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Partial characterization of enzymes of nitrogen metabolism in *Chlorella autotrophica* Shihira & Krauss

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SUMMARY

Chlorella autotrophica shows a major decline in the intracellular concentrations of glutamine synthetase (GS) isoenzymes, GS1 and GS2, and a marked increase in the concentration of NADPH-dependent glutamate dehydrogenase (GDH) when nitrate is replaced by ammonium as the sole nitrogen source. NADH-dependent GDH shows very little change under these conditions. The apparent K_m for ammonium of both GS isoenzymes is around 50 μM and that of NADPH–GDH is around 15 mM. There is little change in the latter when the NADPH concentration in the assay medium is decreased, or when cells are harvested at different growth phases. *In vitro* kinetic analysis of GS1 and GS2 indicate low affinities of these isoenzymes for substrates other than ammonium. Both GS1 and GS2 require about 10 mM ATP, 20 mM Mg^{2+} and 70 mM glutamate for maximum activities. The rate response curves obtained for GS2 show nonlinear kinetics for glutamate and Mg^{2+}. The double reciprocal plots for activity *vs.* substrate concentration for GS2 show dual kinetics with respect to glutamate concentrations, with a low K_m value of about 10 mM and a high K_m value of 40 mM. The rate response curve for GS2 for Mg^{2+} is sigmoidal. The K_m values for glutamate measured for aspartate aminotransferase (AsAT) and alanine aminotransferase (AlAT) are in the same range as those measured for the two GS isoenzymes. The algal aminotransferases have very low K_m values (18–55 μM) for 2-oxoglutarate.

Key words: Enzyme kinetics, glutamate dehydrogenase, glutamine synthetase, nitrogen assimilation, *Chlorella autotrophica*.

INTRODUCTION

Glutamate is the primary product of inorganic nitrogen metabolism in eukaryotic organisms. Both higher plants and green algae possess two distinct pathways for incorporation of ammonium into glutamate, namely the glutamate dehydrogenase (GDH) pathway and the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle (Miflin & Lea, 1980; Stewart, Mann & Fentem, 1980; Syrett, 1981; Hellebust & Ahmad, 1989). The GDH pathway leads to the direct synthesis of glutamate by reductive amination of 2-oxoglutarate, whereas in the GS/GOGAT cycle, ammonium is first incorporated by GS as amide nitrogen into glutamine which then in the second step, catalyzed by GOGAT, is transferred to the α-amino position of 2-oxoglutarate to form glutamate. In higher plants, a great body of data obtained by ^{15}N tracer analysis, enzyme characterization and enzyme-specific inhibitor studies supports the view that the GS–GOGAT pathway is the primary route for the synthesis of glutamate (Miflin & Lea, 1980; Stewart *et al.*, 1980). The relative importance of the two alternative pathways of ammonium assimilation in eukaryotic microalgae including green unicells is, however, not clear.

One approach in comparing the efficiency of the GDH pathway and GS/GOGAT cycle in the assimilation of inorganic nitrogen in plants and microorganisms is to measure K_m values of ammonium for the aminating activities of the two pathways. With the exception of a high-affinity NADPH-dependent GDH in the green microalga *Stichococcus bacillaris* (Everest & Syrett, 1983; Ahmad & Hellebust, 1986*b*), GDHs from various plant and algal sources exhibit high K_m values (10–80 mM) for ammonium (Stewart *et al.*, 1980; Ahmad & Hellebust, 1984*b*). In contrast, the K_m values for ammonium of GS from various sources are in the micromolar range (Stewart & Rhodes, 1977; Miflin & Lea, 1980; Stewart *et al.*, 1980). Since accumulation of cytosolic ammonium above 2 mM is considered disruptive in photosynthetic organisms, the apparent K_m values of GDH for ammonium appear to preclude its role in ammonium assimilation by most green eukaryotes. Nonetheless, the NADPH-GDH in the marine microalga, *Chlorella*

autotrophica, despite exhibiting an apparent K_m for ammonium of about 15 mM, is induced by the presence of ammonium in the external medium and becomes the predominant enzyme of nitrogen assimilation under conditions of high ammonium availability (Ahmad & Hellebust, 1984*b*). Furthermore, inhibition of GS by methionine sulphoximine (MSX) has little effect on the assimilation of ammonium by *C. autotrophica* (Ahmad & Hellebust 1985*a*, 1988*a*). Interestingly, NADPH-GDH in *Chlorella sorokiniana* shows a complex pattern of accumulation and also an alteration of its affinity for ammonium under different growth conditions (Tischner & Lorenzen, 1980; Tischner, 1984; Bascomb & Schmidt, 1987). It would therefore be interesting to examine the kinetic properties of NADPH-GDH from *C. autotrophica* under various growth conditions.

The kinetic data available for GS also present some difficulties as effective operation of this enzyme does not seem to be always compatible with the cytosolic environment in green eukaryotes. Firstly, the K_m for glutamate of GS from various sources is in the range 1–15 mM (Stewart *et al.*, 1980) and thus exceeds the concentration of free glutamate found in non-vacuolated green unicells such as *Brachiomonas submarina* (Ahmad & Hellebust, 1985*b*) and *Chlamydomonas pulsatilla* (Ahmad & Hellebust, 1986*a*). Furthermore, the K_m values for glutamate obtained for angiosperm GS vary with ATP and Mg^{2+} concentrations (Stewart *et al.*, 1980). The lowest K_m values (highest affinity) for glutamate appear to require about 50 mM Mg^{2+}, a concentration unlikely to be present in the cytosolic environment of eukaryotic cells (Ahmad & Hellebust, 1984*a*, 1985*b*, 1986*a*). Some of these uncertainties regarding the validity of *in vitro* kinetic data for evaluating the functional efficiency of the GS enzymes *in vivo* may be resolved by studying GS from green unicells with known intracellular concentrations of amino acids and cations.

A high K_m of GS for glutamate may be compatible with the cell's intermediary nitrogen metabolism as it is suggested to decrease competition for glutamate between GS and other glutamate-requiring reactions (Stewart *et al.*, 1980). This hypothesis, however, suffers from the lack of comparable kinetic data for GS and other glutamate-dependent enzymes within a given cellular environment. Comparison of the K_m values for glutamate of GS and non-mitochondrial, anionic aminotransferases in green unicells could offer useful information about potential competition for glutamate among these enzymes.

MATERIALS AND METHODS

Algal cultures and growth conditions

Axenic cultures of *Chlorella autotrophica* Shihira and Krauss (Clone 580, obtained from Dr R. R. L. Guillard, Woods Hole, MA, USA, culture collection) were grown at 18 °C on a 12:12 h light-dark cycle with 24 W m^{-2} cool white light in 33% (v/v) artificial seawater (132 mM NaCl, 3·3 mM KCl, 3·3 mM $CaCl_2$, 6·6 mM $MgCl_2$ and 6·6 mM $MgSO_4$), with nutrient enrichments as described previously (Ahmad & Hellebust, 1989). The media nitrogen source was 2 mM of either $NaNO_3$ or NH_4Cl unless stated otherwise and the pH of the media was adjusted to 7·6.

Harvesting

Cells were harvested 3–4 h aftr the start of the light period, and washed in nitrogen-free 33% (v/v) artificial seawater (ASW) as described by Ahmad & Hellebust (1984*b*). Cell densities were determined as described previously (Ahmad & Hellebust, 1984*a*).

Preparation of cell-free extracts

Cells from 1 to 4 l of culture were suspended in 2 to 4 ml of extraction buffer, pH 7·6, containing 50 mM Tris, 10 mM $MgSO_4$, 1 mM dithiothreitol, 10 mM mercaptoethanol, 0·5 mM EDTA, 1% (w/v) glycinebetaine and 300 mM sorbitol. For GDH, the extraction buffer also contained 250 mM ammonium sulphate. The preparation was disrupted by passing twice through a French pressure cell at 200 MPa and 4 °C. An aliquot of 0·1 ml was removed for protein determination (Hartree, 1972) and the remainder of the extract was clarified by centrifugation at 30000 ***g*** for 30 min at 4 °C, followed by a passage through a 0·22 μm filter assembly. A 2 ml aliquot was loaded onto a Pharmacia Mono Q anion exchange column attached to a Pharmacia fast protein liquid chromatography (FPLC) system in order to fractionate various enzyme activities.

Fast protein liquid chromatography

The FPLC media employed were prepared according to Ahmad & Hellebust (1987, 1989) with the addition of thiol reagents (1 mM dithiothreitol, 10 mM mercaptoethanol) for all enzyme purifications except for that of the GS1 isoenzyme. The salt gradient and fraction collection were as described by Ahmad & Hellebust (1987).

Enzyme assays

The amination reaction of NADH-GDH and NADPH-GDH was determined spectrophotometrically (Ahmad & Hellebust, 1984*b*, 1985*a*, 1986*b*, 1988*a*). Biosynthetic activity of GS was determined by a modification of the coupled assay described by Stewart & Rhodes (1977). The reaction mixture (1 ml) contained 50 mM imidazole, 80 mM glutamate, 50 mM $MgCl_2$, 20 mM ATP,

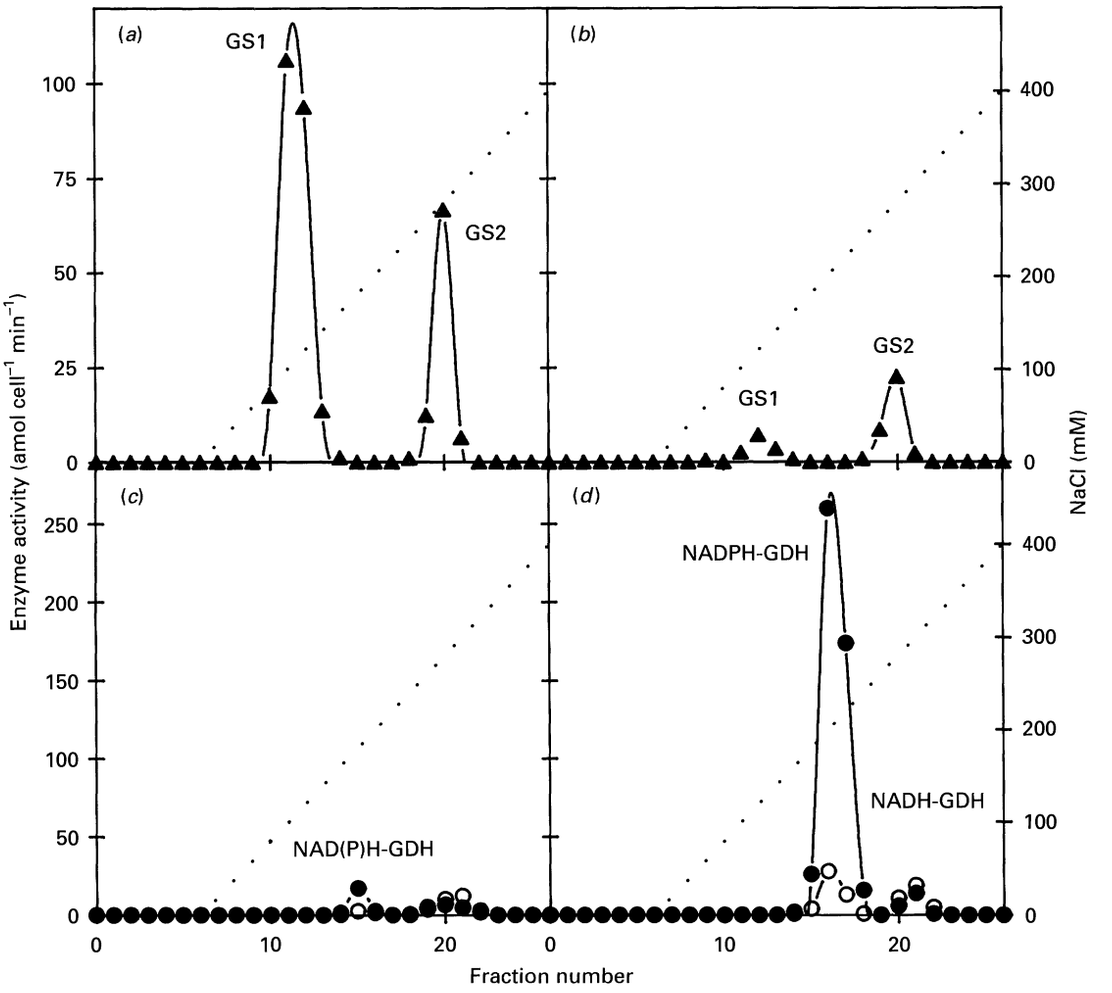

Figure 1. FPLC fractionation of GS (*a*, *b*) and GDH (*c*, *d*) activities by Mono Q anion-exchange chromatography. Each profile shown is typical of 3–5 chromatographs, where the variation in the concentration of NaCl for the given peaks was less than 10%. (*a*, *c*) Nitrate-grown cells of *C. autotrophica*. (*b*, *d*) Ammonium-grown cells. NADPH-GDH (●); NADH-GDH (○). The dotted line indicates the concentration of NaCl in the eluting solution.

50 mM NH_4Cl, 50 mM KCl, 0·2 mM NADH, 1 mm phosphoenolpyruvate, 2 units lactate dehydrogenase and 5 units pyruvate kinase at a final pH of 7·6. The mixture was pre-incubated for 10 min before initiation of the reaction by addition of 0·1 ml extract. The aminating activity of GS was measured following the stoichiometric oxidation of NADH at 340 nm. Alanine- and aspartate-dependent activities of alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT), respectively, and the glutamate-dependent activity of both aminotransferases were determined spectrophotometrically as described by Ahmad & Hellebust (1989).

RESULTS

Fractionation of GS, GDH and aminotransferases

Figure 1 shows elution profiles for the fractionation of GS and GDH in *C. autotrophica* growing exponentially (cell densities 1×10^6 to $2{\cdot}5 \times 10^6$ cells ml^{-1}) with 2 mM of either ammonium chloride or sodium nitrate as sole nitrogen source. The two molecular forms of GS observed have been designated according to the sequence of their elution on the anion-exchange column as GS1 and GS2. The alga also possesses two forms of GDH; the less negatively charged form, which elutes first and is designated NADPH-GDH, shows very high specificity for NADPH (NADPH:NADH activity ratio = 11:1), whilst the more negatively charged form, designated NADH-GDH, shows only about 30% preference for NADH over NADPH (Fig. 1*d*) The elution profiles were almost identical for GS and GDH isoenzymes isolated from cells grown over a range of nitrate or ammonium concentrations (2–25 mM), or from cells at different growth phases in batch cultures (not shown).

One of the striking features of the expression of GS and GDH activities in *C. autotrophica* is the

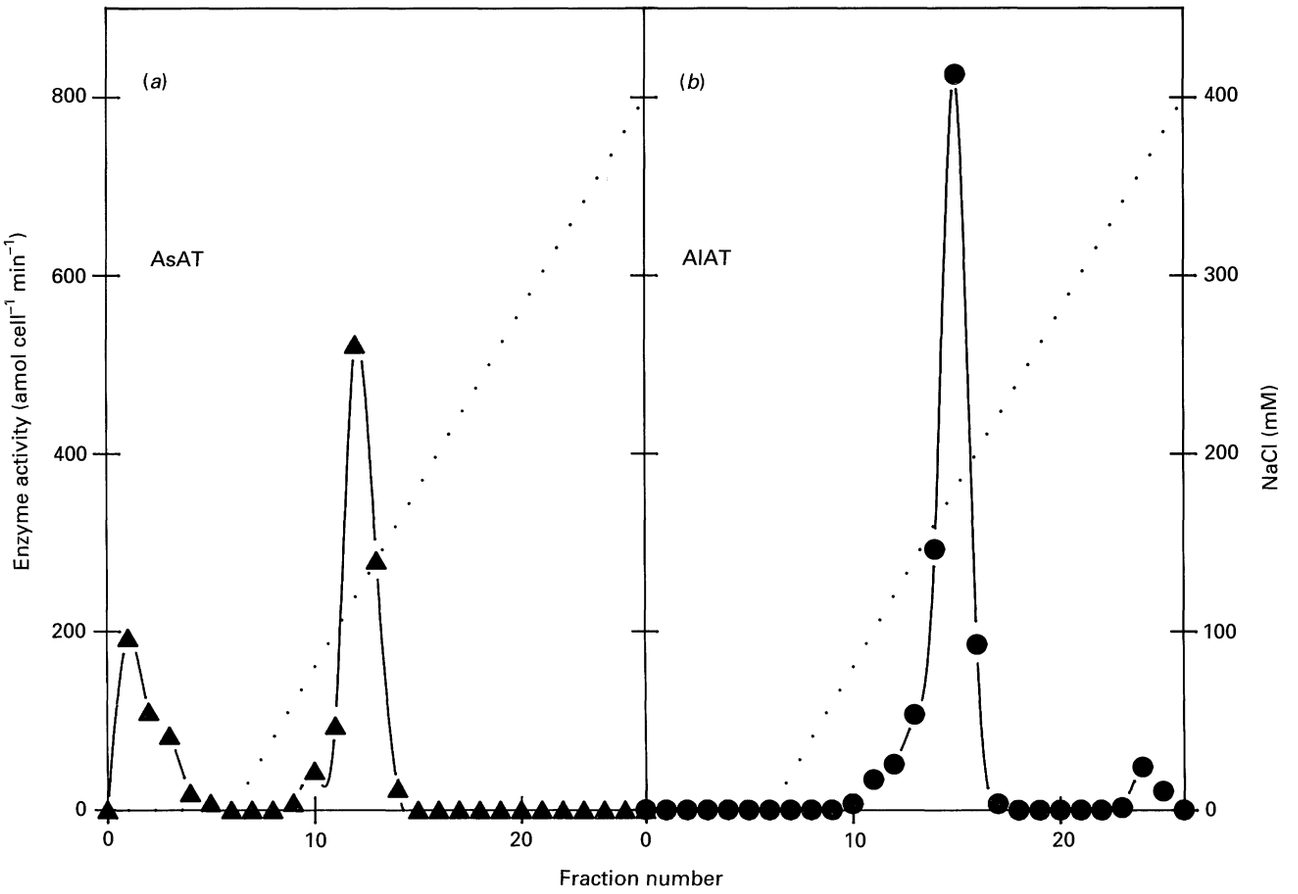

Figure 2. FPLC fractionation of AsAT (*a*) and AlAT (*b*) activities from nitrate-grown cells of *C. autotrophica* by Mono Q anion-exchange chromatography. Each profile shown is typical of 2–5 chromatographs, where the variation in the concentration of NaCl (dotted line) for the given peaks was less than 15 %.

marked change in intracellular concentration of these enzymes in response to changes in nitrogen source (Fig. 1 *a–d*). Both GS isoforms are present at markedly higher concentrations in nitrate-grown cells (Fig. 1 *a*) than in ammonium-grown cells (Fig. 1 *b*), while the reverse is true for the distribution of NADPH-GDH activity in these cells (Fig. 1 *c*, *d*). In nitrate-grown cells, GS1 accounts for most of the GS activity (Fig. 1 *a*). However, the decrease in GS activity in the presence of ammonium is more marked for GS1 than for GS2 and as a consequence, GS2 becomes the dominant GS isoenzyme in ammonium-grown cells (Fig. 1 *b*).

Extracts from cultures of *C. autotrophica* grown exponentially in the presence of 2 mM NO_3^- contain a major anionic form and a minor cationic form of AsAT (Fig. 2). Extracts also contain two molecular forms of AlAT, both of which are anionic. The less negatively charged form is, however, by far the most predominant of the two isoenzymes.

Characterization of GDH enzymes

The amination reaction for NADPH-GDH is optimal around pH 7·8, whereas that for NADH-GDH is optimal around pH 8·8, with activity at pH 7·0 only about 5 % of that at pH 8·8. A double reciprocal plot of NADPH-GDH activity *vs.* ammonium concentration indicated a K_m value of about 15 mM NH_4^+ for the enzyme in both the clarified crude extract and that purified by anion-exchange chromatography. In both preparations, this K_m value was similar, whether NADPH was present at 0·03 or 0·25 mM. Similarly, no significant change in the K_m value for NH_4^+ of NADPH-GDH from *C. autotrophica* was found in extracts prepared from algal cultures grown to different cell densities corresponding to exponential and post-exponential growth phases, respectively. The K_m of NADPH-GDH for NADPH was 5 μM and that for 2-oxoglutarate was 0·3 mM. The K_m values of NADH-GDH for NH_4^+, 2-oxoglutarate and NADH were 25 mM, 0·32 mM and 4 μM, respectively.

Characterization of GS isoenzymes

Both GS1 and GS2 exhibit a pH optimum around pH 7·6. However, the activity profiles for the two GS enzymes showed a broader pH range for GS1 activity than that of GS2. Activity at pH 6 was 37 % whilst that at pH 9 was 45 % of that at pH 7·6 in the case of GS1. The equivalent relative activities for GS2 were

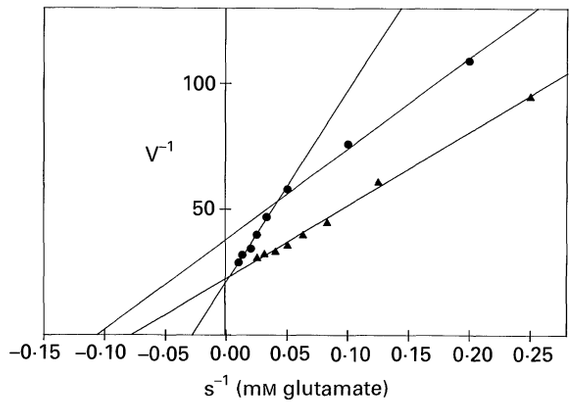

Figure 3. Double reciprocal plots of GS1 activity (▲) and GS2 activity (●) (two lines – see text) as a function of glutamate concentration. The reaction mixture (1 ml) contained 50 mM imidazole, 50 mM ammonium chloride, 20 mM ATP, 50 mM magnesium chloride, 50 mM potassium chloride, 0·2 mM NADH, 1 mM phosphoenolpyruvate, 2 units lactate dehydrogenase and 5 units pyruvate kinase at a final pH of 7·6. The reaction was initiated by addition of 0·1 ml of extract, and amination activity (V = fmol ml^{-1} min^{-1}) was measured following the stoichiometric oxidation of NADH. The values plotted are means of 3–4 experiments.

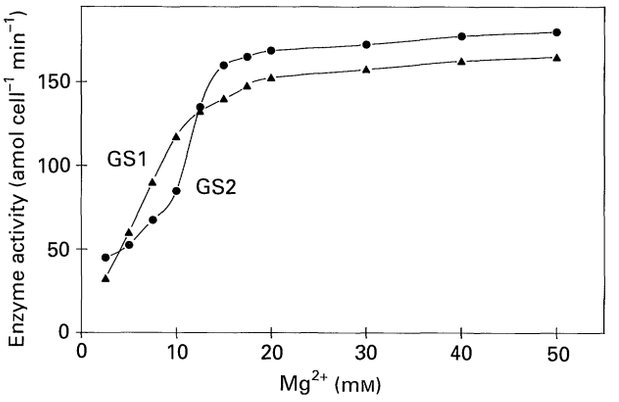

Figure 4. Effects of Mg^{2+} concentration on GS1 (▲) and GS2 (●) activities. The reaction mixture (1 ml) contained 50 mM imidazole, 50 mM ammonium chloride, 80 mM glutamate, 20 mM ATP, 50 mM potassium chloride, 0·2 mM NADH, 1 mM phosphoenolpyruvate, 2 units lactate dehydrogenase and 5 units pyruvate kinase at a final pH of 7·6. The reaction was initiated by addition of 0·1 ml of extract, and amination activity (amol $cell^{-1}$ min^{-1}) was measured following the stoichiometric oxidation of NADH. The values plotted are means of 3–4 replicates.

23 and 5 % respectively. The K_m values for NH_4^+ of both GS isoenzymes were around 50 μM, and those for ATP were around 0·7 and 0·9 mM for GS1 and GS2 respectively. While GS1 exhibits simple kinetics with respect to glutamate concentration with a K_m of about 12 mM, the rate response curve of GS2 for glutamate concentration is complex, with two distinct K_m values of about 5 and 40 mM glutamate, respectively. (Fig. 3). The double reciprocal plots for glutamate concentration of both GS activities were not found to change significantly from those shown in Figure 3 when the concentration of ATP in the assay mixture was decreased from 20 to 3 mM. The rate response curve for Mg^{2+} of GS1 is hyperbolic over the given range of Mg^{2+} concentrations, while that for GS2 is sigmoidal (Fig. 4). It can be seen from Figure 4 that both GS isoenzymes require more than 15 mM Mg^{2+} for maximal activities.

Table 1. K_m *values of aminotransferases in* Chlorella autotrophica*

Aminotransferase	Form	Substrate	K_m values
AsAT	Cationic	Glutamate	12 ± 1·5 mM
		Aspartate	2 ± 0·4 mM
		2-Oxoglutarate	55 ± 6·0 μM
AsAT	Anionic	Glutamate	15 ± 2·2 mM
		Aspartate	5 ± 0·3 mM
		2-Oxoglutarate	25 ± 3·5 μM
AlAT	Anionic	Glutamate	18 ± 2·1 mM
		Alanine	13 ± 1·5 mM
		2-Oxoglutarate	18 ± 1·4 μM

* Cells from 1 l culture were suspended in 3 ml of extraction buffer, pH 7·6, disrupted and the cell free preparation clarified by centrifugation followed by a passage through a 0·22 μm filter assembly. A 2 ml aliquot was fractionated for aminotransferase activities by anion-exchange chromatography using a salt gradient at pH 7·6 (Fig. 2). Aminotransferase activities were assayed using coupled-dehydrogenase procedures. The K_m values were calculated from double reciprocal plots and are shown as means ± SE of four experiments.

Characterization of aminotransferase activities

Table 1 summarizes the kinetic parameters for AsAT and AlAT measured at pH 7·6 with purified anionic and cationic forms as indicated. For anionic AsAT and AlAT, because of their overlap in elution during FPLC fractionation, care was taken to use FPLC fractions of each enzyme with only a residual (less than 2 %) presence of the other. In reactions catalyzing the transfer of amino nitrogen from aspartate and alanine to 2-oxoglutarate, by AsAT or AlAT respectively, the K_m values of the aminotransferases for 2-oxoglutarate are in the range of 18–55 μM, whereas their K_m values for aspartate and alanine are in the range of 2–13 mM. For the reverse reactions of these aminotransferases, the K_m values for glutamate of AsAT and AlAT were found to be in the range of 12–18 mM.

DISCUSSION

The results obtained here offer some useful information about the potential metabolic efficiencies of various enzymes of nitrogen assimilation in the green microalga *Chlorella autotrophica*. The pH profiles for optimal activity of NADPH-GDH and

both GS isoenzymes, GS1 and GS2, indicate that these enzymes could function well in the pH environments of both the cell cytoplasm (*c.* pH 7) and the chloroplastic stroma (*c.* pH 8). In higher plants GS1 is a cytoplasmic isoenzyme present in both chlorophyllous and non-chlorophyllous tissues, whereas GS2 is localized in the chloroplast (McNally *et al.*, 1983; Casselton *et al.*, 1986). Furthermore, NADPH-GDH in most green organisms is considered to be a chloroplastic enzyme (Stewart *et al.*, 1980; Prunkard *et al.*, 1986). The NADH dependent activity of higher plant GDH is considered to be localized in the mitochondrial fraction, and it is interesting that the pH profile of NADH-GDH activity in *C. autotrophica* shows its maximum activity over the pH range of 8·5–9·0.

In *C. autotrophica*, the nitrogen source has a profound effect on the expression of NADPH-GDH and the two GS isoenzymes. In cultures growing on nitrate, cells maintain high activities of both GS isoenzymes and low activity of NADPH-GDH. In contrast, cells growing on ammonium show a major decline in the activity of GS isoenzymes while inducing a high level of NADPH-GDH activity. Considering that NADPH-GDH shows a much higher K_m for ammonium than do the two GS isoenzymes in this alga, the alteration in the expression of ammonium-assimilating enzymes in response to changes in nitrogen sources may be related to the size of endogenous ammonium pool under these conditions. Cells growing with ammonium probably maintain a higher level of endogenous ammonium than those growing with nitrate. In nitrate-grown cells, the uptake of nitrate and its reduction to ammonium is regulated by the incorporation of ammonium into amino acids (Syrett, 1981; Hellebust & Ahmad, 1989) thus preventing an accumulation of ammonium in these cells. The observed diversity of regulation of the nitrogen assimilating enzymes in this alga is probably associated with the maintenance of an efficient metabolic machinery for ammonium assimilation in response to a changing nitrogen supply. However, any attempt to determine the metabolic function of the two alternative pathways in ammonium assimilation should incorporate other kinetic parameters of GS and GDH isoenzymes in addition to their apparent affinities for ammonium.

It is not certain that the measured K_m values for ammonium of NADPH-GDH reflect the true *in vivo* affinity of the enzymes for their substrate. There have been reports of a similar inducible NADPH-GDH in several microorganisms (Tischner & Lorenzen, 1980; Everest & Syrett, 1983; Martin, Msatef & Botton, 1983; Ahmad & Hellebust, 1986*b*; Bascomb & Schmidt, 1987; Jennings, 1989; Ahmad *et al.*, 1990; Schwartz, Kusnan & Fock, 1991). Interestingly, in the green alga *Stichococcus bacillaris* (Everest & Syrett, 1983; Ahmad & Hellebust, 1986*b*) NADPH-GDH exhibits a normal rate response curve for ammonium concentration with a single K_m value of 1–2 mM, whereas in another green alga, *Chlorella sorokiniana* (Tischner, 1984; Bascomb & Schmidt, 1987), The NADPH-GDH exhibits dual kinetics with respect to ammonium concentration, showing a low K_m of < 5 mM and a high K_m of > 20 mM. It has been suggested that the high K_m value for ammonium in the kinetic measurements of NADPH-GDH in *C. sorokiniana* is associated with post-translational modifications of the low K_m enzyme that occur when the culture ages or when the concentration of ammonium in the medium is high (Prunkard *et al.*, 1986). Interestingly, the NADPH-GDH enzymes in two mycorrhizal fungi, *Laccaria bicolor* (Ahmad *et al.*, 1990) and *Cenococcum graniforme* (Martin *et al.*, 1983), also show low and high K_ms for ammonium, and in *L. bicolor* the kinetics of NADPH-GDH are not influenced by culture conditions (I. Ahmad & J. A. Hellebust, unpublished results). Similarly, our present study shows no appreciable change in the measured kinetic parameters of NADPH-GDH in *C. autotrophica* under various growth conditions. The appearance of a single high K_m value for ammonium in the case of NADPH-GDH in *C. autotrophica* therefore cannot be attributed to molecular and/or metabolic modifications associated with the alga's growth conditions. One can argue that the observed differences in the kinetic properties of NADPH-GDH from various microorganisms are true physiological variations and thus are indicative of the differences in the metabolic efficiency of this enzyme in closely related algal species. Nonetheless, the possibility that the measurements of a high K_m value for ammonium of NADPH-GDH in certain algal and fungal species may result from a change in the enzyme affinity during *in vitro* enzyme isolation and purification cannot be excluded.

The remarkably high affinity for ammonium of both GS isoenzymes present in *C. autotrophica* is accompanied by low affinities (high K_m values) for glutamate and Mg^{2+}. Our results show that the two GS isoenzymes require 40–70 mM glutamate and about 20 mM Mg^{2+} for full activities. The reaction catalyzed by GS2 shows dual saturation kinetics for glutamate with a low K_m value of about 5 mM indicating that this GS isoenzyme may function effectively at low intracellular glutamate concentrations. However, this GS isoenzyme also shows a sigmoidal response to the concentration of Mg^{2+} where the reaction rate increases rapidly when the concentration of the divalent cation is increased above 10 mM. It is interesting since the rate response curve for ATP for the aminating activity of GS in this alga shows the activity to be saturated below 10 mM ATP, implying that less than 10 mM magnesium is required to form an enzyme–Mg^{2+}–ATP complex for maximal GS activity. Our results,

therefore, support the suggestion that the stimulation of certain GS activities by high levels of magnesium is associated with the build up of a free divalent cation pool in excess of that required to saturate the enzyme–Mg^{2+}–ATP complex (Stewart *et al.*, 1980).

If the above *in vitro* kinetic data of GS1 and GS2 are true indications of saturation kinetics of these activities in *C. autotrophica*, then the alga may not always maintain maximum GS activities. This is because *C. autotrophica* has an intracellular solute composition of glutamate and magnesium in the range of 30–40 mM and 10–25 mM (Ahmad & Hellebust, 1984*a*), respectively. These considerations are important since under some conditions this alga contains GS levels that, even when measured with saturating concentrations of all substrates, barely match measured nitrogen assimilation rates (Ahmad & Hellebust, 1984*b*). The above comparison of the characteristics of GS isoenzymes and NADPH-GDH, therefore, suggests that in *C. autotrophica*, the two pathways may be functioning together, albeit at different rates in media of different nitrogen sources, to maintain the assimilation of nitrogen under various conditions.

In *C. autotrophica*, the presence of GS activities with a high K_m for glutamate does not appear to confer a competitive advantage to its intermediary amino acid metabolism as both AsAT and AlAT in this alga also have low affinities for glutamate. Conceivably, simultaneous operation of the glutamate-generating GDH and glutamate-consuming GS pathways for assimilation of ammonium may at least in part alleviate the competition for glutamate between ammonium assimilation and transamination reactions. This suggests that nitrogen metabolism in the green alga *Chlorella autotrophica* may differ in the integration of its various processes from that of higher plants, where primary ammonium assimilation is carried out exclusively via the GS/GOGAT pathway. Among the striking features of intermediary nitrogen metabolism in *C. autotrophica* are the high affinities (K_m = 18–55 μM) of AsAT and AlAT for 2-oxoglutarate. Aminotransferases in plants have much lower K_m values for their keto acid substrates than for their amino acid substrates (Givan, 1980), and this feature may account for the pattern of intracellular distribution of amino acids and keto acids in plant cells.

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