problems in chloroplast isolation. The variability of enzyme activities in synchronized cultures and the time of sampling is addressed in more detail under “Discussion.”

All enzyme activities measured in isolated protoplasts and chloroplasts are listed in Tables I and II for nitrate- and ammonium-grown cells, respectively. The localization of enzymes, derived from these data, is described below.

Chloroplastic Enzyme Activities in Cells Grown on Nitrate. In nitrate-grown cells (Table I) both glutamate synthases (NADH- and ferredoxin-GOGAT) and the glutamate dehydrogenase were found exclusively in the chloroplast of C. reinhardtii, while a nitrate reductase activity could not be detected in this organelle. The chloroplast also contained most of the nitrite reductase activity (about 80%) and a portion of the total cellular activity of the glutamine synthetase (about 40%).

Ferredoxin-GOGAT, which was usually assayed with methylviologen as electron donor, was also measured with reduced ferredoxin from spinach (not shown). The specific activity with this electron donor was only about one-third of the methylviologen activity, which is probably due to the low affinity of the enzyme from Chlamydomonas for the spinach ferredoxin. The compartmentation of glutamate synthase, determined with spinach ferredoxin, was the same as with methylviologen in nitrate-grown as well as in ammonium-grown cells (not shown).

Enzyme Activities in Ammonium-grown Cells. Unlike nitrate, ammonium cannot be excluded from the cells because it is able to penetrate membranes as ammonia. Therefore, in a medium with ammonium the cells have to deal with a pH-dependent continuous inflow of nitrogen, even in the dark, when nitrate uptake is always halted. Under our conditions, growth of the algae in a medium with ammonium instead of nitrate as sole nitrogen source resulted in distinct changes of the total cellular activity of nitrate reductase, glutamine synthetase, NADH-GOGAT, and glutamate dehydrogenase, while nitrite reductase and ferredoxin-GOGAT appeared to be unaffected (protoplast values in Tables I and II). With ammonium, a complete loss of nitrate reductase activity and a small decrease (~10%) of GS activity could be observed. The total cellular activity of NADH-GOGAT almost doubled, and glutamate dehydrogenase increased about 30% under these conditions. It should be noted that in our synchronized cultures the total cellular activity of many enzymes varies during the cell cycle. The specific activities reported in this communication are valid for the time of sampling, that is at 9 h in the dark. In the light, total cellular activities are generally higher (see “Discussion”).

Compartmentation in Ammonium-grown Cells. Although the total cellular activity of some nitrogen-assimilating enzymes differed significantly between nitrate- and ammonium-grown algae, there was almost no difference in the distribution of these activities among the chloroplast and the rest of the cell (Tables I and II). Only the plastidic portion of the glutamine synthetase increased to about 80% in ammonium-grown cells (compared to 40% in nitrate-grown cells). All other enzyme activities showed only minor and insignificant changes in their compartmentation. The increase in the plastidic activity of glutamine synthetase, which goes in parallel with the increase of NADH-GOGAT and glutamate dehydrogenase activity (Table II), probably reflects a response to the continuous inflow of ammonia and may help the chloroplast to cope with higher levels of ammonia, which is toxic especially for this organelle due to its uncoupling effect on plastidic electron transport (2).

DISCUSSION

On the Method. This communication describes the location of nitrogen-assimilating enzyme activities in the chloroplast of
C. reinhardtii under dark conditions. One drawback of the method is certainly the limitation of the results to the situation in the dark, where no reduction of nitrate occurs and ammonia assimilation is not high. As mentioned above, our protoplasts and chloroplasts were isolated from cells after approximately 9 h in the dark phase because at this time in the cell cycle we obtained the maximum amount of intact chloroplasts (20). It is known, however, that the activity of some nitrogen-assimilating enzymes is not high in the dark. Nitrate reductase (14), glutamine synthetase (37), and ferredoxin-GOGAT (36) are deactivated under dark conditions. They can be reactivated by light. We repeated these measurements with our synchronized cultures and found a similar variation for the activities of nitrate reductase and glutamine synthetase during the light/dark cycle, that is a low activity in the dark and a high activity (severalfold higher, depending on the enzyme) in the light (not shown). Therefore, due to the use of dark-adapted cells for protoplast and chloroplast isolation, we detected relatively low activities of some enzymes in our fractions. In general, however, there was sufficient activity present to obtain reliable measurements for subsequent calculations of enzyme localization. Only the nitrate reductase had to be activated by light.

We believe that the observed compartmentation in the dark is actually not much different, if at all, from that in the light. Evidence for a similar distribution under dark and light conditions is the immediate increase in the activity of some nitrogen-assimilating enzymes in the light (nitrate reductase [14], glutamine synthetase [37], ferredoxin-GOGAT [36]), which points to an activation of these enzymes at the onset of light rather than to a major de novo synthesis or a rearrangement across the chloroplast envelope. The curve for the increase of nitrate reductase activity in the light presented in this communication (Fig. 1) has a shape typical for activation but not for induction of enzymes. Nevertheless, a different compartmentation of nitrogen-assimilating enzymes at other stages of the cell cycle cannot be completely ruled out on the basis of our results.

An advantage of the method is the simplicity and exactness of the procedure for locating an enzyme in the chloroplast. In contrast to higher plant systems, the specific activities of algal protoplast and chloroplast suspensions can be directly compared even if the suspensions have an extremely different particle concentration. This is due to the fact that Chlamydomonas has only one chloroplast. Hence one algal protoplast contains the same amount of Chl as one chloroplast and calculations of enzyme localization in the algal chloroplast are easy but still exact if the data are related to Chl.

**Enzyme Distribution.** The location of nitrate reductase in the cytoplasm is in accordance with several investigations on its compartmentation in higher plant cells. However, recently the enzyme was localized by immuno-gold labeling in spinach chloroplasts (17) and in the pyrenoid of several green algae, including C. reinhardtii (26). We could not find any nitrate reductase activity in the isolated chloroplasts of the alga. Since the enzyme is known to be sensitive to inactivation it is possible that the activity was lost during chloroplast isolation. However, chloroplast isolation from protoplasts took only about 15 min, which should not be sufficient to inactivate the enzyme completely. Protoplasts, which were kept on ice in the dark during chloroplast isolation, retained their nitrate reductase activity during this period. Even in a crude extract of the alga, an inactivation does not proceed that fast. Furthermore, if the enzyme is located in the pyrenoid, as reported (26), it should be more protected against inactivation than a stroma- or membrane-bound enzyme. Although a rapid inactivation process is not ruled out by our results,
the above considerations lead us to assume that the nitrate reductase detected in the algal pyrenoid by immuno-gold labeling may not be active in vivo. This might be the reason why an activity could not be recorded with our method. In this context it is of interest to note that large quantities of ribulosebisphosphate carboxylase were spotted also in the pyrenoid of Chlamydomonas (22). In this case, too, the enzyme seemed to be without function at this location (22). We believe that the results support our notion that the algal pyrenoid represents a protein pool for the cell that can be mobilized when needed, for example under nitrogen-limited conditions. However, it remains to be explained why nitrate reductase and ribulosebisphosphate carboxylase are major components of this pool.

Nitrite reductase is generally believed to be a solely plastidic enzyme (30, 41), although an association with the cytoplasm was also described (11). Our results indicate that in Chlamydomonas a small portion (about 20%) of the nitrite reductase activity is not located in the chloroplast (Tables I and II). We know from several experiments with chloroplast marker enzymes, for instance the NADP-dependent triosephosphate dehydrogenase, that the experimental error in our method for an enzyme of normal stability is around 5%. That is, the mean value determined for the plastidic location of a chloroplast marker was always found in a range between 95 and 105% (19, 20). Therefore, we have to conclude in this report that some nitrite reductase activity is apparently located in the cytoplasm. However, we have no idea which could be the appropriate cytoplasmic electron donor for this enzyme. Reduced ferredoxin, which is its electron donor in plastids, may not occur in the cytoplasm, and another donor system could not yet be discovered in Chlamydomonas. Therefore, we are still cautious to state an involvement of an extraplastidic nitrite reductase in nitrogen assimilation of Chlamydomonas.

Glutamine synthetase was located inside as well as outside the chloroplast of Chlamydomonas (Tables I and II). In fact, like in higher plants (28), two isoforms of the enzyme, differing in their biochemical properties, were described and characterized in Chlamydomonas (8). It was suggested that one form occurs in the chloroplast and the other in the cytoplasm of the alga, as it was already shown for the two glutamine synthetases in higher plants. Such a location could be confirmed by our study, although we did not assign one form to a specific compartment. Our results also indicate that the synthesis of the two isoforms of glutamine synthetase is obviously regulated independently for the two compartments. Pointing to this conclusion is the increase only of the plastidic portion of the enzyme in cells grown on ammonium, while the total cellular activity slightly decreased (Table II).

Both glutamate synthases (NADH- and ferredoxin-GOGAT) of C. reinhardtii (4) are located in the chloroplast (Tables I and II). In this case we could not confirm the speculation of a separate location of the two enzymes, namely an association of the NADH-GOGAT with the cytoplasm and of the ferredoxin-GOGAT with the algal chloroplast (8). Our results rather show that in C. reinhardtii only the chloroplast contains a complete GS/GOGAT cycle. As both glutamate synthases and in addition the glutamate dehydrogenase are located exclusively in the chloroplast (Tables I and II), a net assimilation of nitrogen can occur only in this organelle. Although the presence of an extraplastidic glutamine synthetase makes possible an assimilation of ammonia in the cytoplasm, it cannot be regarded as true primary nitrogen assimilation because of its dependency on the export of glutamate from the chloroplast. However, since all intermediates of the cycle are able to cross the chloroplast envelope (10, 44), both compartments can continuously exchange these compounds and thereby interact during nitrogen assimilation.

The two glutamate synthases are probably not active all the time but depend strongly on the plastidic concentration of their electron donor. Thus, the prevailing activity in the light should be that of the ferredoxin-GOGAT while in the dark the NADH-GOGAT should be more active.

In higher plants two forms of glutamate dehydrogenase were described, an NAD- and an NADP-dependent enzyme. In most investigations the NAD-specific form was localized in mitochondria (29, 30, 34, 41) while the other form seemed to be located in the chloroplast (23, 32). In Chlamydomonas only one glutamate dehydrogenase appears to occur, which does not show any specificity for NAD or NADP (3). In our study this enzyme was localized exclusively in the chloroplast of the alga (Tables I and II). Here it may be involved in protein degradation or amino acid turnover (3), but its physiological role is still not completely clarified.

We tried to summarize the results of this investigation in a scheme for the flow of nitrogen from nitrate or ammonia to glutamate in C. reinhardtii (Fig. 2). According to our conception, nitrate, which is taken up by the cells with a permease (7), is reduced outside the chloroplast (probably in the cytoplasm) to nitrite. A small amount of the resulting nitrite (about 20%) may be reduced to ammonia in the cytoplasm, but the major site of nitrite reduction is the chloroplast. Besides the production of ammonia by reduction of nitrite, additional sources of ammonia may be diffusion from outside, photosynthesis, and protein turnover. In Chlamydomonas, ammonia can be assimilated in the chloroplast as well as in the cytoplasm by isoforms of the glutamine synthetase. The glutamine formed by this reaction may pass the chloroplast envelope (10) so that cytoplasmic glutamine may also be used as substrate in the next step catalyzed by the chloroplastic glutamate synthase. This reaction is obviously confined to the chloroplast in Chlamydomonas because both isoforms of GOGAT are located in this organelle, which makes it the key site of nitrogen assimilation in the alga. The resulting glutamate can either react with another molecule of ammonia in the chloroplast or be transported again by the dicarboxylate translocator into the cytoplasm (44). A specific
translocator may also be responsible for the supply of the chloroplast with 2-oxoglutarate (44).

The scheme does not include the glutamate dehydrogenase because at the moment it is difficult to assign a role to this enzyme in nitrogen metabolism. Also not addressed in the scheme are the cosubstrates and effectors of enzymes because this was beyond the scope of our investigation. It should be kept in mind, however, that the flow of nitrogen in *Chlamydomonas* is highly influenced by different physiological conditions and that intracellular effectors probably play the major role in the regulation of nitrogen assimilation.

**LITERATURE CITED**


8. FLORENCIO FJ, JM VEGA 1983 Separation, purification, and characterization of two isoforms of glutamine synthetase from *Chlamydomonas reinhardtii*. Z Naturforsch 38c: 531–538


28. MCNALLY SF, B BURIS, P GADAL, AF MANN, GR STEWART 1983 Glutamine synthetase of higher plants. Evidence for a specific isoform content related to their possible physiological role and their compartmentation within the leaf. Plant J 72: 22–25


33. RHODES D, GA RENDON, GR STEWART 1975 The control of glutamine synthetase level in *Leguminosa*. Planta 125: 201–211


38. ULLRICH WR, PJ APARICIO, PJ SYRETT, F CASTILLO, eds 1987 Inorganic Nitrogen Metabolism. Springer-Verlag, Berlin


44. WOO KC, UI FLOEGE, HW HELDT 1987 A two-translocator model for the transport of 2-oxoglutarate and glutamate in the chloroplasts during ammonia assimilation in the light. Plant Physiol 84: 624–632