JOURNAL OF BACTERIOLOGY, Oct. 1983, p. 122–129
0021-9193/83/100122-08$02.00/0

Vol. 156, No. 1

Glutamine and Glutamate Transport by *Anabaena variabilis*

JOHN S. CHAPMAN AND JOHN C. MEEKS*
Department of Bacteriology, University of California, Davis, California 95616

Received 1 November 1982/Accepted 15 July 1983

Anabaena variabilis, a dinitrogen-fixing cyanobacterium, has high- and low-affinity systems for the transport of glutamine and glutamate. The high-affinity systems have K_m values of 13.8 and 100 μM and maximal rates of 13.2 and 14.4 nmol · min^{-1} · mg of chlorophyll a^{-1} for glutamine and glutamate, respectively. The low-affinity systems have K_m values of 1.1 and 1.4 mM and maximal rates of 125 and 100 nmol · min^{-1} · mg of chlorophyll a^{-1} for glutamine and glutamate, respectively. Glutamine was unable to support growth of *A. variabilis* in the absence of any other nitrogen source, and glutamate alone at 500 μM was inhibitory to its growth. The analog L-methionine-DL-sulfoximine (MSX) was transported by a high-affinity system with a K_m of 34 μM. Competition experiments and the transport characteristics of a specific class of MSX-resistant mutants imply that glutamine, glutamate, and MSX share a common component for transport. A second class of MSX-resistant mutants had a glutamine synthetase activity with altered affinity constants for glutamine and glutamate relative to the wild-type enzyme.

Glutamine, or a derivative of it, has been implicated in the regulation of heterocyst differentiation and aerobic dinitrogen fixation in cyanobacteria (1, 16, 26) and of nitrate reductase in *Neurospora* sp. (20). Mutations which alter glutamine metabolism or transport would be of value in studying the physiological role of this amino acid in the regulation of enzyme expression in cyanobacteria. The glutamine synthetase-glutamate synthase pathway is the predominant route of assimilation of exogenous or dinitrogen-derived ammonium in dinitrogen-fixing cyanobacteria (16). Therefore, if glutamine synthetase were completely inactivated, the end product of the reaction, glutamine, and, directly or indirectly, the end product of the pathway, glutamate, would have to be transported at rates sufficient to satisfy essentially all of the nitrogen requirements for growth.

Relatively little is known about transport of amino acids by cyanobacteria. In *Anacystis nidulans*, amino acid transport is an energy-requiring process (13), and some amino acids share common transport pathways (14). *A. nidulans* will transport α-aminoisobutyric acid by high- and low-affinity systems at maximal rates of 12 and 40 nmol · h^{-1} · mg of chlorophyll *a* (Chl *a*)$^{-1}$, respectively (14). Heterocyst-forming *Anabaena cylindrica* was reported to assimilate glutamine at a rate of about 50 nmol · min^{-1} · mg of Chl a^{-1} (22). In *Anabaena variabilis*, methionine, uracil, adenine, biotin, and nicotinic acid are assimilated at rates sufficient to support growth of the respective auxotrophic mutant strains (7).

We have examined the transport of glutamine and glutamate by *A. variabilis* to determine whether isolation of mutants requiring glutamine could be hindered, in part, by their rates of uptake. In *Salmonella typhimurium*, L-methionine-DL-sulfoximine (MSX) is transported by a high-affinity methionine and glutamine transport system (4), and glutamine is reported to reverse the in vivo inhibition of glutamine synthetase activity by MSX in *Anabaena* sp. strain 7120 (11). Therefore, we isolated MSX-resistant mutants for their possible use in characterizing glutamine transport by *A. variabilis*.

MATERIALS AND METHODS

Growth conditions. *A. variabilis* ATCC 29413 was cultured in the medium of Allen and Arnon (2), diluted eightfold. When supplemented with organic or inorganic combined nitrogen, the medium was buffered with 5 mM sodium *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonate (TES) or sodium morpholinepropanesulfonate, pH 7.2 to 7.5. Stock cultures were grown in the absence of combined nitrogen under air at room temperature with fluorescent illumination in a semicontinuous culture mode and maintained at a density of 3.5×10^6 to 6.9×10^6 cells per ml by dilution. For transport experiments, 20- to 30-ml samples were aseptically removed with a suction-operated sampler. Contamination was routinely checked microscopically and by streaking samples onto nutrient agar plates.

Growth rates were determined by following changes in light scattering at 750 nm or in Chl *a* as described

previously (17) or by protein content. In experiments in which both growth and glutamine synthetase activity were determined, the cultures were incubated at 30°C with growth-limiting fluorescent illumination in 50 ml of medium in 125-ml Erlenmeyer flasks on an orbital shaker. For protein determinations, cells were lysed by cavitation for 60 s/ml of suspension (model W-225R sonicator, Heat Systems Inc., Plainview, N.J.). The extract was centrifuged at 15,000 × *g* for 10 min, and protein in the supernatant was determined by the method of Lowry et al. (15) with bovine serum albumin as the standard.

The amount of glutamine in uninoculated medium incubated under normal growth conditions was determined by titration, using purified glutamine synthetase in the γ-glutamyltransferase assay (24), with known concentrations of freshly prepared glutamine as the standards. Samples were incubated with the enzyme in the reaction mixture for 60 min. Purified glutamine synthetase from *Escherichia coli* was a gift from S. G. Kustu.

Transport of [^{14}C]glutamine and [^{14}C]glutamate. [*U*-^{14}C]glutamine (261 mCi/mmol) and [*U*-^{14}C]glutamate (293 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass. Except for glutamine, nonradioactive amino acids and analogs were obtained from Sigma Chemical Co., St. Louis, Mo. High-purity glutamine was obtained from Calbiochem, La Jolla, Calif. All solutions were made in 50 mM morpholinepropanesulfonate, pH 7.5, and diluted 10-fold for an assay. The transport assays were done at 30°C under fluorescent illumination in rapidly shaking test tubes holding 6.7 ml of cell suspension at a density of 1 to 2 μg of Chl *a* per ml, 0.3 ml of the appropriate amino acid solution, and 10 or 15 μl of ^{14}C-labeled amino acid. Aliquots of 1 ml were removed at 10-min intervals for up to 30 min (or 20-min intervals for up to 60 min in experiments with greater than 0.5 mM glutamate), filtered onto filter disks (0.45-μm pore size; Millipore Corp., Bedford, Mass.), and immediately washed with 20 ml of basal medium or, in some glutamine transport assays, a solution of 5 mM glutamine. Radioactivity on the filter disks was determined in 10 ml of Aquasol (New England Nuclear) with a Beckman model LS7500 liquid scintillation system. Boiled cells showed no accumulation of label. In experiments in which inhibition of [^{14}C]glutamine transport was examined, the glutamine concentrations were 10 and 50 μM and that of the inhibiting amino acid was 5 to 500 μM. In similar experiments for [^{14}C]glutamate, the glutamate concentrations were 10, 50, and 250 μM and the inhibiting glutamine concentration was 5 to 500 μM

Glutamine synthetase assays. Glutamine synthetase activity was measured by the transferase and biosynthetic reactions in crude extracts or in toluene-treated whole cells. Crude extracts were prepared by sonic cavitation for 60 s/ml of cell suspension in 10 mM imidazole buffer (pH 7.5)–1 mM dithiothreitol (Sigma)–2 mM $MnCl_2$. The extract was centrifuged at 15,000 × *g* for 10 min, and the supernatant fraction was used for enzyme and protein assay. Whole cells were rendered permeable by vigorously mixing 0.5 ml of culture with 0.2 ml of toluene and allowing the mixture to stand for 5 min before adding 0.5 ml of the glutamine synthetase transferase reaction mixture (24). The transferase activity of glutamine synthetase in toluene-treated cells was linear with time and comparable with activity in crude extracts. The biosynthetic assay was by the method of Elliot (8) and contained 100 mM buffer (imidazole or TES; pH 7.5), 24 mM glutamate, 4 mM dithiothreitol, 50 mM $MgCl_2$, 10 mM ATP, 50 mM hydroxylamine, and crude extract in a total volume of 1.25 ml.

Inhibition of glutamine synthetase activity in vitro by low concentrations of MSX required preincubating with the inhibitor. Crude cell extracts were incubated for 30 min with 50 μM MSX and the transferase assay mixture, with glutamine and hydroxylamine deleted. The transferase reaction was then initiated by adding glutamine and hydroxylamine and was terminated after 45 min.

Transport of MSX. In vivo inhibition of glutamine synthetase activity by MSX involves transport of the inhibitor followed by binding to the enzyme. Because MSX irreversibly inhibits glutamine synthetase (6), the relative rate of its transport could be determined by measuring residual enzyme activity in lysed or toluene-treated cells after in vivo exposure to the inhibitor. We emphasize that this approach gives a relative, not absolute, measure of MSX transport. This method will tend to underestimate MSX transport if there is not a rapid, stoichiometric inhibition of glutamine synthetase activity by intracellular MSX. In direct transport experiments, cells were incubated for up to 90 min in various concentrations of MSX. At 15-min intervals, subsamples were treated with toluene, and glutamine synthetase transferase activity was determined. The percent inhibition per minute was calculated from the slope of the linear line of glutamine synthetase activity versus the time of incubation in various concentrations of MSX. This assay was satisfactory with concentrations of MSX up to 400 μM. At higher concentrations, inhibition of glutamine synthetase by MSX in the suspension medium of toluene-treated cells occurred during enzyme assays. To examine inhibition of MSX transport, cells were incubated for 90 min with 50 μM MSX and 5 to 1,000 μM inhibiting amino acid. The cells were then toluene treated, and glutamine synthetase activity was determined.

Mutagenesis and mutant selection. *A. variabilis* was harvested at a density of 2 × 10^6 cells per ml, concentrated 10-fold by centrifugation, and sonicated in a cleaning bath (Branson Cleaning Equipment Co., Shelton, Conn.) until the filaments were reduced to an average length of 2 cells. The cells were washed twice with basal medium and suspended in 10 mM citrate buffer, pH 6.0, at 10^7 cells per ml. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (nitrosoguanidine; Sigma) was added to a final concentration of 250 μg/ml and the suspension was incubated for 4 h at 30°C in the light with shaking. The treatment was terminated by washing twice with basal medium, and the cells were resuspended in basal medium.

In experiments to isolate MSX-resistant strains, mutagenized cultures were grown photosynthetically for 7 days at 30°C and then plated onto full-strength medium solidified with 1% agar purified by the method of Braun and Wood (5) containing 2.5 mM NH_4Cl, 5 mM TES (pH 7.5), and 50 μM MSX. MSX-resistant colonies were picked after 7 to 10 days of incubation at 30°C in the light, restreaked on selective medium, and cloned.

RESULTS

Transport of glutamine, glutamate, and MSX. In wild-type *A. variabilis*, biphasic curves for transport of glutamine and glutamate were obtained for concentrations from 5 μM to 2.5 mM (Fig. 1). The metabolic fate of the assimilated amino acids was not determined, but [^{14}C]glutamine could not be removed from living cells when filtered samples were washed with 20 volumes of 5 mM glutamine. Incorporation of labeled amino acids was linear throughout the incubation periods. Two transport systems for glutamine and glutamate were clearly distinguished in Lineweaver-Burk plots (Fig. 1). The high-affinity system for glutamine was calculated to have an apparent K_m of 13.8 μM and a V_{max} of 13 nmol · min · mg of Chl a^{-1}. The low-affinity system, not saturated in these experiments, had an apparent K_m of 1.3 mM and a calculated V_{max} of 125 nmol · min^{-1} · mg of Chl a^{-1}. The calculated kinetic constants for glutamate transport by its two systems were apparent K_m values of 100 μM and 1.1 mM and maximal rates of 14 and 110 nmol · min^{-1} · mg of Chl a^{-1} for the high- and low-affinity systems, respectively.

Based on the in vivo rate of inhibition of glutamine synthetase activity, MSX was transported by a high-affinity system with an apparent K_m of 34 μM (Fig. 1).

Growth of wild-type *A. variabilis* in the presence of ammonium, glutamine, and glutamate. Under an atmosphere of Ar-O_2-CO_2, in the absence of combined nitrogen, *A. variabilis* grew exponentially for about 48 h (about two doublings) before entering a stationary phase (Fig. 2). Within 72 h of complete nitrogen deprivation, the heterocyst frequency increased from 7.7% ± 0.5 to 11.6% ± 1.0 of the total cells (mean ± standard error in all cases where variability is given), whereas levels of phycobiliproteins declined. This is a characteristic response of dinitrogen-fixing cyanobacteria to complete nitrogen deprivation (1). The cultures continued to grow under Ar-O_2-CO_2 when supplemented with 2.5 mM NH_4Cl or 25 mM, but not 5 mM, glutamine. As determined by titration, 3.7% ± 0.9 of the 25 mM glutamine was lost per day from uninoculated growth medium under these culture conditions. Assuming that 1 mol of ammonium was released per mol of glutamine lost (10), the 0.7 to 0.9 mM ammonium generated per

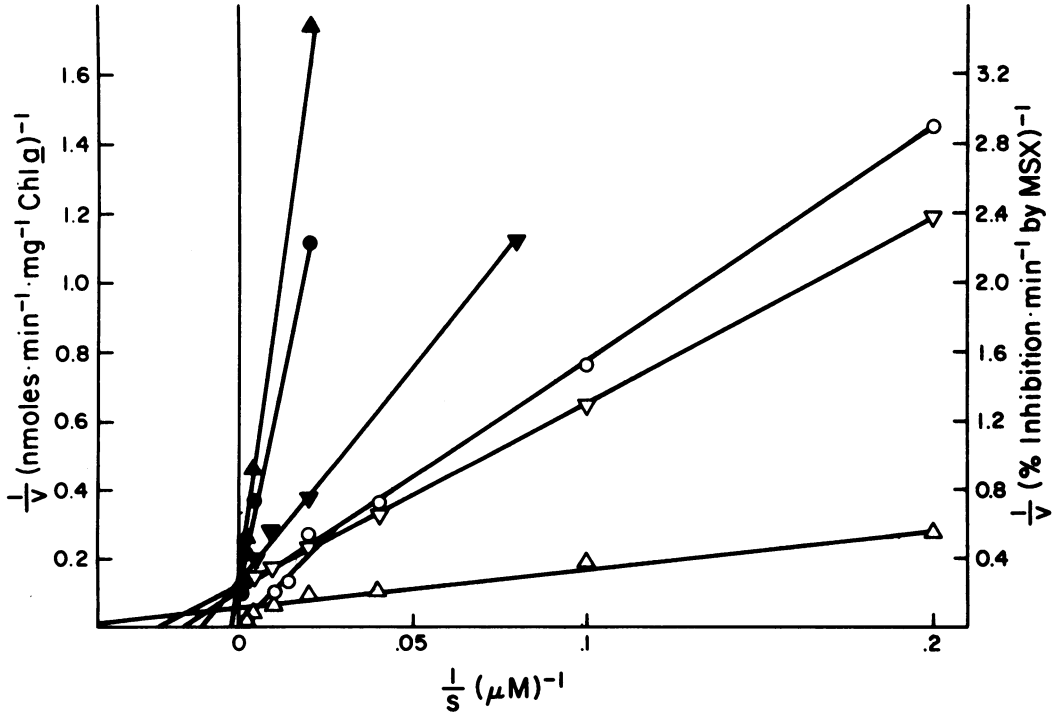

FIG. 1. Reciprocal plot of glutamine (△, ▲), glutamate (○, ●), or MSX (▽, ▼) concentration versus velocity of transport of glutamine or glutamate or in vivo inhibition of glutamine synthetase transferase activity by MSX. Open symbols represent the wild-type strain and closed symbols represent MSX-resistant strain 7-2. Values represent means of three to six experiments. For clarity in presentation, all of the 11 datum points of glutamine or glutamate concentrations were not plotted, although the values were used in calculations of kinetic constants.

day could have supported the growth of *A. variabilis* rather than glutamine. Glutamate alone at 0.5 mM inhibited the growth of *A. variabilis* under air or Ar-O_2-CO_2 (Fig. 2).

Characteristics of MSX-resistant mutants. MSX-resistant mutants were isolated for their possible use in characterizing glutamine transport. Two distinct colonial morphologies were observed under 50 μM MSX selection conditions among the resistant survivors after nitrosoguanidine mutagenesis: large colonies, similar to those of the wild-type strain under nonselective conditions, which were recovered at a frequency of 1×10^{-6} and petite colonies which were recovered at a frequency of 2×10^{-7}.

MSX-resistant strains 7-2 (large-colony phenotype) and 5-1 (petite-colony phenotype) grew in the absence of MSX under dinitrogen-fixing conditions or in medium supplemented with ammonium or nitrate at rates identical to that of the wild-type strain. The presence of 50 μM MSX inhibited the growth of the wild-type strain but had no immediate effect on the growth of strain 7-2 or 5-1 (Fig. 3A). The glutamine synthetase activity of the wild-type strain decreased to undetectable levels within 96 h of in vivo exposure to 50 μM MSX, whereas strains 7-2 and 5-1 retained 40 and 11%, respectively, of their glutamine synthetase activities (Fig. 3A). In the wild-type strain, a partial prevention of MSX inhibition of growth occurred when 0.1 mM glutamine or glutamate was added simultaneously with 50 μM MSX (Fig. 3B). Stimulation of growth was not observed when 0.1 mM glutamine or glutamate was added to MSX-inhibited wild-type cells after the inhibitor had been removed or when these compounds were added to either MSX-resistant strain in the presence or absence of MSX.

Although strain 7-2 grew well in the presence of 50 μM MSX, it grew poorly in MSX concentrations near 200 μM. Strain 7-2 transported MSX at essentially the same maximal rate as did

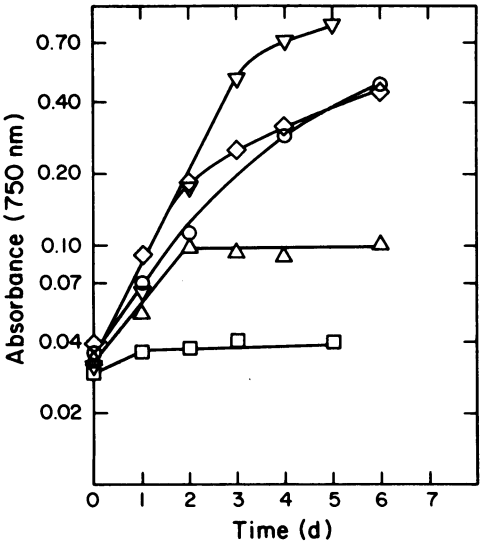

FIG. 2. Growth of *A. variabilis* on basal medium supplemented with different nitrogen sources and incubated with air or Ar-O_2-CO_2 (80:19:1, vol/vol/vol). Symbols: ○, air; △, plus or minus 5 mM glutamine, Ar-O_2-CO_2; ◇, 25 mM glutamine, Ar-O_2-CO_2; ▽, 2.5 mM NH_4Cl, Ar-O_2-CO_2; □, 0.5 mM glutamate, air or Ar-O_2-CO_2. d, Day.

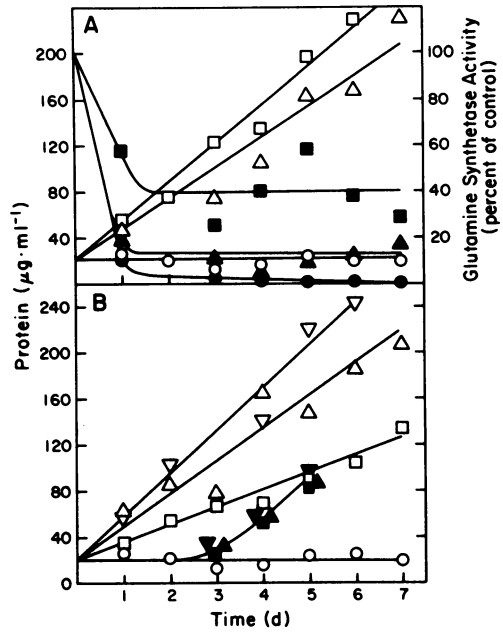

FIG. 3. Effect of MSX, with or without glutamine and glutamate, on growth and glutamine synthetase activity. (A) Growth in the presence of 50 μM MSX of the wild-type strain (○), strain 5-1 (△), and strain 7-2 (□) and glutamine synthetase transferase activity from the wild-type strain (●), strain 5-1 (▲), strain 7-2 (■). (B) Growth of wild-type cells with N_2 alone (▽), N_2 plus 50 μM MSX (○), 0.1 mM glutamine plus 50 μM MSX (△), and 0.1 mM glutamate plus 50 μM MXS (□). After wild-type cells had been exposed to 50 μM MSX under N_2 as the sole source of nitrogen for 48 h and the exogenous MSX had been removed by washing, incubation was continued with N_2 alone (▼), 0.1 mM glutamine (▲), or 0.1 mM glutamate (■). Growth at 30°C under limiting light intensity was monitored by changes in the protein content of subsamples. Glutamine synthetase activity was measured in toluene-treated cells. Values represent means of four experiments. Control values of glutamine synthetase-specific activities were 34 nmol · min^{-1} · mg of $protein^{-1}$ for the wild-type strain, and 35 and 15 nmol · min^{-1} · mg of $protein^{-1}$, respectively, for strains 7-2 and 5-1. d, Day.

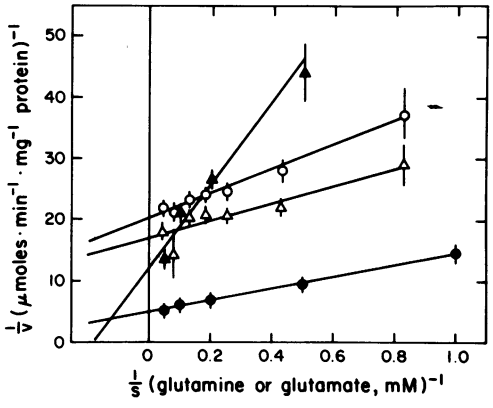

FIG. 4. Reciprocal plot of glutamate or glutamine concentration versus velocity of the glutamine synthetase biosynthetic or transferase reaction, respectively, in crude extracts from cells of the wild-type strain or strain 5-1. Symbols: ○, wild-type strain transferase reaction with glutamine; ●, wild-type strain biosynthetic reaction with glutamate; △, strain 5-1 transferase reaction with glutamine; and ▲, strain 5-1 biosynthetic reaction with glutamate. Values represent means ± standard error of the mean of four to five experiments.

wild-type cells at high inhibitor concentrations, but the apparent K_m value for transport was 80 μM compared with 34 μM in wild-type cells (Fig. 1). The maximal rates of glutamine and glutamate transport by strain 7-2 were 11 and 9 nmol · min^{-1} · mg of Chl a^{-1}, respectively, which are more than 10-fold lower than the rates of wild-type cells. The apparent K_m values of strain 7-2 were 0.9 mM for glutamine and 0.4 mM for glutamate. Thus, strain 7-2 appeared to have altered transport for glutamine, glutamate, and MSX.

The petite-colony phenotype appears to reflect an alteration in the interaction of MSX with glutamine synthetase. Strain 5-1 transported glutamine and glutamate at rates similar to those of the wild-type strain (data not shown). The in vitro glutamine synthetase transferase activity of strain 5-1 was inhibited only 12% by 50 μM MSX, whereas the activities of the wild-type strain and strain 7-2 were inhibited by 88%. In crude extracts, glutamine synthetase from strain 7-2 and the wild-type strain had an apparent K_m value of 2.9 mM ± 0.26 for glutamine in the transferase reaction, whereas that from strain 5-1 was 7.1 mM ± 1.7 (Fig. 4). In the biosynthetic reaction, strain 5-1 and the wild-type strain had apparent K_m values for glutamate of 0.8 mM ± 0.13 and 1.4 mM ± 0.2, respectively (Fig. 4).

Relationship among glutamine, glutamate, and MSX transport in wild-type cells. The altered transport of glutamine and glutamate by MSX-resistant strain 7-2 led us to examine the relationship among transport of these compounds. A Dixon plot shows that glutamate was a competitive inhibitor of glutamine transport through both the high- and the low-affinity systems, with K_i values of ca. 10 and 340 μM, respectively (Fig. 5). Glutamine inhibition of glutamate transport was of a mixed type in both systems, with K_i values in the range of 75 to 100 μM for the high-affinity system and 350 to 500 μM for the low-affinity system (Fig. 6).

Glutamine and glutamate, which were able to partially prevent inhibition of growth by MSX in wild-type cells (Fig. 3B), were different with respect to inhibition of MSX transport. Individually, 25 μM glutamine, histidine, methionine, and asparagine gave a 50% reversal of in vivo inhibition of glutamine synthetase activity by 50 μM MSX (relative MSX transport), and at 1.0 mM nearly 100% of the activity was recovered (Fig. 7). Glutamate, aspartate, citrulline, alanine, and the glutamine analog γ-glutamylhydrazide gave a 50% reversal at about 250 μM each and a 77% reversal at 1 mM. Tryptophan, phenylalanine, or arginine at 1 mM resulted in a 25% reversal, whereas uracil, adenine, and 2-oxoglutarate had no effect on in vivo inhibition of glutamine synthetase by MSX. The fact that glutamine and glutamate fell into distinct groups supports our interpretation that in vivo reversal of MSX inhibition of glutamine synthetase was due to inhibition of MSX transport rather than competition between these amino acids and MSX in binding to glutamine synthetase. Alanine and aspartate are feedback inhibitors of glutamine synthetase in cyanobacteria (22), but there are no indications that the other amino acids in these groups interact directly with glutamine synthetase.

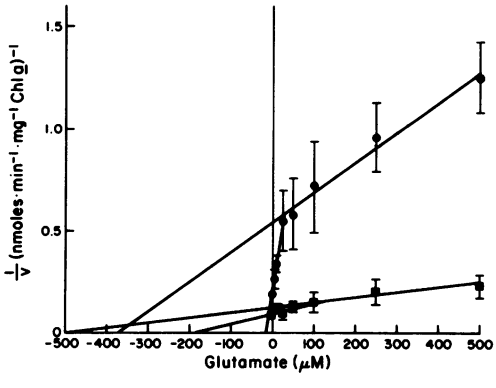

FIG. 5. Dixon plot of glutamate inhibition of glutamine transport in the wild-type strain. Substrate (glutamine) concentrations were 10 μM (●) and 50 μM (■). Values represent means of three to five determinations.

Although not identical, the grouping of compounds that inhibit glutamine transport was similar to that observed in the MSX competition experiments. Methionine and alanine inhibited transport of 50 μM glutamine at concentrations of ca. 20 μM (Fig. 8). Glutamate, asparagine, histidine, and γ-glutamylhydrazide gave a 50% inhibition at concentrations ca. 250 μM, but inhibition did not increase at higher concentrations. MSX, phenylalanine, adenine, and 2-oxoglutarate did not affect transport of glutamine.

DISCUSSION

Glutamine and glutamate are transported by high- and low-affinity systems in *A. variabilis*, whereas MSX is transported by a high-affinity system. The number of potential transport systems for these amino acids cannot be determined from the results obtained, but the following lines of evidence indicate that the measured transport systems at least share a common component.

Resistance to MSX in strain 7-2 was due to a decreased rate of its transport at low concentrations and not to alterations in inhibition of glutamine synthetase activity. Strain 7-2 also showed decreased rates of glutamine and glutamate transport, with affinity constants that were similar to those of the low-affinity systems. Thus, strain 7-2 appeared to have a lesion(s) in a component shared by the transport systems for both amino acids and MSX. Relative to wild-type cells, strain 7-2 grew at the same rates on dinitrogen, nitrate, and ammonium, and its growth was inhibited by the same concentrations of 5-fluorocytosine and 5-fluorouracil (data not shown). These observations indicate that the transport of nitrate, ammonium, and uracil by strain 7-2 had not been altered significantly and that the mutation(s) induced by nitrosoguanidine did not affect general permeability properties.

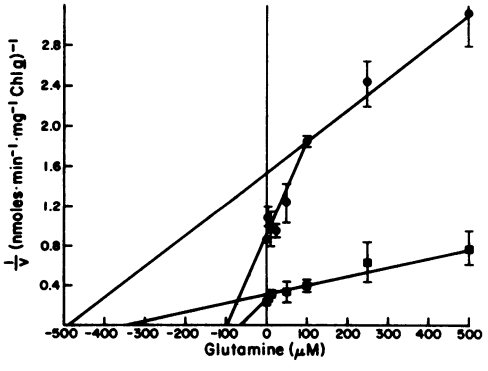

FIG. 6. Dixon plot of glutamine inhibition of glutamate transport in the wild-type strain. Substrate (glutamate) concentrations were 10 μM (●) and 50 μM (■). Values represent means of three to five determinations.

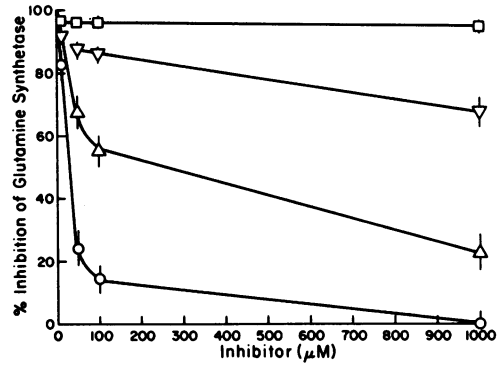

FIG. 7. Effects of amino acids and an analog on the transport of 50 μM MSX. Tranport was determined by the percentage of in vivo inhibition of glutamine synthetase activity. Added: ○, glutamine, methionine, histidine, or asparagine; △, glutamate, aspartate, alanine, citrulline, or γ-glutamylhydrazide; ▽, tryptophan, phenylalanine, or arginine; □, uracil, adenine, or 2-oxoglutarate. Values represent means of three to five determinations for each compound.

Glutamine and glutamate inhibited the transport of each other, although the extent and type of inhibition were not the same. Glutamate appeared to be a competitive inhibitor of glutamine transport, whereas glutamine displayed a mixed type of inhibition of glutamate transport. At low concentrations, glutamate was not as effective an inhibitor of glutamine transport as were other amino acids. Glutamine and glutamate also were distinctly different with respect to the concentra-

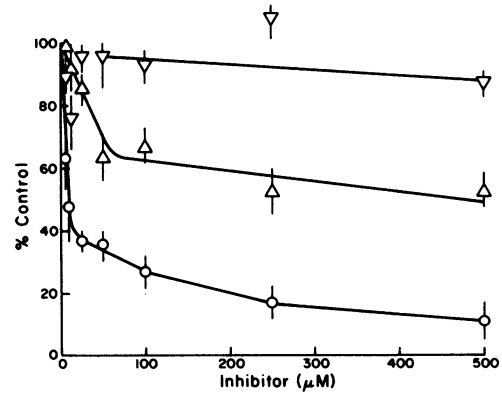

FIG. 8. Effects of amino acids and analogs on the rate of uptake of glutamine. Effect on transport of 50 μM [^{14}C]glutamine by methionine or alanine (○); glutamate, histidine, asparagine, or γ-glutamylhydrazide (△); and MSX (▽). Values are presented as the percentage of glutamine transport in the absence of other compounds and represent means of three to four experiments for each compound.

tion-dependent inhibition of MSX transport, glutamine being more effective. These latter results can be explained by differences in the affinity constants for transport. However, coupled with the competitive versus mixed type of inhibition of glutamine and glutamate transport by each other, the results also imply that glutamate may be transported by more than one system, one of which may share a common component with the glutamine and MSX systems. We are not sure why MSX did not inhibit glutamine transport even though the reciprocal inhibition was extensive. It is probable that a common component would react more strongly with a natural substrate than it would with an analog.

Based on the results of additional experiments on inhibiting transport of glutamine and MSX, the common component also appears to bind or transport alanine, methionine, histidine, asparagine, aspartate, citrulline, and γ-glutamylhydrazide, but in classes with different affinities. Aromatic amino acids and arginine, as well as nucleic acid bases and 2-oxoglutarate, did not inhibit glutamine or MSX transport. The interaction of amino acids from such a variety of classes with a single component is unusual among procaryotes. General amino acid transport systems have been demonstrated in yeasts (9). Enteric bacteria have both specific systems and ones capable of transporting classes of amino acids such as acidic, basic, neutral, and aromatic (3, 19, 21, 23). The unicellular cyanobacterium *A. nidulans* has transport systems resembling the α-aminoisobutyric acid–alanine–glycine and leucine-isoleucine-valine systems of *E. coli* (14).

The maximal rates of glutamine and glutamate transport by *A. variabilis* were similar and nearly threefold higher than that of glutamine transport by *A. cylindrica* (22). Using the maximal rates of transport at saturating substrate concentrations, two nitrogens per mol of glutamine and one nitrogen per mol of glutamate, and assuming that Chl *a* and nitrogen are 1 and 10%, respectively, of the dry weight of a cyanobacterial cell (27), we calculated that exogenous glutamine and glutamate could support generation times of 30 and 66 h, respectively, in the absence of any other nitrogen source. However, glutamate was inhibitory to the growth of *A. variabilis* at 0.5 mM (more than fourfold lower than saturation of the glutamate transport systems). Moreover, under an atmostphere of Ar-O_2-CO_2, *A. variabilis* did not grow in the presence of a concentration of glutamine (5 mM) that saturates the transport systems. Although high concentrations of glutamine (25 mM) appeared to support growth under Ar-O_2-CO_2, we doubt that glutamine was the actual nitrogen source. We assume, based on the report of Gross et al. (10), that the 3.7% glutamine lost per day from uninoculated growth medium gave rise to ammonium at concentrations sufficient to support growth. In fact, a growth curve can be calculated that matches almost exactly the experimental curve obtained with 25 mM glutamine based on the biomass at each time point, the projected concentrations of ammonium released from glutamine, and the previously stated assumptions of Chl *a* and nitrogen content. If glutamine and glutamate could have served as nitrogen sources, MSX-inhibited growth should have recovered more rapidly when they were subsequently added, but it did not. Such recovery does occur in similar experiments with *S. typhimurium* (25). The growth that was observed when MSX and glutamine or glutamate were added simultaneously can be explained by inhibition of MSX transport.

Why *A. variabilis* is apparently unable to grow with glutamine as the sole source of nitrogen is unknown. With respect to the primary route of ammonium assimilation in *A. variabilis* (16), glutamine must be metabolized to glutamate. However, the relationship between measured rates of short-term glutamine transport and glutamine-dependent growth of *A. variabilis* is analogous to that of *S. typhimurium*, where the measured rate of histidine transport appears to be sufficient to support growth but, in fact, does not. Rare mutants able to utilize histidine as a nitrogen source have a mutation in a promoter that allows the histidine-utilizing genes to be induced (18); histidine transport is not affected. An additional example of this type of relationship is provided by *hisP* mutants of *S. typhimurium*; calculations show that arginine transport is sufficient to support growth, but does not (12).

Two results indicate that glutamine synthetase of MSX-resistant strain 5-1 differed from that of wild-type cells. First, 50 μM MSX inhibited in vitro activity 12% in strain 5-1 compared with 88% in wild-type cells. It must be emphasized that, in vivo, MSX eventually inhibited greater than 89% of the glutamine synthetase activity of strain 5-1, and this was probably the reason why strain 5-1 grew as a petite colony under the selection conditions. Second, affinity constants of glutamine synthetase were different in strain 5-1. Relative to wild-type cells, the enzyme from strain 5-1 had less affinity for glutamine in the transferase reaction and greater affinity for glutamate in the biosynthetic reaction.

The presence of a shared transport component for a variety of amino acids, the instability of glutamine over long incubation times, and the apparent inability to grow with glutamine as the sole source of nitrogen may explain why we and others have been unable to isolate glutamine auxotrophs of *A. variabilis* and other dinitrogen-

fixing cyanobacteria in complex segregation media. The altered glutamine synthetase activities of strain 5-1 show that the structural gene for this enzyme is sensitive to mutation, and the resulting mutants can be isolated. We are currently attempting to isolate revertants of MSX-resistant strain 7-2, as well as spontaneous mutants of wild-type cells, in a positive selection for growth on low concentrations of glutamine under Ar-O_2-CO_2. The selection of strains with elevated levels of glutamine transport would increase the probability of isolating glutamine auxotrophs in cyanobacteria.

ACKNOWLEDGMENTS

This work was supported in part by the California Agricultural Experiment Station under project no. CAD*-3620-H and National Science Foundation grant PCM 79-04136.

We thank K. Wycoff for measurements of MSX transport and C. S. Enderlin, C. M. Joseph, G. Cangelosi, and C. P. Wolk for helpful comments during preparation of this manuscript.

LITERATURE CITED

1. **Adams, D. G., and N. G. Carr.** 1981. The developmental biology of heterocyst and akinete formation in cyanobacteria. Crit. Rev. Microbiol. **9**:45–100.
2. **Allen, M. B., and D. I. Arnon.** 1955. Studies on nitrogen-fixing blue-green algae. I. Growth and nitrogen fixation by *Anabena cylindrica* Lemm. Plant Physiol. **30**:366–372.
3. **Ames, G. F.** 1964. Uptake of amino acids by *Salmonella typhimurium*. Arch. Biochem. Biophys. **104**:1–18.
4. **Betteridge, P. R., and P. D. Ayling.** 1972. The role of methionine transport mutants in resistance to methionine sulfoximine in *Salmonella typhimurium*. Mol. Gen. Genet. **138**:41–52.
5. **Braun, A. C., and H. N. Wood.** 1962. On the activation of certain essential biosynthetic systems in cells of *Vinca rosea L.* Proc. Natl. Acad. Sci. U.S.A. **48**:1776–1782.
6. **Brenchley, J. E.** 1973. Effect of methionine sulfoximine and methionine sulfone on glutamate synthesis in *Klebsiella aerogenes*. J. Bacteriol. **114**:666–673.
7. **Currier, T. C., J. F. Haury, and C. P. Wolk.** 1977. Isolation and preliminary characterization of auxotrophs of a filamentous cyanobacterium. J. Bacteriol. **129**:1556–1562.
8. **Elliot, W. H.** 1955. Glutamine synthetase. Methods Enzymol. **2**:337–342.
9. **Grenson, M., C. Hou, and M. Crabeel.** 1970. Multiplicity of the amino acid permeases in *Saccharomyces cerevisiae*. IV. Evidence for a general amino acid permease. J. Bacteriol. **103**:770–777.
10. **Gross, M., A. J. Cooper, and A. Meister.** 1976. On the utilization of L-glutamine by glutamate dehydrogenase. Biochem. Biophys. Res. Commun. **70**:373–380.
11. **Haselkorn, R., B. Mazur, J. Orr, D. Rice, N. Wood, and R. Rippka.** 1980. Heterocyst differentiation and nitrogen fixation in cyanobacteria, p. 259–278. *In* W. E. Newton and W. H. Orme-Johnson (ed.), Nitrogen fixation, vol. 2. University Park Press, Baltimore, Md.
12. **Kustu, S. G., and G. F. Ames.** 1973. The *hisP* protein, a known histidine transport component in *Salmonella typhimurium*, is also an arginine transport component. J. Bacteriol. **116**:107–113.
13. **Lee-Kaden, J., and W. Simonis.** 1979. Photosystem I and photophosphorylation dependent leucine incorporation in the blue-green alga *Anacystis niculans*. Plant Cell Physiol. **20**:1179–1190.
14. **Lee-Kaden, J., and W. Simonis.** 1982. Amino acid uptake and energy coupling dependent on photosynthesis in *Anacystis nidulans*. J. Bacteriol. **151**:229–236.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurements with the Folin phenol reagent. J. Biol. Chem. **93**:265–275.
16. **Meeks, J. C.** 1981. Radiotracer studies of nitrogen metabolism in cyanobacteria, p. 269–294. *In* J. W. Root and K. A. Krohn (ed.), Recent advances in chemistry series, no. 197, short-lived radionuclides in chemistry and biology. American Chemical Society, Washington, D.C.
17. **Meeks, J. C., K. L. Wycoff, J. S. Chapman, and C. S. Enderlin.** 1983. Regulation of expression of nitrate and dinitrogen assimilation by *Anabaena* species. Appl. Environ. Microbiol. **45**:1351–1359.
18. **Meiss, H. K., W. J. Brill, and B. Magasanik.** 1969. Genetic control of histidine degradation in *Salmonella typhimurium* LT-2. J. Biol. Chem. **244**:5382–5391.
19. **Piperno, J. R., and D. L. Oxender.** 1968. Amino acid transport systems in *Escherichia coli*. J. Biol. Chem. **243**:5914–5920.
20. **Premakumar, R., G. J. Sorger, and D. Gooden.** 1980. Repression of nitrate reductase in *Neurospora* studied by using L-methionine-DL-sulfoximine and glutamine auxotroph gln-1b. J. Bacteriol. **143**:411–415.
21. **Rosen, B. P.** 1971. Basic amino acid transport in *Escherichia coli*. J. Biol. Chem. **246**:3653–3662.
22. **Rowell, P., S. Enticott, and W. D. P. Stewart.** 1977. Glutamine synthetase and nitrogenase activity in the blue-green alga *Anabaena cylindrica*. New Phytol. **79**:41–54.
23. **Schellenberg, G. S., and C. Furlong.** 1977. Resolution of the multiplicity of the glutamate and asparate transport systems in *Escherichia coli*. J. Biol. Chem. **252**:9055–9064.
24. **Stadtman, E. R., A. Ginsburg, J. E. Ciardi, J. Yeh, A. B. Henning, and B. M. Shapiro.** 1970. Multiple molecular forms of glutamine synthetase produced by enzyme catalyzed adenylylation and deadenylylation reactions. Adv. Enzyme Regul. **8**:99–118.
25. **Steimer-Veale, K., and J. E. Brenchley.** 1974. Characterization of *Salmonella typhimurium* strains sensitive and resistant to methionine sulfoximine. J. Bacteriol. **119**:848–856.
26. **Thomas, J., J. C. Meeks, C. P. Wolk, P. W. Shaffer, S. M. Austin, and W.-S. Chien.** 1977. Formation of glutamine from [^{13}N]ammonia, [^{13}N]dinitorgen, and [^{14}C]glutamate by heterocysts isolated from *Anabaena cylindrica*. J. Bacteriol. **129**:1545–1555.
27. **Wolk, C. P.** 1973. Physiology and cytological chemistry of blue-green algae. Bacteriol. Rev. **37**:32–101.