Purification and Properties of Glutamine Synthetase from *Methylococcus capsulatus* (Bath)

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(Received 5 July 1982; revised 13 October 1982)

The obligate methanotroph *Methylococcus capsulatus* (Bath) was grown in batch and continuous culture with ammonia, nitrate or dinitrogen as the sole nitrogen source. Cell-free extracts from these cultures all contained the ammonia assimilation enzyme glutamine synthetase. During growth on ammonia, however, the glutamine synthetase was biosynthetically inactive. Isolation of glutamine synthetase from *M. capsulatus* (Bath) resulted in a 133-fold purification with a yield of 11%. The purified enzyme had a molecular weight of 617 000 and a subunit size of 60 000 Dal. The metal ion and nucleotide specificity of glutamine synthetase was studied using both a biosynthetic assay and an artificial assay – the γ -glutamyltransferase assay. Feedback inhibition by several end-products of glutamine metabolism was observed and their effects were cumulative. Control of glutamine synthetase activity by adenylation/deadenylation was demonstrated by snake venom phosphodiesterase treatment of the enzyme isolated from cells grown with different nitrogen sources.

INTRODUCTION

Methylococcus capsulatus (Bath) is an obligate methanotroph which utilizes methane as its sole source of carbon and energy. It grows with ammonia or nitrate as nitrogen sources and can also fix dinitrogen (N_2) at reduced oxygen tensions. Both nitrate and dinitrogen are reduced to ammonia which is then assimilated into the cell via glutamine synthetase.

The most extensive studies on the enzyme glutamine synthetase (GS) have been carried out in *Escherichia coli* [reviewed by Ginsburg & Stadtman (1973) and Tempest *et al.* (1973)]. They established that GS in *E. coli* would respond to changes in extracellular concentrations of ammonia. When the ammonia concentration was high, GS rapidly lost its ability to form glutamine in the biosynthetic reaction:

although the enzyme still retained γ -glutamyltransferase activity in the artificial assay:

The ability of GS to form glutamine in the biosynthetic reaction was rapidly decreased by the attachment of adenylyl groups to the enzyme. Removal of these adenylyl groups resulted in restoration of biosynthetic activity observed during growth in medium containing low concentrations of ammonia. The reader is referred to the reviews by Tyler (1978) and Dalton (1979) for a detailed description of the adenylation/deadenylation cascade mechanism found in *E. coli* and many other organisms including *Klebsiella aerogenes* (Bender *et al.*, 1977),

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Azotobacter vinelandii (Siedel & Shelton, 1979) and *Rhodopseudomonas capsulata* (Johansson & Gest, 1977).

A second means of GS regulation is cumulative feedback inhibition by the end-products of glutamine metabolism, first observed in *E. coli* by Woolfolk & Stadtman (1964) and subsequently found to occur in several other organisms including *Pseudomonas fluorescens*, *Salmonella typhimurium* and *Clostridium pasteurianum* (Hubbard & Stadtman, 1967a) and *A. vinelandii* (Kleinschmidt & Kleiner, 1978). In organisms for which there is no evidence for control of GS by adenylation, it is thought that feedback inhibition is the main mechanism by which GS activity is regulated, e.g. as in *Bacillus subtilis* (Deuel & Stadtman, 1970), *Bacillus stearothermophilus* (Wedler *et al.*, 1976) and *Anabaena* CA (Stacey *et al.*, 1979).

Although the presence of GS has been reported in several methanotrophs (Shishkina & Trotsenko, 1979; Bailey *et al.*, 1978) very little was known about the properties and regulation of this enzyme in these organisms. This paper describes the purification and properties of GS from *M. capsulatus* (Bath). The effects of metal ions in both the GS biosynthetic and γ -glutamyl-transferase assays are reported and the control of enzyme activity by feedback inhibition and covalent modification are discussed.

METHODS

Growth of organism and preparation of cell-free extracts. *Methylococcus capsulatus* (Bath) (Whittenbury *et al.*, 1970) was grown in continuous culture in a 2.5 l fermenter (L. H. Engineering, Stoke Poges, Bucks., U.K.) on mineral salts (MS) medium (Dalton & Whittenbury, 1976) supplemented with 1 g potassium nitrate l⁻¹ (NMS medium) or 1 g ammonium chloride l⁻¹ (AMS medium). The chemostat was operated under oxygen limitation with methane [20% (v/v) in air at a flow rate of 50 ml min⁻¹] as carbon source. Temperature and pH were maintained at 45 °C and 6.8, respectively. A dilution rate (*D*) of 0.05 h⁻¹ was used and at steady-state the dry weight of the culture was 1.0–1.5 mg ml⁻¹, depending on the nitrogen source and on the growth medium.

At steady-state, bacteria were harvested by centrifugation for 10 min at 20000 *g* (4 °C). After washing once with ice-cold 25 mM-imidazole/HCl buffer pH 7.15 (buffer A), the cells were resuspended in 25 mM-imidazole/HCl buffer pH 7.15, containing 5 mM-magnesium chloride, 10 mM-sodium glutamate, 5 mM-2-mercaptoethanol and 1 mM-EDTA (buffer B). Cell extracts were prepared by two passages of the cell suspension through a pre-cooled French pressure cell at 137 MPa, followed by centrifugation at 38000 *g* for 30 min. The resulting clear red supernate was decanted off and this soluble extract was immediately frozen in pellet form by drop-wise addition into vials standing in liquid nitrogen. These pellets were stored at –80 °C.

Large quantities of whole cells of *M. capsulatus* (Bath) for preparation of cell extracts for enzyme purification were grown at 45 °C in batch cultures on MS, NMS or AMS medium in a 100 l fermenter (L. H. Engineering). The fermenter was inoculated with 10 l of a continuous culture overflow. After 18–24 h, when the cell density had reached approximately 2 mg ml⁻¹, the culture was harvested using a Westfalia continuous centrifuge (Westfalia Separator, Wolverton, Bucks., U.K.). Cell-free extracts were then prepared as described above.

Enzyme assays. Glutamine synthetase (GS) [EC 6.3.1.2; L-glutamate:ammonia ligase (ADP-forming)] was assayed using two assay systems.

(1) GS γ -glutamyltransferase activity was assayed by the method of Bender *et al.* (1977). The concentrations of reactants in this assay mixture (total volume 0.5 ml) were: imidazole/HCl buffer pH 7.15, 135 mM; manganese chloride, 0.27 mM; potassium arsenate, 25 mM; sodium-ADP, 0.36 mM; and cell extract. Any inhibitors etc. were added to the reaction mixture prior to initiation of the reaction with L-glutamine (final concentration 20 mM). After 10 min the reaction was terminated by the addition of 1.0 ml 'stopmix' containing 55 g ferric chloride, 20 g TCA and 21 ml concentrated HCl (l water)⁻¹. Samples were then centrifuged to remove any precipitate before the *A*₅₄₀ was measured. A standard curve for γ -glutamyl hydroxamate was prepared using commercial γ -glutamyl hydroxamate. (1 μ mol γ -glutamyl hydroxamate gave an *A*₅₄₀ of 0.63).

(2) GS biosynthetic activity in cell extracts was assayed by a method similar to that used by Woolfolk *et al.* (1966). The concentrations of reactants in the assay mixture were: imidazole/HCl buffer pH 7.15, 50 mM; sodium glutamate, 100 mM; ammonium chloride, 50 mM and magnesium chloride 50 mM. The total assay volume including enzyme was 0.5 ml. Any inhibitors etc. were added to the reaction mixture prior to initiation of the reaction with ATP (1 mM final concentration). After 10 min, the reaction was terminated by the addition of 1.0 ml 10% (w/v) TCA. Samples were then centrifuged to remove any precipitate, and the inorganic phosphate released from ATP was measured by the method of Baginski *et al.* (1967). All enzyme assays were done at 45 °C and enzyme activities are given as nmol product min⁻¹ (mg protein)⁻¹.

Purification of GS. All procedures were carried out at 4 °C. Elution of protein from columns was monitored at 206 nm by a Uvicord S UV monitor (LKB). *Step 1:* 100 ml of crude, soluble cell-free extract was applied to a column (4 × 30 cm) of DEAE-cellulose, previously equilibrated with buffer B. The column was eluted with a 0 to

1.2 M-sodium chloride gradient at a flow rate of 100 ml h⁻¹. Fractions (5 ml) were collected and those showing significant GS activity were pooled and dialysed to remove sodium chloride. The pooled fractions were then concentrated by ultramembrane filtration to give a final volume of 5 ml. *Step 2*: GS protein from the DEAE-cellulose chromatography step was applied to a column (3 × 100 cm) of Sepharose 4B, previously equilibrated with buffer B. The column was eluted with the same buffer at a flow rate of 20 ml h⁻¹. GS-active fractions were pooled. *Step 3*: pooled fractions from step 2 were applied to a column (4 × 20 cm) of DEAE-Sephacel, previously equilibrated with buffer B. The column was eluted with a 0 to 0.5 M-sodium chloride gradient at a flow rate of 50 ml h⁻¹. Active fractions were pooled and dialysed to remove sodium chloride. Pooled fractions were concentrated, as in step 1, to a volume of 2 ml. *Step 4*: pooled fractions from step 3 were applied to a column (1 × 30 cm) of Sephacryl S-300, previously equilibrated with buffer B. The column was eluted with the same buffer at a flow rate of 5 ml h⁻¹, and 1 ml fractions were collected. GS-active fractions were pooled and frozen by drop-wise addition into vials standing in liquid nitrogen. The resultant pellets were stored at -80 °C.

PAGE. The procedure of O'Farrell (1975) was followed for the preparation and running of 18 × 15 × 0.15 cm slab polyacrylamide gels. The discontinuous buffer system of Laemmli (1970) was used. The molecular weight of the GS subunit was estimated by SDS-PAGE. The stacking gel, resolving gel and running buffer contained SDS at 0.1% (w/v) and 2-mercaptoethanol at 10 mM. Enzyme purity was assessed with non-denaturing tube polyacrylamide gels (Gabriel, 1971), using the buffer system described above, by staining the Coomassie blue. GS was located in unstained tube gels by the method of Kleinschmidt & Kleiner (1978).

Molecular weight determinations. The molecular weight of GS was determined by gel filtration on a column of Sepharose 4B, equilibrated at 4 °C with buffer B and run at a flow rate of 20 ml h⁻¹. Rabbit muscle phosphorylase kinase (1 300 000), *E. coli* β-galactosidase (540 000), jack bean urease (480 000), horse spleen apoferritin (443 000), bovine liver catalase (232 000) and rabbit muscle aldolase (160 000) were used as molecular weight standards.

The subunit size of GS was determined by SDS-PAGE using the molecular weight markers rabbit muscle phosphorylase b (94 000), BSA (67 000), ovalbumin (43 000), soybean trypsin inhibitor (20 100) and bovine α-lactalbumin (14 400). Relative mobilities were measured with respect to bromophenol blue.

Snake venom phosphodiesterase (SVP) treatment of GS. The reaction was carried out in 100 mM Tris/HCl buffer pH 7.8, containing 20 mg of purified enzyme and 20 mg SVP. After 1 h incubation at 37 °C, the reaction was terminated by the addition of 1 ml 10% TCA and precipitated proteins were removed by centrifugation. AMP was identified by TLC using polyethyleneimine cellulose and nucleotide monophosphate standards (Randerath & Randerath, 1967). The solvent used for ascending elution was 1 M-acetic acid.

RESULTS AND DISCUSSION

Purification of GS from M. capsulatus (Bath)

It is not possible to purify GS by affinity chromatography using the affinity ligand Cibacron blue covalently linked to agarose, and so a conventional purification procedure was adopted, the protocol for which is shown in Table 1 and described in Methods. The cell-free extract was prepared from approximately 25 g dry wt cells from a 100 l batch culture of *M. capsulatus* (Bath) grown on NMS medium. The purification procedure gave an 11% yield with an overall 133-fold purification of the enzyme (Table 1). The enzyme was judged to be better than 95% pure following electrophoresis in polyacrylamide gels under denaturing and non-denaturing conditions. Therefore, GS constituted about 0.7% (w/w) of the total soluble protein in the cell extract.

Enzyme eluted from DEAE-cellulose, Sepharose 4B, DEAE-Sephacel and Sephacryl S-300 as a single peak of activity when measured by both the γ-glutamyltransferase assay and the GS biosynthetic assay, indicating, on the basis of both charge and size, the presence of only one form of GS in *M. capsulatus* (Bath).

In the presence of buffer B, containing the stabilizing ligands EDTA, glutamate, 2-mercaptoethanol and Mg²⁺, pure GS was completely stable for several months at -80 °C and could also be stored at 4 °C for several hours with almost no loss of activity. The stabilizing effects of these ligands is similar to that found with GS from *Anabaena cylindrica* (Sampaio *et al.*, 1979).

Molecular weight and subunit of GS

The molecular weight of GS was estimated by gel filtration to be 617 000 (Fig. 1). This is comparable with molecular weights of GS from several bacteria including *A. cylindrica*, *E. coli*, *A. vinelandii* and *Bacillus* species. However, the subunit molecular weight of GS from *M.*

Table 1. *Purification protocol for GS from M. capsulatus (Bath)*

Step	Total protein (mg)	Total activity (units)*	Specific activity [units (mg protein) ⁻¹]*	Yield (%)	Purification factor
Crude extract	5000	11 500	2.3	100	1
DEAE-cellulose eluate	619	6871	11.1	60	5
Sephacrose 4B eluate	101	4848	48.0	42	21
DEAE-Sephacel eluate	25	1770	70.8	15	31
Sephacryl S-300 eluate	4	1224	306.0	11	133

* One enzyme unit = 1 μ mol γ -glutamyl hydroxamate produced min^{-1} .

Fig. 1. Molecular weight estimation of GS on a Sepharose 4B column. The K_{av} values were calculated from $K_{av} = (V_e - V_o) / (V_t - V_o)$ where V_e is the elution volume, V_o the void volume and V_t the bed volume. The standards used were: aldolase (●), catalase (○), ferritin (▲), urease (△), β -galactosidase (■) and phosphorylase kinase (□). See Methods for details.

capsulatus (Bath) of 60000 as estimated using SDS-PAGE (Fig. 2) is considerably higher than values reported for GS enzymes from other organisms and suggested a decameric structure for the enzyme.

Properties of GS

The pH optimum for GS activity measured by both the γ -glutamyltransferase assay and the GS biosynthetic assay was found to be pH 7.15. The optimum temperature for activity was 45 °C, the growth temperature of the organism. All subsequent assays were done under these optimum conditions.

K_m values of substrates of GS in both the GS biosynthetic assay and γ -glutamyltransferase assay were determined using purified enzyme from nitrate-grown *M. capsulatus* (Bath) (Table 2). The K_m for hydroxylamine was estimated to be 14.0 mM which is considerably higher than the K_m value of 0.3 mM reported by Bailey *et al.* (1978) for the GS from *Methylococcus* NCIB 11803. The K_m value for ammonia was low and is in agreement with values obtained for GS enzymes of many other prokaryotes. The high affinity of GS from *M. capsulatus* (Bath) provides good evidence for ammonia assimilation by this enzyme during growth conditions in which the extracellular concentration of ammonia is low. K_m values for glutamate, ATP, glutamine and ADP confirmed that concentrations of these reactants used routinely in GS assays were in excess and not rate-limiting.

Nucleotide specificity

The nucleotide specificity of GS was investigated using cell extracts of *M. capsulatus* (Bath) grown on nitrate-supplemented medium (Table 3). The enzyme showed a marked dependence

Fig. 2. SDS-PAGE of GS and molecular weight standards on a 5% to 30% (w/v) polyacrylamide gradient gel (see Methods for details). The standards used were: (1) phosphorylase b (94000); (2) BSA (67000); (3) ovalbumin (43000); (4) carbonic anhydrase (30000); (5) trypsin inhibitor (20000); (6) α -lactalbumin (14400). Track A, standards (100 μ g protein); track B, GS (50 μ g protein); track C, GS (100 μ g protein).

Table 2. K_m values for substrates of GS

The K_m values were determined using an excess of all substrates other than the substrate in question. Initial velocities were measured and K_m values determined from Lineweaver-Burk plots. Linear regression analysis of plots gave correlation coefficients greater than 0.987 in all cases.

Assay	Substrate	K_m (mM)
γ -Glutamyltransferase assay	Hydroxylamine	14.00
	Glutamine	4.87
	ADP	0.15
Biosynthetic assay	Ammonia	0.72
	Glutamate	2.25
	ATP	0.60

for ATP in the biosynthetic assay although other nucleotide triphosphates, particularly GTP, could substitute for ATP to varying extents. These effects have also been noted with GS enzymes from many other bacteria including *E. coli* (Woolfolk *et al.*, 1966), *B. stearothermophilus* (Wedler & Hoffmann, 1974) and *Rhodospseudomonas palustris* (Alef *et al.*, 1981). GS from *M. capsulatus*

Table 3. *Nucleotide specificity of GS*

Nucleotide concentrations were 12 mM for the biosynthetic assay [control activity = 76 nmol min⁻¹ (mg protein)⁻¹] and 0.36 mM for the γ -glutamyltransferase assay [control activity = 4.7 μ mol min⁻¹ (mg protein)⁻¹].

Nucleotide	GS	GS
	biosynthetic activity (percentage of control)	γ -glutamyltransferase activity (percentage of control)
ATP	100	14
GTP	59	11
ITP	32	12
TTP	10	12
UTP	10	9
CTP	0	11
ADP	0	100
GDP	0	67
IDP	0	41
TDP	0	34
UDP	0	27
CDP	0	24
AMP	0	19
None	0	0

Table 4. *Feedback inhibition of GS activity*

Inhibitor	Inhibitor concn (mM)	GS			GS		
		biosynthetic activity (percentage of control)*			γ -glutamyltransferase activity (percentage of control)†		
		1	5	10	1	5	10
L-Alanine		64	28	14	48	20	10
L-Glutamine		48	10	6	ND	ND	ND
Glycine		93	70	34	83	47	35
L-Histidine		98	94	89	100	99	76
L-Serine		89	33	24	85	82	62
L-Tryptophan		98	98	97	99	96	95
Glucosamine 6-phosphate		94	83	78	100	98	92
AMP		84	57	38	90	75	62
Carbamyl phosphate		ND	ND	49	100	92	75

ND, Not determined.

* Control GS biosynthetic activity = 97 nmol min⁻¹ (mg protein)⁻¹.

† Control GS γ -glutamyltransferase activity = 9.4 μ mol min⁻¹ (mg protein)⁻¹.

(Bath), like the enzyme from *E. coli* (Woolfolk *et al.*, 1966), showed maximum activity with ADP and good activity with GDP in the γ -glutamyltransferase assay.

Feedback inhibition of GS

The possibility of feedback regulation of GS by various amino acids and other potential end-products of glutamine metabolism, e.g. glucosamine 6-phosphate, carbamyl phosphate and AMP was investigated using partially purified GS from nitrate-grown *M. capsulatus* (Bath) (Table 4).

When tested individually, several end-products of glutamine metabolism caused limited degrees of inhibition of GS activity. When two or more inhibitors were present in the assay, their effects were cumulative. This suggested that inhibitors were acting independently and that each may have occupied a separate binding site on the GS enzyme.

Feedback inhibition of GS by the above inhibitors has been reported in many bacteria including *R. capsulata* (Johansson & Gest, 1976), *K. aerogenes* (Bender *et al.*, 1977) and *Bacillus licheniformis* (Hubbard & Stadtman, 1967*b*). In *E. coli*, the sensitivity of GS towards feedback inhibition was found to be altered by adenylylation (Kingdon & Stadtman, 1967). The

Table 5. *Effects of feedback inhibitors on GS activity from dinitrogen- and ammonia-grown cells*

The specific activities of GS in dinitrogen-grown (MS) and ammonia-grown (AMS) extracts prepared from cells grown to steady-state were 5.4 and $2.3 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$, respectively.

Inhibitor (10 mM)	Percentage of control activity	
	MS extract	AMS extract
None	100	100
L-Tryptophan	94	77
L-Histidine	78	64
AMP	57	42
CTP	98	99

Fig. 3. Inhibition of GS activity by Mg^{2+} in the γ -glutamyltransferase assay.

adenylylated form of the enzyme, from cells grown in medium containing high extracellular levels of ammonia, was found to be more susceptible to feedback inhibition by L-histidine, L-tryptophan, CTP and ATP than the deadenylylated form (from cells grown in the absence of ammonia), when assayed by the γ -glutamyltransferase assay. Therefore, GS enzymes in cell-free extracts of dinitrogen- and ammonia-grown *M. capsulatus* (Bath) were compared for inhibition by these metabolites in the transferase assay (Table 5). GS from ammonia-grown cells was inhibited by L-tryptophan, L-histidine and AMP to a higher degree than the GS from dinitrogen-grown cells, suggesting that GS from ammonia-grown cells might have been adenylylated. In order to establish whether GS activity in *M. capsulatus* (Bath) was regulated by the system of adenylylation/deadenylylation observed in several *Enterobacteriaceae*, the effects of Mg^{2+} and Mn^{2+} ions were studied.

Effect of metal ions on GS activity

In *E. coli*, GS has been shown to be affected by metal ions when the γ -glutamyltransferase assay was used to determine activity: Mn^{2+} ions supported activity of both adenylylated (biosynthetically inactive) and deadenylylated (biosynthetically active) enzyme although the pH optimum varied considerably with the state of adenylylation (Shapiro & Stadtman, 1970). Furthermore, the addition of Mg^{2+} ions to the Mn^{2+} -dependent transferase assay inhibited the activity of the adenylylated subunits only (Stadtman *et al.*, 1970) and so the relative state of adenylylation can be measured by comparing the activity in the presence of Mn^{2+} ions with the activity in the presence of both Mn^{2+} and Mg^{2+} ions.

Activity of the enzyme from *M. capsulatus* (Bath) was completely dependent on Mn^{2+} , showing a maximum activity at 0.3 mM-Mn^{2+} in the assay mixture. No other divalent cation tested, including Mg^{2+} , Co^{2+} , Ni^{2+} , Ca^{2+} , Fe^{2+} , Zn^{2+} and Cu^{2+} would support γ -glutamyltransferase activity. Of these divalent cations (5 or 10 mM), only Mg^{2+} showed any significant inhibition of Mn^{2+} -supported transferase activity in nitrate-grown cells (Fig. 3).

Table 6. Inhibition of GS activity by Mg^{2+} in cell-free extracts of *M. capsulatus* (Bath)

Extracts were prepared from chemostat-grown cells grown with ammonia, nitrate or dinitrogen as the nitrogen source. All assays contained Mn^{2+} (0.27 mM) and γ -glutamyltransferase activity was measured as described in Methods.

Nitrogen source for growth	$MgCl_2$ concn (mM)	GS γ -glutamyltransferase activity [$\mu\text{mol min}^{-1}$ (mg protein) $^{-1}$]					
		0	2	5	10	15	20
Ammonia		1.10	0.50	0.18	0.10	0.02	0
Nitrate		2.30	1.08	0.35	0.21	0.05	0
Dinitrogen		6.60	3.04	0.79	0.53	0.20	0

Fig. 4. Effect of SVP on the Mg^{2+} -dependent biosynthetic activity of GS. Cells were grown in steady-state oxygen-limited chemostat culture. The enzyme was extracted, partially purified and assayed for activity in the presence (○; SVP-treated) and absence (●; control) of SVP. (a) Cells grown on ammonia-supplemented medium; (b) cells grown on nitrate-supplemented medium; (c) cells grown on dinitrogen-based medium.

In similar experiments it was observed that GS from *M. capsulatus* (Bath) showed absolute specificity for Mg^{2+} in the biosynthetic assay. Of all the divalent cations tested, only Mn^{2+} caused inhibition of Mg^{2+} -dependent biosynthetic activity (50% inhibition with 2 mM- $MnCl_2$ present in the assay mixture).

When cell extracts from ammonia-, nitrate- or dinitrogen-grown cells harvested from steady-state cultures were assayed for Mg^{2+} -dependent biosynthetic activity, values of 0, 64 and 98 nmol min^{-1} (mg protein) $^{-1}$, respectively, were observed. However, when these extracts were assayed for γ -glutamyltransferase activity in the presence of 20 mM- Mg^{2+} to inhibit non-biosynthetic activity, none of the extracts showed any activity although they all contained GS at varying levels (Table 6). Furthermore, decreasing the Mg^{2+} concentration for each extract showed the same relative increase in transferase activity, indicating that GS in each extract was equally adenylylated [according to the formula devised by Stadtman *et al.* (1970)]. Since the measured biosynthetic activities for these extracts indicated a large variance in the state of adenylylation for the enzyme according to growth conditions, it was clear that the γ -glutamyltransferase assay could not be used reliably to determine the state of adenylylation in this organism.

Demonstration of adenylylation of GS by SVP treatment

In vitro deadenylylation of adenylylated enzyme by SVP has been described for a number of bacterial GS enzymes, including *E. coli* (Stadtman *et al.*, 1970), *A. vinelandii* (Siedel & Shelton, 1979), *Pseudomonas aeruginosa* (Janssen *et al.*, 1980) and '*Streptomyces cattleya*' (Streicher & Tyler, 1981). In each case SVP cleaved off AMP groups from the adenylylated enzyme rendering it biosynthetically active. Partially purified GS preparations (after the Sepharose 4B step in

purification) from *M. capsulatus* (Bath) grown in continuous culture on ammonia-, nitrate- and dinitrogen-based medium were each treated with SVP as described in Methods. Samples were taken from each incubation mixture at 5 min intervals and assayed for Mg^{2+} -dependent biosynthetic activity (Fig. 4). SVP treatment of GS from ammonia-grown cells resulted in the appearance of Mg^{2+} -dependent biosynthetic activity, suggesting that the enzyme had been deadenylylated. Identical treatment of GS from nitrate-grown cells caused an increase in biosynthetic activity, indicating that the enzyme from these cells was initially partially adenylylated. Activity of GS from dinitrogen-grown cells was unaffected by SVP showing that the enzyme from N_2 -fixing cells was not adenylylated but was present in its fully biosynthetically active form. AMP cleaved off GS from ammonia-grown cells was identified by TLC, confirming that in *M. capsulatus* (Bath) glutamine synthetase activity is controlled not only by feedback inhibition of the end-products of glutamine metabolism but also by the system of adenylylation/deadenylylation which has been found in many other Gram-negative bacteria.

We wish to thank the Science Research Council for the award of a research studentship to J.C.M.

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