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SOME PROPERTIES OF GLUTAMINE SYNTHETASE AND GLUTAMATE SYNTHASE FROM *DERXIA GUMMOSA*

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Key Word Index—*Derxia gummosa*; Azotobacteraceae; nitrogen-fixing bacteria; ammonia assimilation; glutamine synthetase; glutamate synthase.

Abstract—The incorporation of ^{15}N into washed cells of *Derxia gummosa* from labelled-$(NH_4)_2SO_4$ and -KNO_3 respectively was inhibited by both L-methionine-DL-sulphoximine and azaserine. Glutamine synthetase purified to homogeneity from this bacterium had a molecular weight of 708 000 and was composed of 12 similar subunits each of 59 000. The enzyme assayed by γ-glutamyltransferase method had K_m values for L-glutamine and hydroxylamine of 12.5 and 1.2 mM, respectively. Optimal pH values for adenylylated and deadenylylated forms were pH 7.0 and pH 8.0, respectively. The adenylylated enzyme was deadenylylated by treatment with snake venom phosphodiesterase. The inhibitions by both glutamate and ammonia were competitive. The activity was markedly inhibited by L-methionine-DL-sulphoximine, alanine, glycine and serine and to a lesser extent by aspartate, phenylalanine and lysine. Various tri-, di- and mono-phosphate nucleotides, organic acids (pyruvate, oxalate and oxaloacetate) were also inhibitory. Glutamate synthase purified 167-fold had specific requirements for NADH, L-glutamine and 2-ketoglutarate. The K_m values for NADH, glutamine and 2-ketoglutarate were 9.6, 270 and 24 μM respectively. Optimal pH range was 7.2–8.2. The enzyme was inhibited by azaserine, methionine, aspartate, AMP, ADP and ATP.

INTRODUCTION

The assimilation of ammonia in plants and microorganisms is carried out either via glutamine synthetase (GS)/glutamate synthase (GOGAT) or glutamate dehydrogenase (GDH) pathway [1]. The GS/GOGAT route usually operates at low levels of ammonia whereas GDH is important at higher concentrations. In microorganisms and plants, nitrate is reduced to ammonia which is then assimilated into cell nitrogen.

Regulation of GS has been the subject of several papers and reviews [2–6]. The enzyme has been characterized in various bacteria [2, 7–11], higher plants [1] and animals [12]. Glutamine synthetase has been purified to homogeneity from *Rhodopseudomonas palustris* and *R. capsulata* [9, 13]. The purified enzyme is a dodecamer and is regulated by adenylylation/deadenylylation which is dependent on the ammonia concentration in cells. AMP attached to glutamine synthetase in its adenylylated state can be removed by treating the enzyme with snake venom phosphodiesterase resulting in a deadenylylated active enzyme [14, 15].

Glutamate synthase is NADPH-dependent in *Azospirillum brasilense* [16] and in purple non-sulphur photosynthetic bacteria [17–20] and NADH-dependent in purple and green sulphur bacteria [10, 17, 21]. Glutamate synthase has been purified from a variety of bacteria and found to be an iron–sulphur flavoprotein [10, 16, 22, 23].

Derxia gummosa, an obligate aerobic bacterium [24, 25] fixes dinitrogen at low oxygen tensions [26, 27]. It can grow autotrophically with H_2 as the energy source in the absence of an organic carbon substrate [28]. Little is known about the way in which ammonia is assimilated into amino acids in this bacterium. We now report on studies on the assimilation of ^{15}N-labelled compounds, i.e. $(^{15}NH_4)_2SO_4$ and $K^{15}NO_3$, into washed cells of *Derxia*. Some properties of purified glutamine synthetase and glutamate synthase are also described.

RESULTS

Purification of glutamine synthetase

The enzyme was purified 128-fold with a 23% recovery as shown in Table 1. Washed cells in 10 mM Tris–HCl buffer (pH 7.0) containing 1 mM $MnCl_2$ (Buffer A) disrupted by two passages through a French Pressure Cell were centrifuged at 20 000 g for 15 min. The supernatant (S_{20}; Fraction 1, Table 1) was adsorbed onto a 1.6 × 12 cm column of Blue Sepharose CL-6B, which had been equilibrated with Buffer A. The column was washed with the same buffer until the OD_{280} was close to zero and then eluted with the buffer containing 2 mM ADP. Active fractions were pooled (Fraction 2, Table 1) and then loaded onto a DE-52 column (1.5 × 15 cm) pre-equilibrated with buffer A. The column was developed with a linear gradient of NaCl, 0–0.5 M in 300 ml Buffer A, at a flow rate of 50 ml/hr. Active fractions were pooled (Fraction 3, Table 1) and dialysed against 3 l Buffer A for 15 hr at 4°. The dialysed Fraction 3 was then loaded onto a second Blue Sepharose CL-6B column (1.2 × 10 cm), which had been equilibrated with Buffer A and purified as described above. Active fractions were pooled and dialysed as described above to remove ADP. The purified enzyme could be stored at 4° for two months with little loss of activity.

Table 1. Purification of glutamine synthetase

Fraction	Total protein (mg)	Total activity (units*)	Recovery (%)	Specific activity (units/mg protein)	Purification (fold)
1. Supernatant left after centrifuging at 20000 *g*/15 min	1064	279	100	0.26	1
2. Fraction 1, affinity chromatography, Blue Sepharose CL-6B column (1.6 × 12 cm), eluted with 2 mM ADP in 10 mM Tris–HCl buffer containing 1 mM $MnCl_2$ (pH 7.0)	15	204	73	13.4	51
3. Fraction 2 loaded onto a DE-52 column (1.5 × 15 cm) eluted with 0–0.5 M NaCl linear gradient (300 ml 10 mM Tris–HCl buffer, containing 1 mM $MnCl_2$, pH 7.0)	10.4	192	69	18.5	71
4. Fraction 3, dialysed against 10 mM Tris–HCl buffer, containing 1 mM $MnCl_2$ (pH 7.0) for 15 hr and then loaded onto a Blue Sepharose CL-6B column, eluted with 2 mM ADP in 10 mM Tris–HCl buffer containing 1 mM $MnCl_2$ (pH 7.0)	1.9	65	23	33.5	128

*1 unit activity = 1 nmol γ-glutamylhydroxamate produced/min.
The enzyme activity assayed as described in Experimental.

Purification of glutamate synthase

The enzyme was purified 167-fold with an 82% recovery as shown in Table 2. Washed cells in 50 mM Tris–HCl buffer (pH 7.5) (Buffer B) disrupted by two passages through a French Pressure Cell were centrifuged at 10000 *g* for 15 min. The supernatant (S_{10}; Fraction 1, Table 2) was centrifuged at 144000 *g* for 90 min in a Beckman L8-70 ultracentrifuge (rotor 70.1 Ti). The supernatant (S_{144}; Fraction 2, Table 2) was treated with saturated ammonium sulphate solution and the precipitate between 35–50% saturation was dissolved in 5 ml Buffer B and desalted by passing through a Sephadex G-25 column (2.5 × 15 cm) which had been equilibrated with Buffer B. The desalted fraction (Fraction 3, Table 2) was then loaded onto a Blue Sepharose CL-6B column (1.8 × 12 cm) which had been equilibrated with Buffer B. The column was eluted with a linear gradient, 100 ml 0–0.5 mM NADH in Buffer B. Active fractions were pooled and stored under argon at 4°.

Effects of L-*methionine*-DL-*sulfoximine* (*MSX*) *and azaserine on the incorporation of* ^{15}N-*labelled compounds into washed cells*

The results in Table 3 show that MSX and azaserine markedly inhibited ^{15}N incorporation from $(^{15}NH_4)_2SO_4$ by 52 and 60%, respectively and the incorporation of ^{15}N from $K^{15}NO_3$ was inhibited by 33 and 36% respectively.

Table 2. Purification of glutamate synthase.

Fraction	Total protein (mg)	Total activity (units*)	Recovery (%)	Specific activity (units/mg protein)	Purification (fold)
1. S_{10} supernatant after centrifuging cell-extracts at 10000 *g* for 15 min	1260	140	100	0.096	1
2. S_{144}, supernatant after centrifuging S_{10} at 144000 *g* for 90 min	950	138	100	0.145	1.5
3. Fraction 2 precipitated between 35–50% saturation of $(NH_4)_2SO_4$. Desalted by passing through a Sephadex G-25 column (15 × 2.5 cm)	550	130	93	0.236	2.5
4. Fraction 3 loaded onto a Blue Sepharose CL-6B column (15 × 1.5 cm), eluted with 0–0.5 mM NADH linear gradient in 50 mM Tris–HCl buffer (pH 7.5).	7.3	116	82	16.0	167

*1 unit = 1 μmol NADH oxidized/min.
The enzyme was assayed as described in Experimental.

Table 3. Effects of MSX and azaserine on the incorporation of ^{15}N from $(^{15}NH_4)_2SO_4$ and $K^{15}NO_3$ respectively into washed cells

N-source in culture	^{15}N-Compound supplied to washed cells	^{15}N Incorporation* (μg N/mg cell nitrogen)		
		Without inhibitor	MSX (2 mM)	Azaserine (2 mM)
10 mM NH_4Cl	5 mM $(^{15}NH_4)_2SO_4$	113	54 (52)	45 (60)
10 mM $NaNO_3$	10 mM $K^{15}NO_3$	147	98 (33)	94 (36)

*Values in brackets indicate % inhibition.
Details are given in Experimental.

Molecular weight and subunits of glutamine synthetase

The purified enzyme (Fraction 4, Table 1) produced one band in 0.1% (w/v) SDS polyacrylamide gel electrophoresis (Fig. 1). By comparison with known standard proteins, the molecular weight of this band was calculated to be 59 000. In linear gradient polyacrylamide gel electrophoresis, a maximum molecular weight of 708 000 was recorded for the same preparation, indicating that the enzyme was composed of 12 subunits (Table 4). Protein bands at molecular weights of 237 000 and 135 000 were also recorded in the pore gradient gel.

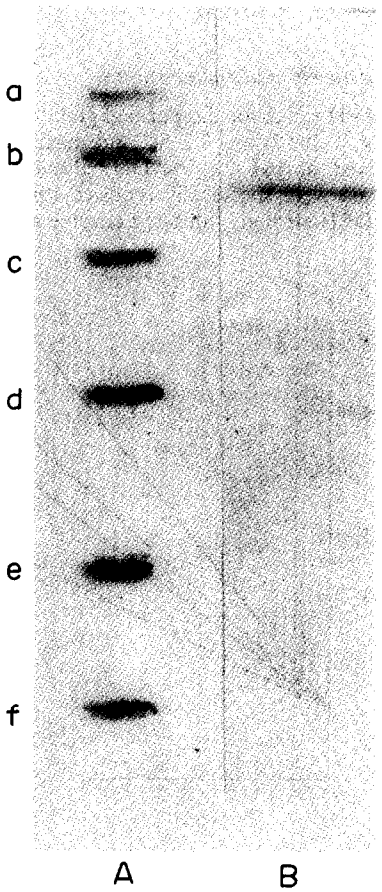

Fig. 1. SDS-polyacrylamide gel electrophoresis of purified glutamine synthetase. (A) Protein molecular markers. a, Phosphorylase *b* (94 K); b, albumin (67 K); c, ovalbumin (43 K); d, carbonic anhydrase (30 K); e, trypsin inhibitor (20 K) and f, α-lactalbumin (14 K); (B) purified enzyme (Fraction 4, Table 1).

Some properties of the purified glutamine synthetase

Optimal γ-glutamyltransferase activity was observed at pH 7.0 with the adenylylated enzyme (Fraction 4, Table 1). A deadenylylated enzyme, prepared by incubating with snake venom phosphodiesterase, had an optimal activity at pH 8.0.

No activity was observed when either glutamine, hydroxylamine, ADP, arsenate or Mn^{2+} was omitted from the reaction mixture. The K_m values for glutamine and hydroxylamine were 15 and 1.9 mM, respectively analysed by a computer method [29]. Various divalent cations including Mn^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+} and Hg^{2+} were tested in the γ-glutamyltransferase assay but only Mn^{2+} (> 5 μM) resulted in activity.

Adenylylation/deadenylylation

The transferase activity of the purified glutamine synthetase (Fraction 4, Table 1) was inhibited (82%) by 60 mM $MgCl_2$ and an adenylylation of 10 subunits was calculated for this preparation by the formula of Shapiro and Stadtman [2]. Incubation of the adenylylated enzyme with snake venom phosphodiesterase decreased the Mg^{2+} inhibition of transferase activity (Fig. 2), indicating that the enzyme was deadenylylated.

Inhibitor studies with glutamine synthetase

γ-Glutamyltransferase activity was inhibited competitively by both the substrates of the biosynthetic assay, viz. NH_4Cl and L-glutamate as shown in Fig. 3A,B: MSX

Table 4. Molecular weight determinations of purified glutamine synthetase (Fraction 4, Table 1)

Electrophoresis conditions	Bands on gel in order of size	Molecular weight ($\times 10^{-3}$)	Proposed subunit assembly
Pore gradient gel electrophoresis, non-denaturing conditions (4–30% (w/v) polyacrylamide gel)	a	708	12
	b	237	4
	c	135	2
SDS-PAGE		59	1

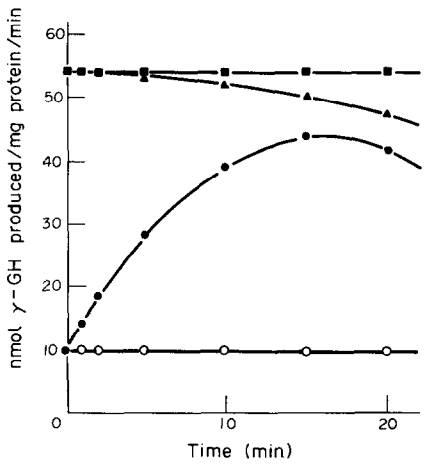

Fig. 2. Effects of incubating purified glutamine synthetase (Fraction 4, Table 1) with snake venom phosphodiesterase. Experimental details as described in Experimental. ■, Enzyme assayed without $MgCl_2$. ○, Enzyme assayed in the presence of 60 mM $MgCl_2$. ▲, Enzyme incubated with phosphodiesterase and then assayed without $MgCl_2$. ●, Enzyme incubated with phosphodiesterase and then assayed in the presence of 60 mM $MgCl_2$.

inhibited γ-glutamyltransferase activity with a K_i value of 4 mM, calculated from a double reciprocal plot. The transferase activity was also markedly inhibited by alanine, serine and glycine and to a lesser extent, by aspartate, phenylalanine and lysine each at 5 mM final concentration (Table 5). Little or no inhibition was observed for asparagine, cysteine, arginine, iso-leucine, tryptophan, threonine, proline, histidine and valine each at 5 mM. Various combinations of amino acids which inhibited transferase activity singly resulted in cumulative inhibition calculated by the method by Woolfolk and Stadtman [30].

Table 5. Effects of various L-amino acids on γ-glutamyltransferase activity of glutamine synthetase (Fraction 4, Table 1)

Amino acids (5 mM)	nmol γ-GH produced /min/mg protein	Inhibition (%)
None	54	—
Alanine	6	90
Glycine	25	53
Serine	27	50
Aspartate	40	27
Phenylalanine	45	17
Lysine	48	12

Amino acids were at 5 mM (final concentration). The enzyme activity was assayed as described in Experimental.

Tri-phosphate nucleotides, viz. ATP, UTP, GTP, CTP and ITP (each at 5 mM) inhibited enzyme activity to a greater extent than di-phosphate nucleotides, whereas mono-phosphate nucleotides (5 mM) were only slightly inhibitory (< 20% inhibition). Various combinations of nucleotides gave cumulative inhibition as did combinations of nucleotides with amino acids. Pyruvate, oxalate and oxaloacetate inhibited enzyme activity by 71, 76 and 50% respectively at 10 mM.

Some properties of glutamate synthase

Optimal pH range for the purified glutamate synthase (Fraction 4, Table 2) was 7.2–8.2 and maximum activity was observed at pH 7.5. NADH, glutamine and 2-ketoglutarate were specifically required for enzyme activity. NADPH strongly inhibited enzyme activity (50%

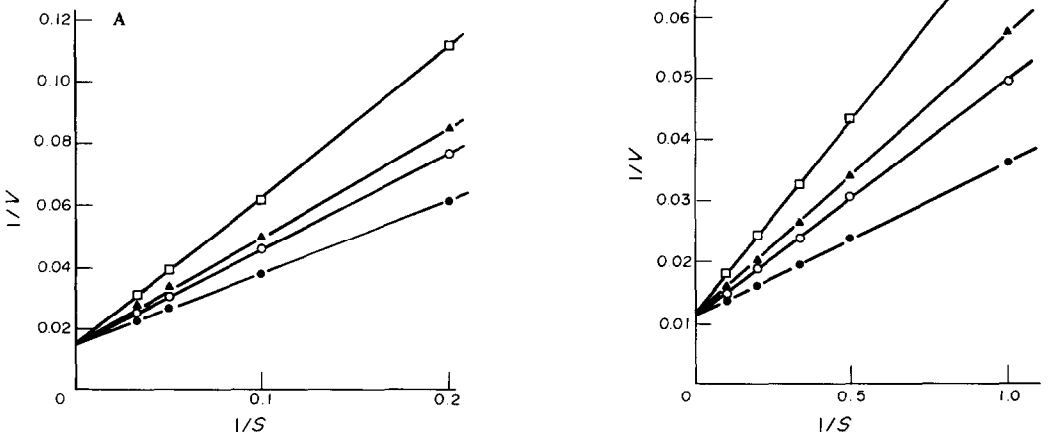

Fig. 3. (A) Lineweaver–Burk plots of the effects of various concentrations of glutamate on activity of purified glutamine synthetase (Fraction 4, Table 1). Enzyme activity assayed as described in Experimental. ●, No glutamate; ○, 10 mM glutamate; ▲, 20 mM glutamate; □, 40 mM glutamate; $1/S$, (glutamine mM)$^{-1}$; $1/V$, (nmol γ-glutamylhydroxylamate produced/min·mg protein)$^{-1}$. (B) Lineweaver–Burk plots of the effects of various concentrations of ammonium chloride on activity of purified glutamine synthetase (Fraction 4, Table 1). Enzyme activity was assayed as described in the Experimental. ●, No NH_4Cl; O, 5 mM NH_4Cl; ▲, 10 mM NH_4Cl; □, 20 mM NH_4Cl; $1/S$ (hydroxylamine mM)$^{-1}$ $1/V$ (nmol γ-glutamylhydroxamate produced/min·mg protein)$^{-1}$.

inhibition at 200 μM). The apparent K_m values were for NADH, 9.6 μM, for glutamine, 270 μM and for 2-ketoglutarate, 24 μM, calculated from double reciprocal plots.

Inhibitor studies with glutamate synthase

Azaserine inhibited glutamate synthase with a K_i value of 0.8 mM, calculated from a double reciprocal plot. Methionine and aspartate markedly inhibited glutamate synthase activity at 5 and 10 mM, respectively (Table 6). Alanine, glycine, serine, lysine, proline, cysteine, valine, leucine, iso-leucine, threonine, phenylalanine, histidine, tryptophan, asparagine and arginine did not inhibit enzyme activity at either 5 or 10 mM. ATP, ADP and AMP markedly inhibited enzyme activity at 10 mM and ADP only at 5 mM (Table 6). No inhibition of the enzyme was observed with UTP, ITP and GTP.

DISCUSSION

The glutamine synthetase/glutamate synthase pathway is the major route whereby ammonia is assimilated in many heterotrophic and N_2-fixing bacteria and in blue–green algae [6, 10, 17, 31, 32]. The results presented herein for *Derxia gummosa* are in agreement with these findings; thus the incorporation of ^{15}N from $(^{15}NH_4)_2SO_4$ and $K^{15}NO_3$ respectively into washed cells was markedly depressed by MSX and azaserine, the inhibitors of glutamine synthetase and glutamate synthase respectively. Glutamine synthetase and glutamate synthase purified from this bacterium have similar physical and biochemical properties to those reported for these enzymes from other microorganisms [5, 8–10].

Thus glutamate synthase from *Derxia* is composed of 12 similar subunits as for the enzyme from other microorganisms [8, 9, 11]. The molecular weight (708K) of the enzyme is comparable with that for *Rhodopseudomonas* (670K) [9] and *Rhizobium* (720K) [11], but higher than those for *Escherichia coli* (590K) [2] and *Nitrosomonas* (440K) [8] and twice that for rat liver (352K) [33]. In non-denaturing pore gradient PAGE, two protein bands of the purified enzyme with molecular weights of 237K and 135K were recorded besides the one corresponding to the dodecamer. These are most probably 4- and 2-subunit assemblies, indicating that the subunit binding is not very strong. Protein bands corresponding to monomer and hexamer assemblies were not detected in these gels as were reported for the enzyme from *Rhodopseudomonas palustris* [9].

Table 6. Effects of L-amino acids and nucleotides on glutamate synthase (Fraction 4, Table 2)

Amino acids or nucleotides	% Inhibition	
	5 mM	10 mM
Methionine	37	47
Aspartate	49	60
Glutamate	15	24
ATP	17	61
ADP	38	84
AMP	10	37

Amino acids and nucleotides were each at 5 mM and 10 mM final concentrations. Results are expressed as % inhibition compared with the control.

The requirements for Mn^{2+} and arsenate for activity indicate that the production of γ-glutamylhydroxamate is catalysed by γ-glutamyltransferase type reaction rather than either aminohydrase [34] or glutaminase [3] reactions. Mn^{2+} is specifically required for transferase activity by the *Derxia enzyme*. By contrast, in *Nitrosomonas*, Mn^{2+} can be partly replaced by Cu^{2+}, Fe^{2+}, Mg^{2+} and Co^{2+} respectively [8], in *Chlorobium*, Mg^{2+}, Fe^{2+} and Ni^{2+} are effective [10] and in *Rhizobium*, Ca^{2+}, Mg^{2+}, Ni^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} activate the enzyme [11].

The inhibition of γ-glutamyltransferase activity by both substrates of the biosynthetic reaction, viz. L-glutamate and ammonium chloride, is competitive for glutamine and hydroxylamine, respectively. The competitive inhibition by ammonium chloride indicates that hydroxylamine binds to the ammonia site on glutamine synthetase. This contrasts with the data for *Nitrosomonas europaea* [8], *Chlorobium vibrioforme* f. *thiosulfatophilum* [10], and *Rhizobium japonicum* [11], where the inhibition by ammonium chloride is non-competitive.

The adenylylated enzyme purified from *Derxia* is deadenylylated by treatment with snake venom phosphodiesterase, in agreement with the data for the enzyme from other sources [5, 15, 35]. The two forms of enzyme have different optimal pH values; thus the adenylylated form has a lower pH optimum, in agreement with the data of Bender *et al.* [36].

The purified glutamine synthetase from *Derxia* is sensitive to various feedback inhibitors, viz. amino acids, nucleotides and organic acids. The enzyme is inhibited by alanine, glycine, serine, aspartate and lysine, as in *Nitrosomonas europaea* [8]. Nucleotide inhibition of transferase activity was in the order tri- > di- > monophosphate nucleotides as for *Nitrosomonas europaea* and *Rhizobium japonicum* [8, 11]. Inhibitions by various combinations of amino acids, and with nucleotides were cumulative for the enzyme from *Derxia*, in accord with the results for other bacteria [8, 10, 11, 34]. Cumulative inhibitions by various combinations of nucleotides were also observed. These results indicate that various nucleotides as well as various amino acids have separate binding sites on the enzyme for each of the feed-back inhibitors [37].

Glutamate synthase has been purifed from a variety of bacteria and shown to be an iron–sulphur flavoprotein [22, 38] composed of two dissimilar subunits [16, 23]. The purified enzyme from *Derxia* has a high affinity for substrates. Thus the K_m values for NADH (9.6 μM), 2-ketoglutarate (24 μM) and glutamine (270 μM) are lower than those for *Chlorobium* (13.5, 270 and 769 μM respectively [10]. These results contrast with those for *Klebsiella pneumoniae* [39] and *Thiobacillus thioparus* [23] in which NH_4Cl can partly replace glutamine for activity. The wide pH range for optimal activity is in accord with that for *Thiobacillus* [23].

Methionine, ATP, ADP and AMP inhibited glutamate synthase, in agreement with the data for *Chlorobium* [10]. However, serine, leucine, and glycine which inhibited glutamate synthase from *Chlorobium* [10] did not inhibit the *Derxia* enzyme.

EXPERIMENTAL

Materials. A culture of *Derxia gummosa* was kindly supplied by Professor Y. T. Tchan, Department of Microbiology, University of Sydney, Australia. Various amino acids, nucleotides, γ-glutamylhydroxamate, organic acids, L-methionine-DL-sulphoximine, azaserine and bovine serum albumin were purchased from Sigma. Blue Sepharose CL-6B, polyacrylamide gradient gel and molecular weight calibration kits for various protein markers were purchased from Pharmacia. Snake venom phosphodiesterase was obtained from Worthington and DE-52 cellulose ion exchanger from Whatman. All other chemicals used in these investigations were of analytical grade.

Growth conditions. The culture medium used was as follows (per l): K_2PO_4, 2 g; KH_2PO_4, 1 g; glucose, 10 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.02 g; Fe·Na·EDTA, 0.02 g; $Na_2MoO_4 \cdot 2H_2O$, 2 mg; $MnSO_4 \cdot H_2O$, 3.5 mg; H_3BO_3, 2.8 mg; $CuSO_4 \cdot 5H_2O$, 0.08 mg and $ZnSO_4 \cdot 7H_2O$, 0.24 mg. Phosphate and glucose were autoclaved separately in 10 × concentrated solutions and were added aseptically to the rest of the medium after cooling. Cells were grown in 10 l. flasks containing 6 l. of the 10 mM NH_4Cl medium. The culture solution inoculated with 5% (v/v) 20 hr-grown cells was then sparged with sterile air at 30° for 24 hr. Cells harvested by continuous flow centrifugation were washed twice in 10 mM Tris–HCl buffer (pH 7.0) for studies with glutamine synthetase and with 50 mM Tris–HCl buffer (pH 7.5) for glutamate synthase. In preparing cells for ^{15}N incorporation studies, 1 l. of the culture medium containing 10 mM either NH_4Cl_4 or $NaNO_3$ was dispensed into 2 l. flasks. The cultures were inoculated with 5% (v/v) inoculum from cultures grown with either NH_4Cl (10 mM) or $NaNO_3$ (10 mM) and then placed on a gyrotory shaker (120 rpm) at 30° for 20 hr. Cells harvested at 10 000 g for 20 min (GSA rotor) were washed twice with 100 mM K-phosphate buffer (pH 7.5).

Incorporation of ^{15}N into washed cells from ^{15}N-labelled compounds. Washed cells, grown in media containing either 10 mM NH_4Cl or 10 mM $NaNO_3$, were suspended in fresh culture medium without combined nitrogen. Cell suspensions (200 mg cell wet weight/5 ml) were incubated for 1 hr with and without MSX and azaserine respectively (2 mM final concentration) and then for a further 2 hr with either $(^{15}NH_4)_2SO_4$ (5.25% ^{15}N, 5 mM final concn) for NH_3-grown cells or $K^{15}NO_3$ (30.8% ^{15}N, 10 mM final concn) for nitrate-grown cells. The cells were then centrifuged at 20 000 *g* for 10 min and washed 3 × 100 mM K-phosphate buffer (pH 7.5). Samples were transferred into 100 ml-micro Kjeldahl flasks for acid digestion and then aliquots for ^{15}N enrichment were assayed in a 602E mass spectrometer (ISOMASS, Cheshire, U.K.) [40].

Enzyme assays. Glutamine synthetase was assayed at 37° by γ-glutamyltransferase method [2] except that 0.3 mM $MnCl_2$ was used. γ-Glutamylhydroxamate was used as a standard. Glutamate synthase was assayed at 30° by measuring the initial rates of oxidation of NADH at 340 nm in a 1 cm cuvette in a recording spectrophotometer (Varian, model 635, Melbourne, Victoria, Australia). The reaction mixture in 3 ml contained NADH, 200 μM; glutamine, 3 mM; 2-ketoglutarate, 1 mM; Tris–HCl buffer (pH 7.5), 50 mM and 0.1 ml enzyme (10 μg protein). The reactions were started by adding 2-ketoglutarate.

Polyacrylamide gel electrophoresis (PAGE). Native PAGE was carried out in a 4–30% linear gradient polyacrylamide gel. The electrode buffer was 0.09 M Tris, 0.08 M boric acid and 0.93 g/l Na_2 EDTA (pH 8.4). Electrophoresis was carried out at 150 V for 16 hr. Thyroglobin (669K), ferritin (440K), catalase (232K), LDH (140K) and albumin (67K) were used as markers. The molecular weight of enzyme subunits was determined by discontinuous SDS PAGE [41] with the following proteins as markers: phosphorylase *b* (94K), albumin (67K), ovalbumin (43K), carbonic anhydrase (30K), trypsin inhibitor (20.1K) and α-lactalbumin (14.4K). 10 μg of the purified GS (Fraction 4, Table 1) was loaded into the sample well.

Deadenylylation of glutamine synthetase. Purified enzyme (120 μg Fraction 4, Table 1) was incubated at 30° with 30 μg of snake venom phosphodiesterase in Buffer A. Samples were taken at various time intervals and the enzyme assayed with and without 60 mM $MgCl_2$. Aliquots of untreated enzyme were used as controls.

Protein assay. Protein was determined by the method of Bradford [42] using bovine serum albumin as a standard protein.

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