Assimilation of Ammonia by *Bacteroides amylophilus* in Chemostat Cultures

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The size of the amino acid pool in *Bacteroides amylophilus*, a rumen bacterium, was limited by the availability of ammonia in the growth medium. Alanine was the major pool constituent irrespective of the growth-limiting nutrient. In steady-state ammonia-limited chemostat cultures, glutamine synthetase (GS) activity was tenfold higher and glutamate dehydrogenase (GDH) activity up to fivefold lower than in maltose-limited cultures. No glutamate synthase (GOGAT) activity was demonstrated. When excess ammonia was pulsed into an ammonia-limited chemostat culture, the glutamine pool expanded rapidly and GS was inactivated. This was consistent with the observation that, in a number of bacteria, GS functions to assimilate ammonia when the prevailing concentration is low. However, GDH was always very active in extracts of *B. amylophilus* and its Michaelis constant for ammonia was relatively low (1 to 2 mM). This suggested that GDH could continue to function biosynthetically when GS was derepressed.

INTRODUCTION

Although there is considerable interest in the mechanisms of protein degradation and synthesis in the rumen and especially in the use of non-protein nitrogen as a source of nitrogen for ruminants (Buttery & Annison, 1973), there is surprisingly little information on the mechanisms of amino acid biosynthesis by pure cultures of rumen bacteria. The peptides, amino acids and ammonia which arise as end-products of dietary protein breakdown act as nitrogen sources for the rumen microbes (see, for example, Lewis & Swan, 1971). However, it is generally accepted that ammonia is quantitatively the most important nitrogenous nutrient for the rumen bacteria and for many species it is essential for growth (Allison, 1970).

The various routes by which ammonia may be assimilated by bacteria have been discussed by Brown *et al.* (1974). It is apparent that neither aspartase nor alanine dehydrogenase generally has a biosynthetic function and also that glutamate dehydrogenase may not function effectively except when the environmental concentration of ammonia is relatively high (Meers *et al.*, 1970). Under conditions of low ammonia availability, the two enzymes glutamine synthetase and glutamate synthase together provide a route for the incorporation of ammonia in a wide variety of bacteria (Meers *et al.*, 1970; Brown & Stanley, 1972; Dainty, 1972; Tyler, 1978).

Glutamate dehydrogenase activity has been detected in nine species of rumen bacteria which were considered representative of about 40% of the rumen microbial population (Joyner & Baldwin, 1966). It was assumed that a pathway involving this enzyme was the major route by which ammonia was fixed by the rumen bacterial population (Allison, 1970).

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Glutamine synthetase activity was detected in extracts of mixed rumen bacteria (Chalupa *et al.*, 1970) and this enzyme might also operate to assimilate ammonia to provide glutamine which serves as the nitrogen donor in the biosynthesis of many diverse metabolites (Shapiro & Stadtman, 1970a).

Bacteroides amylophilus is an obligately anaerobic, Gram-negative rod which may constitute about 10% of the rumen bacterial population in animals fed certain diets (Hunigate, 1966). Ammonia is essential for growth although there is some evidence for limited amino acid uptake (Hullah & Blackburn, 1971). The work reported in this paper examines some of the physiological aspects of the assimilation of ammonia by this organism. When suspended in a medium devoid of ammonia, the cells became swollen and leaked nitrogen-containing compounds but remained intact (Henderson *et al.*, 1969). Under ammonia limitation in a chemostat ($D < 0.08 \text{ h}^{-1}$), a considerable amount of cell debris was microscopically visible in suspension. Nitrogen limitation was evidently severe in this organism and the possibility that this might be reflected by the mode of ammonia incorporation or regulation of nitrogen metabolism was investigated.

METHODS

Abbreviations. GDH, Glutamate dehydrogenase; GOGAT, glutamate synthase; GS, glutamine synthetase; γ GT, γ -glutamyltransferase; MDH, malate dehydrogenase; ADH, alanine dehydrogenase; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase.

Organism. *Bacteroides amylophilus* strain WP91, kindly supplied by Dr P. N. Hobson, Rowett Research Institute, Bucksburn, Aberdeen, was maintained at 4 °C in MT agar [containing maltose (3 g l⁻¹), Tryptone (3 g l⁻¹) and Ionagar (1.5 %, w/v)] and was subcultured every 3 weeks. Lyophilized suspensions, prepared according to Blackburn (1968), were reconstituted at intervals.

Culture media. *Bacteroides amylophilus* was grown in a basal medium which, unless otherwise stated, contained (g l⁻¹ in distilled water): K₂HPO₄, 0.45; KH₂PO₄, 0.45; NaCl, 0.90; (NH₄)₂SO₄, 0.90; MgSO₄ · 7H₂O, 0.09; CaCl₂, 0.09; resazurin, 0.001; NaHCO₃, 5.0; L-cysteine hydrochloride, 0.08 (Blackburn, 1965; Hobson & Summers, 1967). The mineral ingredients were stored as concentrated solutions at 4 °C. The phosphate components were added as a heat-sterilized solution (121 °C, 15 min) to the autoclaved bulk medium. Resazurin, bicarbonate and cysteine (reducing agent) solutions were prepared on the day of use. For continuous cultures, maltose limitation was obtained with 3 g l⁻¹ in basal medium, and ammonia limitation with (NH₄)₂SO₄ at 0.143 g l⁻¹ and maltose at 4.5 g l⁻¹. The redox potential (E_h) of reduced medium was about -250 mV as measured with a calomel electrode.

Anaerobic methods. Liquid media were prepared according to Latham & Sharpe (1971). Broth cultures were transferred with sterile plastic disposable syringes fitted with size 21 gauge, 4 cm Luer hypodermic needles by inoculating through the rubber stoppers sealing the growth vessels. Cultures were grown in 100 ml medium in 150 ml medical flat bottles fitted with black butyl rubber bungs (A. R. Horwell, Grangeway, Kilburn High Rd, London). For continuous cultures, medium was autoclaved at 121 °C for 1 h and equilibrated overnight under CO₂ prior to the addition of sterile solutions of phosphate buffer, maltose, bicarbonate and reducing agent (Hobson, 1965). All cultures were incubated at 39 °C under CO₂. The initial pH was 6.6 to 6.8.

Continuous cultures. Cultures (2 l) were grown in a stirred fermenter (FL103; Biotec, Stockholm, Sweden) with the pH controlled at 6.2 by automatic addition of 10% (w/v) NaOH and the temperature held at 39 °C. The organisms were grown to late-exponential phase as a batch culture in the fermenter prior to commencing medium input. A positive pressure was maintained in the vessel by sparging with sterile O₂-free CO₂ (50 to 100 ml min⁻¹). This, in combination with a slow impeller speed (80 rev. min⁻¹), effected complete mixing. Sterile feed medium was pumped from a 20 l storage reservoir by a Micropump Series II (Metering Pumps, Ealing, London) fitted with either a size 4 or size 2 pumphead; the latter was used for input rates of less than 300 ml h⁻¹. The pumphead was cooled with a cold water jacket during operation to avoid the formation of CO₂ bubbles in the medium lines. About five volume changes of medium were passed through the cultures to establish steady states before the dilution rate or medium mixture was changed.

Culture analysis. Bacterial growth was monitored turbidimetrically at 660 nm with a Pye Unicam SP600 Series II spectrophotometer. Dry weight was determined by centrifuging a measured volume (about 40 ml) of culture (15000 g, 15 min, 4 °C), resuspending the pellet in about 30 ml 0.15 M-NaCl in 0.02 M-KH₂PO₄/K₂HPO₄ buffer (pH 6.4) and recentrifuging. After a final wash with 0.015 M-NaCl, the bacteria were dried to constant weight at 85 °C.

Maltose in cell-free culture supernatants was determined by the method of Dubois *et al.* (1956) using 5% (w/v) phenol. Ammonia was estimated by micro-diffusion (Conway, 1962).

Amino acid pools. To assess the intracellular free amino acid pool content and composition of *B. amylophilus*, bacteria were extracted with HClO_4 as described by Tempest *et al.* (1970). Similar results were obtained when bacteria were extracted with boiling water for 10 min (Dawson, 1965). Basic amino acids were analysed with a Locarte Mk IV amino acid analyser; acidic and neutral amino acids were analysed with an EEL model 294 analyser converted to Locarte specification (Atkin & Ferdinand, 1970). The amino acids were assayed with ninhydrin and quantified by comparing the peak areas from the chart recordings with those of internal standards. The total amino acid pool was assessed by summing the concentrations of those amino acids whose concentration was greater than or equal to 0.1 mM. To determine the amino acid content of culture supernatants, samples were deproteinized with 0.25 M- HClO_4 , centrifuged, neutralized and applied to the columns of the analysers.

When the pools were extracted from a number of samples simultaneously, bacteria were harvested by membrane filtration (Millipore, 0.45 μm pore size) and the filters were suspended in 4 ml ice-cold 0.25 M- HClO_4 . Similar results were obtained for the content and composition of the pools extracted from bacteria harvested by centrifugation or by filtration.

Extraction of enzymes. Culture samples (10 to 20 mg dry wt cells) were collected in an ice-cold receiver, centrifuged (15000 *g*, 5 min, 2 °C) and then the pellet was dispersed in about 30 ml 50 mM-Tris/HCl buffer (pH 7.6) containing 10 mM-2-mercaptoethanol (Meers *et al.*, 1970). After recentrifuging, the bacteria were resuspended to approximately 2 to 5 mg dry wt ml⁻¹ in similar buffer. They were disrupted either (i) in a Hughes pressure cell (Hughes, 1951), the crushed material, after thawing, being centrifuged at 20000 *g* for 20 min at 4 °C or (ii) with an MSE ultrasonic disintegrator (1 cm diam. probe, 60 W, 5 min), a crude extract being obtained as before. The latter method gave slightly lower activities than the former and was only used when a number of samples required disruption consecutively. The enzyme activities in the crude extracts were measured as soon as possible after preparation. Protein concentrations were determined by the method of Lowry.

Determination of enzyme activity. Glutamate dehydrogenase [GDH; EC 1.4.1.4; L-glutamate:NADP⁺ oxidoreductase] activity in crude extracts was measured spectrophotometrically from the rate of NADPH oxidation at 340 nm. The assay mixture (3 ml) was similar to that of Meers *et al.* (1970) except that the buffer was 50 mM-Tris/HCl, pH 8.0. GDH activity was also measured by the rate of glutamate oxidation as described by Chalupa *et al.* (1970). Glutamate synthase [GOGAT; EC 1.4.1.13; L-glutamate:NADP⁺ oxidoreductase (transaminating)] activity was measured spectrophotometrically from the rate of NAD(P)H oxidation at 340 nm; the components of the assay mixture (3 ml) were similar to those of Cole *et al.* (1974) except that the buffer was 50 mM-Tris/HCl, pH 8.0. GDH and GOGAT activities were measured at 22 °C.

Glutamine synthetase [GS; EC 6.3.1.2; L-glutamate:ammonia ligase (ADP-forming)] was measured by its synthetase and transferase activities. Synthetase activity was determined by the method described by Rowe *et al.* (1970). The assay mixture (2 ml) for the measurement of γ -glutamyl transferase (γ GT) activity contained (mM): imidazole/HCl, pH 7.0, 50; sodium arsenate, 40; L-glutamine, 40; hydroxylammonium chloride, 12; MnCl_2 , 10; ADP (sodium salt), 8 (Woolfolk *et al.*, 1966). After 30 min at 37 °C, the reaction was stopped by adding 2 ml 24% (w/v) trichloroacetic acid/6 M-HCl/10% (w/v) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (in 0.02 M-HCl)/ H_2O (1:1:1:4.5, by vol.). The absorbance at 510 nm was referred to that of a standard L-glutamic acid γ -monohydroxamate solution with FeCl_3 reagent.

Malate dehydrogenase [MDH; EC 1.1.1.37; L-malate:NAD⁺ oxidoreductase] activity was estimated as described by Reeves *et al.* (1971) at 22 °C and pH 7.4. Alanine dehydrogenase [ADH; EC 1.4.1.1; L-alanine:NAD⁺ oxidoreductase] activity was determined as described by Griffith & Carlsson (1974). Glutamate-oxaloacetate transaminase [GOT; EC 2.6.1.1; L-aspartate:2-oxoglutarate aminotransferase] and glutamate-pyruvate transaminase [GPT; EC 2.6.1.2; L-alanine:2-oxoglutarate aminotransferase] activities were measured by the 2,4-dinitrophenylhydrazine reaction (Sigma Technical Bulletin no. 505, 1977).

The enzyme activities were determined as far as possible under near optimal conditions. Reaction velocities were proportional to the enzyme concentration and initial rates were determined where appropriate. Estimates were corrected for endogenous rates measured in the absence of substrates. NAD(P)H oxidase activity in cell extracts was, however, usually negligible. Specific enzyme activity was expressed as nmol product formed min⁻¹ (mg protein)⁻¹, assuming a value of ϵ_{340} for NADPH and NADH of 6200 l mol⁻¹ cm⁻¹ and a value of ϵ_{510} for L-glutamic acid γ -monohydroxamate of 520 l mol⁻¹ cm⁻¹.

Chemicals. Ionagar and Tryptone were from Oxoid and buffers, substrates and nucleotides were from Sigma, except for the sodium salts of ADP and ATP which were obtained from Boehringer. Other materials were of analytical reagent grade quality.

Table 1. *Effect of dilution rate and nature of nutrient limitation on the intracellular free amino acid pool content and composition during growth of B. amylophilus in chemostat cultures*

Pool amino acid concentrations are expressed as mM, assuming a cell water content of four times the dry weight. Ammonia-limited cultures washed out at $D > 0.29 \text{ h}^{-1}$.

Dilution rate (h^{-1}) ...	Maltose limitation			Ammonia limitation		
	0.08	0.15	0.39	0.04	0.09	0.15
Aspartate	0.3	0.4	0.5	ND	ND	0.2
Threonine	0.3	0.2	0.3	ND	ND	0.7
Serine	0.2	0.5	0.5	ND	ND	0.5
Glutamate	2.3	1.9	1.7	0.6	1.0	2.1
Glutamine	0.4	0.2	0.5	ND	ND	0.1
Proline	ND	ND	ND	ND	ND	ND
Glycine	ND	0.6	0.8	ND	ND	0.4
Alanine	4.6	9.0	7.3	0.9	3.4	5.0
Valine	2.3	1.9	2.4	0.8	0.3	0.4
Methionine	ND	ND	0.1	ND	ND	ND
Isoleucine	ND	ND	0.2	ND	ND	0.3
Leucine	ND	ND	0.2	ND	ND	ND
Tyrosine	ND	ND	0.1	ND	ND	ND
Lysine	2.0	2.8	3.0	0.3	1.2	2.4
Histidine	ND	ND	ND	ND	ND	ND
Arginine	ND	ND	ND	ND	ND	ND
Total pool	12.4	17.5	17.6	2.6	5.9	12.1
Glutamate portion (%)	18.5	10.8	9.6	23.0	16.9	17.3
Glutamine portion (%)	3.2	1.8	2.8	—	—	0.8
Alanine portion (%)	37.1	51.4	41.5	34.6	57.6	41.3
Extracellular ammonia (mM)	11.5	10.9	11.2	0.13	0.08	0.09

ND, Not detectable ($< 0.1 \text{ mM}$).

RESULTS

Intracellular free amino acid pool

The concentration of amino acids in the intracellular pool extracted from *B. amylophilus* in the early-stationary phase of growth in batch cultures was 6 to 15 mM. This is similar to the range reported for other Gram-negative bacteria by Brown & Stanley (1972) and considerably smaller than the range in Gram-positive bacteria (Tempest *et al.*, 1970). However, the size (15 mM) and the proportion of glutamate (7%) in the intracellular pool after growth was limited by the availability of maltose were different from the size (6 mM) and the proportion of glutamate (22%) after ammonia-limited growth. The pool size varied with the population density; the concentration of the growth-limiting nutrient was therefore adjusted so that a similar bacterial density was obtained in each case.

Tempest *et al.* (1970) reported that the nature of the growth-limiting nutrient had little influence on the total intracellular free amino acid concentration in *Aerobacter aerogenes* during growth in chemostat cultures in which the environment was strictly regulated. It was possible that the pool size was much lower at peak growth in ammonia-limited batch cultures of *B. amylophilus* because, under these conditions, lysis was rapid. However, the pool extracted from *B. amylophilus* growing under ammonia limitation in chemostat culture was always smaller than that from bacteria growing under maltose limitation at the same dilution rate (Table 1). The effect of increasing D was, nevertheless, to effect an

Table 2. Influence of dilution rate and nature of nutrient limitation on the specific activity of glutamate dehydrogenase (GDH), glutamine synthetase (GS), malate dehydrogenase (MDH), glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) during growth of *B. amylophilus* in chemostat cultures

Bacterial extracts were prepared with a Hughes pressure cell. Specific enzyme activities are expressed as nmol product formed min⁻¹ (mg protein)⁻¹. Ammonia-limited cultures washed out at $D > 0.29 \text{ h}^{-1}$.

Nutrient limitation	Dilution rate (h ⁻¹)	Specific enzyme activity				
		GDH	GS	MDH	GOT	GPT
Ammonia	0.043	747	NM	NM	NM	NM
Ammonia	0.080	760	113	NM	139	69
Ammonia	0.120	725	133	NM	83	NM
Ammonia	0.150	633	138	3707	254	NM
Ammonia	0.204	691	NM	NM	159	58
Maltose	0.033	1575	62	NM	NM	—
Maltose	0.080	1973	37	NM	140	NM
Maltose	0.150	2840	10	2919	182	22
Maltose	0.390	2771	12	3923	206	NM

NM, Not measured.

increase in pool size. Much of this increase could be attributed to alanine which always predominated.

At $D = 0.09 \text{ h}^{-1}$, the concentration of amino acids in the supernatant was about 30% of that in the intracellular pool during ammonia-limited growth, and about 4% of that in the pool during maltose-limited growth. The low concentration of intracellular amino acids in ammonia-limited cultures might have been partly due to amino acid excretion and cell lysis. The amino acid composition of the supernatants reflected that of the cell pools so there was no evidence for selective excretion. Furthermore, the amount and composition of the amino acids in the cell pools and culture supernatants were unaffected when the cysteine in the culture medium was replaced by ascorbate (0.5 g l^{-1}) and $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (0.2 g l^{-1}).

Specific enzyme activities

The activities of GDH, GOGAT, GS, MDH, ADH, GOT and GPT were measured with bacterial extracts prepared with a Hughes pressure cell from organisms growing under maltose or ammonia limitation in a chemostat (Table 2). There was an inverse relationship between the specific activities of GDH and GS, and a low concentration of ammonia (about 0.1 mM) promoted an increase in specific GS activity. The specific activities of MDH, GOT and GPT did not vary significantly with D or with the nature of the growth-limiting nutrient, although specific GPT activity was often lower when maltose limited growth. Neither GOGAT nor ADH activities were detected even under a wide range of assay conditions.

GDH was NADP⁺-specific; the activity with NAD⁺ was about 5% of that with NADP⁺. The activity of GDH with NADP⁺ as measured by glutamate oxidation at pH 8.0 was 10 to 15% of that measured by glutamate formation.

GS activity was measured by the rate of γ -glutamyl hydroxamate formation; with the biosynthetic reaction (synthetase) the activity was only 5 to 10% of that of the transferase reaction. The γ GT activity in extracts of *B. amylophilus* was unaffected or occasionally slightly stimulated by the presence of 60 mM-Mg^{2+} .

Fig. 1. Changes in the intracellular free amino acid pool content and composition of *B. amylophilus* following an ammonia 'pulse' to an ammonia-limited chemostat culture ($D = 0.15 \text{ h}^{-1}$). At time zero, the ammonia concentration in the culture was increased from $< 0.1 \text{ mM}$ to approx. 7 mM by the rapid addition of sterile ammonium sulphate solution. Bacteria were harvested by filtration and their free amino acid content was assessed as described in Methods: ○, glutamate; ●, glutamine; □, alanine; ■, valine; △, total pool.

Fig. 2. Changes in the specific activities of some enzymes in *B. amylophilus* following an ammonia 'pulse' to an ammonia-limited chemostat culture ($D = 0.15 \text{ h}^{-1}$). At time zero, the ammonia concentration in the culture was increased from $< 0.1 \text{ mM}$ to approx. 7 mM by the rapid addition of sterile ammonium sulphate solution. About 1 h after the pulse, the medium entering the growth vessel was changed to one which led to maltose-limited growth. Bacteria were harvested by centrifugation at 4°C , resuspended in buffer and ultrasonically disrupted as described in Methods. Enzyme specific activity is expressed as $\text{nmol product formed min}^{-1} (\text{mg protein})^{-1}$: ○, glutamate dehydrogenase (GDH); ●, glutamine synthetase (GS); □, glutamate-oxaloacetate transaminase (GOT); ■, malate dehydrogenase (MDH).

Routes of ammonia assimilation

To investigate further the routes by which ammonia might be assimilated, ammonia was pulsed into an ammonia-limited chemostat culture ($D = 0.15 \text{ h}^{-1}$) and the subsequent changes in amino acid pool content and specific activities of GDH, GS, MDH and GOT were recorded. About 1 h after the pulse, the medium entering the growth vessel was changed to one which led to maltose limited growth.

The pulse of excess ammonia (final concn 7 mM) effected a 10-fold increase in the pool

concentration of glutamine within 20 s which decayed to less than half the peak value by 40 s (Fig. 1). There was also a threefold increase in the valine concentration after 40 s and a twofold increase in the glutamate concentration over a period of 90 s. The high activity of GDH in extracts at all times indicated that when ammonia was available glutamate could be synthesized rapidly. The increases in alanine and valine might be due to their ease of formation from the intermediary metabolites, such as pyruvate, formed during maltose breakdown. The possibility that alanine was formed from glutamine by a reaction analogous to that catalysed by GOGAT was investigated, but no activity could be demonstrated.

The specific GS activity (γ GT) decreased to about 30% of the initial value within 5 min (Fig. 2) while the specific GDH activity increased fivefold over a 24 h period. By this time the culture was maltose-limited and the GS activity was very low.

Effect of pH, NaCl, cations and amino acids on GDH activity

The pH for optimum GDH activity was 8.2 to 8.3. At pH 6.5 the activity was 40% of the optimum, and at pH 9.5 the activity was negligible. Activity was stimulated twofold by 50 mM-NaCl or -KCl and reached a maximum (about 2.5 fold stimulation) at 100 mM-NaCl; the same effects were observed with Tris/HCl at 100 and 150 mM. Activity was relatively unaffected by the addition of 100 mM-aspartate or -alanine but 50 mM-glutamate gave 35% inhibition.

Michaelis constant for ammonia of GDH

From Lineweaver-Burk plots, the K_m value for ammonia of GDH at optimum pH was 1.7 mM with 50 mM-Tris/HCl buffer in the reaction mixture, 2.1 mM with 50 mM-KH₂PO₄/KOH buffer, and 1.0 mM at pH 7.6 with 150 mM-Tris/HCl buffer.

DISCUSSION

The amino acid pool content of *Bacteroides amylophilus* differed from that of some other bacteria (Tempest *et al.*, 1970; Brown & Stanley, 1972) in that it was markedly influenced by the environmental ammonia concentration. The decrease in pool content under ammonia limitation may have been due to some extent to cell lysis which was evident under these conditions (since pool concentrations were expressed in mM on the basis of bacterial dry weight measurements) or to amino acid leakage (Henderson *et al.*, 1969). Both of these factors might have accounted for some of the amino acids in the ammonia-limited culture supernatants, but the extent of protein breakdown, which could account for a major part of these amino acids, was not investigated.

The present results do not allow any firm conclusions to be drawn as to the role that any one pool amino acid might play in the physiology of growth of this organism. However, the rather broad pH-activity profile of GDH might not be consistent with the mechanism of osmoregulation proposed by Brown & Stanley (1972) in which glutamate may function. The predominance of alanine under all growth conditions was probably not due to the fixation of ammonia through pyruvate because no ADH activity was detected. A transamination through glutamate seemed unlikely to account for the high alanine content of the pool because GPT was also inactive. If, however, a large proportion of the alanine arose from a transamination of pyruvate with aspartate, then this might explain the high activities of GOT and MDH. This scheme would also suggest a direct involvement of CO₂ in amino acid biosynthesis (see Caldwell *et al.*, 1969).

The amount of GS in many bacteria is dependent upon the nitrogen source and this enzyme may be essential for nitrogen assimilation when the available nitrogen is present at very low concentrations (Magasanik *et al.*, 1974). The results presented would indicate that in *B. amylophilus*, GDH functions to assimilate ammonia when the availability is high

(> 1 mM) and GS is repressed. The specific activity of GDH may have increased concomitantly with growth rate under these conditions so that the nitrogen requirement for the increased rate of protein synthesis was met. When the concentration of ammonia is low (< 0.5 mM) and the intracellular free amino acid concentration is depressed, GS is depressed. Assimilation of ammonia may then proceed with the formation of glutamine. However, the absence of GOGAT would require that glutamate be provided for glutamine synthesis by some other reaction. In view of the relatively low K_m of the GDH for ammonia compared, for example, with that of *Escherichia coli* (Umbarger, 1969), the activity of GDH in extracts of *B. amylophilus* growing at $D = 0.15 \text{ h}^{-1}$ in 0.1 mM-ammonia would be sufficient to provide glutamate at a rate in excess of that required for glutamine synthesis *in vivo*. However, this assumes that enzyme activities are similar *in vitro* and *in vivo*.

It is difficult to assess the significance of the inactivation of GS. It was probably not due to adenylation because the state of adenylation had little effect on the γ GT activity of the *E. coli* enzyme (Wohlhueter *et al.*, 1973). Under slightly modified assay conditions, Mn^{2+} supports the γ GT activity of both the adenylylated (biosynthetically inactive) and the unadenylylated (active) *E. coli* enzyme, but in the presence of 60 mM- Mg^{2+} only the unadenylylated enzyme is active (Shapiro & Stadtman, 1970*b*). Since Mg^{2+} had little effect on the γ GT activity of the *B. amylophilus* enzyme, this might suggest that an adenylation type mechanism did not operate. However, the γ GT activities of GS enzymes from different sources vary markedly in their responses to Mg^{2+} (Bender *et al.*, 1977; Nyberg & Clarke, 1978).

The present results and those of Pulman & Johnson (1978) indicate that the possession of GOGAT may not be as widespread amongst the prokaryotes as was first thought (see Tyler, 1978). Since *B. amylophilus* survives and grows in the rumen despite the lack of GOGAT and the evident inability to fix gaseous nitrogen (Jones & Thomas, 1974), the concentration of ammonia in the rumen is likely to be adequate.

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