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The Enzymes of the Ammonia Assimilation in *Pseudomonas aeruginosa*

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Abstract. Glutamine synthetase from *Pseudomonas aeruginosa* is regulated by repression/derepression of enzyme synthesis and by adenylylation/deadenylylation control. High levels of deadenylylated biosynthetically active glutamine synthetase were observed in cultures growing with limiting amounts of nitrogen while synthesis of the enzyme was repressed and that present was adenylylated in cultures with excess nitrogen.

NADP- and NAD-dependent glutamate dehydrogenase could be separated by column chromatography and showed molecular weights of 110,000 and 220,000, respectively. Synthesis of the NADP-dependent glutamate dehydrogenase is repressed under nitrogen limitation and by growth on glutamate. In contrast, NAD-dependent glutamate dehydrogenase is derepressed by glutamate. Glutamate synthase is repressed by glutamate but not by excess nitrogen.

Key words: Glutamine synthetase – Glutamate synthase – Glutamate dehydrogenase – Ammonia assimilation – *Pseudomonas aeruginosa.*

In bacteria, two pathways are of major importance for the assimilation of ammonia. In the presence of high amounts of ammonia in the culture medium, its incorporation into glutamate is catalyzed by the NADP-dependent glutamate dehydrogenase (GDH; L-glutamate: $NADP^+$ oxidoreductase, EC 1.4.1.4) ("high ammonia pathway"). If there is a low availability of ammonia, the combined action of glutamine synthetase (GS; L-glutamate: ammonia ligase, EC 6.3.1.12) and glutamate synthase (GOGAT; L-glutamine: 2-oxoglutarate aminotransferase, EC 2.6.1.53) is responsible for the synthesis of glutamate ("low ammonia pathway"). These two routes have been demonstrated in enteric bacteria (Meers et al., 1970; Brenchley et al., 1975; Senior, 1975; Pulman and Johnson, 1978), nitrogen fixing bacteria (Nagatani et al., 1971), photosynthetic bacteria (Brown and Herbert, 1977a, b) and are also operative in *Pseudomonas aeruginosa* (Brown et al., 1973).

Glutamine synthetase catalyzes the ATP-dependent incorporation of ammonia in glutamate to form glutamine. Its cellular activity is finely regulated by repression/derepression, by several effectors and by adenylylation/deadenylylation control (Stadtman et al., 1968). The enzyme plays a key role in nitrogen assimilation because it regulates the synthesis of several other enzymes involved (Magasanik et al., 1974; Tyler, 1978). A well documented example is the regulation of the synthesis of the hut enzymes in *Klebsiella aerogenes.* GS causes an escape from catabolite repression of histidine degrading enzymes under nitrogen limiting conditions (Prival et al., 1973). Tyler et al. (1974) have shown that deadenylylated GS stimulates the in vitro transcription of the hut operon. The synthesis of a number of other enzymes is thought to be regulated in a similar way e.g. the enzymes responsible for the utilization of molecular nitrogen (Streicher et al., 1974), asparagine (Resnick and Magasanik, 1976) and urea (Friedrich and Magasanik, 1978). Repression of enzyme synthesis by GS is thought to occur with GDH from *K. aerogenes* (Brenchley et al., 1973).

In *P. aeruginosa*, the ammonia assimilatory enzymes are not well studied. Some evidence for adenylylation/deadenylylation control of GS was presented (Tronick et al., 1973) but its physiological

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Abbreviations. GS = glutamine synthetase; GOGAT = glutamate synthase; GDH = glutamate dehydrogenase; CTAB = cetyltrimethylammoniumbromide; SVD = snake venom phosphodiesterase; MM = minimal salts medium

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significance is unclear (Nyberg and Clarke, 1978). Nothing is known about the possible regulatory role of GS in enzyme synthesis in this organism. We have been studying the metabolism of purines in *P. aeruginosa* (Vogels and van der Drift, 1976) and were interested in the regulation of ammonia assimilation and a possible regulatory role of GS during growth of *P. aeruginosa* on allantoin. As a first step we report here data on the regulation and properties of ammonia assimilatory enzymes in *P. aeruginosa*.

Materials and Methods

Organism

Pseudomonas aeruginosa strain V 3003 was used in all experiments.

Growth Media

Minimal salts medium (MM) contained per 1: 4.3 g $Na_2HPO_4 \cdot 2H_2O$, 2.2 g KH_2PO_4, 100 mg $MgSO_4 \cdot 7H_2O$ and 1 ml of the chloride and sulfate solutions described before (Rijnierse and van der Drift, 1974). The medium was supplemented with a carbon and a nitrogen source at the concentrations indicated. pH after sterilization was 7.0.

Batch Cultures

Media were inoculated from precultures on nutrient broth and the cells were grown at 37° C with rotatory shaking (250 rev/min).

Continuous Cultures

Bacteria were grown under nitrogen or carbon limitation in a chemostat (New Brunswick Scientific Bioflow C 30) and the pH was maintained at 7.0 with HCl by a pH control system (Radiometer TTT 60), equipped with an pH electrode (Ingold). For nitrogen-limited growth, MM was supplemented with 10 mM citrate and a nitrogen source at 1 mM N. Carbon-limited growth was obtained with MM supplemented with 2.7 mM citrate and a nitrogen source at 15 mM N, or with 4 mM allantoin as carbon and nitrogen source. The growth temperature was 37° C and the dilution rate 0.1 or 0.3 h^{-1}. After establishment of the steady state, the cultures were used for enzyme determinations.

Preparation of Cell Extracts

Cultures were harvested by centrifugation (10,000 × *g* for 10 min at 4° C). The cells were washed with 40 mM Na_2HPO_4-KH_2PO_4 buffer (pH 7.0), resuspended in this buffer and disrupted at 4° C with a MSE 150-W ultrasonic disintegrator (10 times 10 s at maximum output with intermittent cooling). After centrifugation (100,000 × *g* for 30 min at 4° C) a crude extract was obtained.

Preparation of Cells for Glutamine Synthetase (GS) Assays

One min prior to harvesting cetyltrimethylammoniumbromide (CTAB) was added to the cultures (final concentration 0.1 mg per ml) to prevent possible changes in adenylylation state (Bender et al., 1977). The cells were collected by centrifugation, washed and suspended in 1% KCl. GS activities were assayed immediately.

Snake Venom Phosphodiesterase (SVD) Treatment of GS

Crude extract was prepared as described above but with 150 mM imidazole · HCl, pH 7.0, replacing the phosphate buffer. Treatment with SVD was performed as described by Tronick et al. (1973).

Determination of GS

Biosynthetic activity of GS, i.e. the ATP-dependent production of glutamine from glutamate and ammonia was measured at 37° C by following the release of inorganic phosphate from ATP as described by Shapiro and Stadtman (1970).

In the transferase assay, the formation of γ-glutamylhydroxamate from glutamine and ammonia is measured. The assay used was adopted from Bender et al. (1977) with 5 mM $MgCl_2$ included in the reaction mixture. This stimulated and lowered the activity of deadenylylated and adenylylated GS, respectively (see Results). Routine assays were performed at pH 7.8, the isoactivity point.

The synthetic reaction is the ATP-dependent formation of γ-glutamylhydroxamate from glutamate and hydroxylamine. It was measured at pH 8 and 37° C as described by Bender et al. (1977).

In enteric bacteria, only the deadenylylated form of GS shows biosynthetic and synthetic activity. The transferase assay measures total GS since both the adenylylated and deadenylylated forms of GS are active, the former being inhibited and the latter unaffected or stimulated by 60 mM Mg^{2+} (Stadtman et al., 1970; Bender et al., 1977).

Further Assays

Glutamate dehydrogenase and glutamate synthase were assayed spectrophotometrically by measuring NAD(P)H oxidation at 340 nm as described by Meers et al. (1970). Incubation temperature was 25° C.

Allantoinase (allantoin amidohydrolase, EC 3.5.2.5) was measured as described before (Rijnierse and van der Drift, 1974).

Urease (urea amidohydrolase, EC 3.5.1.5) was measured at 30° C in an incubation mixture containing 40 µmol urea, 48 µmol phosphate, pH 7.0, and up to 0.2 units of enzyme in a final volume of 1.2 ml. Ammonia production was measured by the phenol-hypochlorite method (Richard, 1965).

Ammonia levels in the culture medium were measured with an ammonium electrode (Orion).

Protein was determined according to Lowry et al. (1951) with bovine serum albumin as a standard.

One unit of enzyme is defined as the activity that forms 1 µmol product per min under the incubation conditions used. Specific activity is expressed as units per mg dry weight (for whole cell assays) or in units per mg protein (for assays of crude extracts or purified proteins).

Ion Exchange Chromatography

Crude extract (30 mg protein) was applied to a column (35 × 1.8 cm) of DEAE-cellulose, previously equilibrated with TM buffer (50 mM Tris · HCl, pH 7.5, containing 1 mM β-mercaptoethanol). Protein was eluted with a 600 ml linear gradient of 0—0.5 M NaCl in TM buffer. Five ml fractions were collected and assayed for enzyme activity.

Gelfiltration

DEAE-cellulose eluate (5 ml) was applied to a Sephadex G-200 column (100 × 2.4 cm) and eluted with TM buffer. Five ml fractions were collected. For molecular weight determination, the column was

calibrated with jack bean urease (483,000), catalase (240,000), alcohol dehydrogenase (148,000), bovine serum albumin (68,000) and cytochrome c (13,500).

Materials

The molecular weight calibration proteins were obtained from Boehringer, Mannheim, FRG, except urease that was obtained from Sigma Chemical Co., St. Louis, MO. Amino acids and allantoin were from Merck, Darmstadt, FRG.

Results

Adenylylation/Deadenylylation Control of Glutamine Synthetase

The effect of an ammonia shock on cells grown under nitrogen limiting conditions shows that *Pseudomonas aeruginosa* glutamine synthetase (GS) is subject to adenylylation/deadenylylation control. For these experiments, cells were grown in batch culture to the end of the exponential growth phase in minimal medium (MM) supplemented with 35 mM citrate and 1 mM KNO_3. Under these conditions high levels of GS were formed. Addition of 100 mM ammonia to these derepressed cells caused a rapid change in pH optimum of the transferase activity from 8.2 to 7.0 and the enzyme was converted from a form with Mg^{2+}-stimulated activity to a Mg^{2+}-inhibited form (Fig. 1). The synthetic activity was reduced from 0.15 to 0.046 units/mg dry weight.

Mn^{2+}-dependent transferase activity of GS from shocked cells was rather low and therefore the transferase assay was more suitable for the determination of total GS in the presence of both Mn^{2+} and Mg^{2+}. With 0.3 mM Mn^{2+} and 5 mM Mg^{2+} included in the reaction mixture, isoactivity of the different forms of GS was found at pH 7.8 (Fig. 1).

AMP residues can be removed from adenylylated GS by snake venom phosphodiesterase (SVD) with a concomitant change in catalytic properties (Stadtman et al., 1970; Tronick et al., 1973). Table 1 shows that SVD treatment of crude extract obtained from ammonia-shocked GS-derepressed cells resulted in a change in pH profile of transferase activity, conversion of Mg^{2+}-inhibited to Mg^{2+}-stimulated transferase activity (at pH 7.8) and a strong increase in synthetic activity.

From these results it can be concluded that changes in adenylylation state regulate the activity of GS in *P. aeruginosa* in a similar way as found in other organisms. The effect of an additional 60 mM Mg^{2+} in the reaction mixture may be used as a measure of adenylylation state; adenylylated GS being inhibited and deadenylylated GS being stimulated.

Regulation of the Ammonia Assimilatory Enzymes

P. aeruginosa is able to incorporate ammonia both by the activity of glutamate dehydrogenase (GDH) and via the GS/glutamate synthase (GOGAT) pathway (Brown et al., 1973). The regulation of the enzymes involved, together with adenylylation state of GS were studied in chemostat experiments (Table 2) and in batch culture experiments (Table 3).

The obtained data show that in chemostat cultures under carbon-limited conditions with ammonia, glutamate or allantoin as nitrogen source, GS was repressed. A 3–10-fold derepression occurred when the cultures were nitrogen-limited with nitrate, ammonia, glutamine or allantoin as nitrogen source. This repression/derepression of GS was more pronounced at dilution rate 0.3 than at 0.1 (Table 2).

In nitrogen-limited cultures GS was deadenylylated as shown by its stimulation by 60 mM Mg^{2+}. Carbon-limitation resulted in adenylylated GS. This further increased the difference in content of biosynthetically active GS between nitrogen-limited and carbon-limited cultures.

A strong derepression of GS synthesis was also observed in batch cultures supplemented with 1 mM KNO_3 as nitrogen source. NH_4^+ and, to a lower extent, also glutamate repressed the synthesis of GS (Table 3).

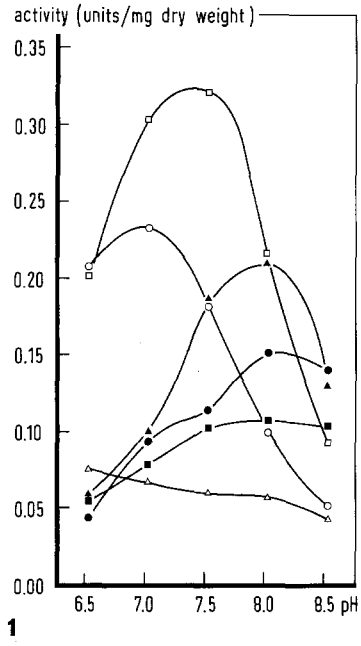
GOGAT activities from nitrogen-limited and carbon-limited cultures did not differ substantially, except at low dilution rate with ammonia as nitrogen source. The enzyme was not repressed under carbon-limited conditions with allantoin as nitrogen source (Table 2). In batch cultures, GOGAT synthesis was repressed by glutamate but not by excess ammonia (Table 3).

P. aeruginosa contains both NAD- and NADP-dependent GDH activities. The former is probably involved in glutamate breakdown while NADP-GDH is biosynthetically active (Brown et al., 1973). In chemostat cultures grown with an excess of nitrogen source, NADP-GDH was derepressed. The highest

Table 1. Effect of snake venom phosphodiesterase treatment on the catalytic properties of glutamine synthetase

	Transferase activity (10^{-3} U/mg)[a]						Synthetic activity (10^{-3} U/mg)[a]
	pH 7.0		pH 7.8		pH 8.1		
Untreated	570[b]	160[c]	240[b]	60[c]	60[b]	30[c]	45
After SVD treatment	250[b]	170[c]	230[b]	300[c]	230[b]	330[c]	210

[a] Activities are expressed in 10^{-3} units/mg protein
[b] Measured in the presence of 5 mM Mg^{2+}
[c] Measured in the presence of 60 mM Mg^{2+}



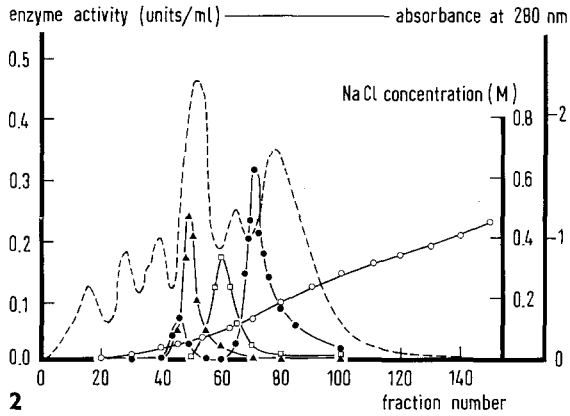


Fig. 1. Effect of an ammonia shock on the pH-activity profiles of glutamine synthetase. Cells were grown to the late exponential phase in MM supplemented with 35 mM citrate and 1 mM KNO_3. Transferase activity was measured at different pH values with untreated cells (*closed symbols*) and with cells shocked by the addition of 50 mM $(NH_4)_2SO_4$ 1 min prior to harvesting (*open symbols*). The incubation mixtures contained no Mg^{2+} (*squares*), 5 mM Mg^{2+} (*circles*) or 60 mM Mg^{2+} (*triangles*)

Fig. 2. Separation of NADP-glutamate dehydrogenase (GDH), NAD-GDH and glutamate synthase (GOGAT) by DEAE-cellulose chromatography. Cells were grown on MM supplemented with 35 mM citrate and 75 mM NH_4Cl. Crude extract was prepared and fractionated by DEAE-cellulose chromatography as described under „*Materials and Methods*". – – – Absorbance at 280 nm; ●——● NADP-GDH activity; ▲——▲ NAD-GDH activity; □——□ GOGAT activity; ○——○ NaCl concentration

Table 2. Ammonia assimilatory enzymes in chemostat cultures of *Pseudomonas aeruginosa* under different growth conditions

Growth conditions			Enzyme levels (10^{-3} U/mg)[a]							Supernatant NH_4^+ (mM)
Limitation	Nitrogen source	Dilution rate (h^{-1})	GS[b]	GS[c]	GOGAT	NADP-GDH	NAD-GDH	Allantoinase	Urease	
N	NO_3^-	0.1	140	240	20	10	20	–[d]	490	0.1
N	NO_3^-	0.3	160	280	67	8	23	–	1,300	0.1
N	NH_4^+	0.1	110	200	86	10	23	–	380	0.06
N	NH_4^+	0.3	130	240	66	6	9	–	1,200	0.17
N	glutamine	0.1	230	340	55	5	23	–	790	0.024
N	allantoin	0.1	160	300	66	4	11	220	1,200	–
N	allantoin	0.3	180	–	103	4	25	450	2,100	0.026
C	NH_4^+	0.1	42	26	22	61	26	–	26	3.7
C	NH_4^+	0.3	14	4	47	71	15	–	4	0.78
C	glutamate	0.1	12	8	37	75	43	–	14	–
C	allantoin	0.1	31	18	63	240	20	2,100	23	1.2
C	allantoin	0.3	34	19	98	365	30	3,100	24	2.2

GS = glutamine synthetase; GOGAT = glutamate synthase; NADP-GDH = NADP-glutamate dehydrogenase; NAD-GDH = NAD-glutamate dehydrogenase

[a] Glutamine synthetase (GS) activities are expressed in 10^{-3} units/mg dry weight, the other enzyme activities in 10^{-3} units/mg protein
[b] GS transferase activity measured in the presence of 5 mM Mg^{2+}
[c] GS transferase activity measured in the presence of 60 mM Mg^{2+}
[d] –; not determined

Table 3. Ammonia assimilatory enzymes in batch cultures of *Pseudomonas aeruginosa* with different growth media

Growth medium	Enzyme levels (10^{-3} U/mg)[a]				
	GS[b]	GS[c]	GOGAT	NADP-GDH	NAD-GDH
35 mM citrate + 75 mM NH_4Cl	32	33	73	115	20
35 mM citrate + 75 mM NH_4Cl + 40 mM glutamate	43	46	33	44	47
35 mM citrate + 40 mM glutamate	100	99	26	15	40
40 mM glutamate	115	110	27	38	54
35 mM citrate + 1 mM KNO_3	343	543	79	7	16

GS = glutamine synthetase; GOGAT = glutamate synthase; NADP-GDH = NADP-glutamate dehydrogenase; NAD-GDH = NAD-glutamate dehydrogenase

[a] GS activities are expressed in 10^{-3} units/mg dry weight, the other enzyme activities in 10^{-3} units/mg protein

[b] GS transferase activity measured in the presence of 5 mM Mg^{2+}

[c] GS transferase activity measured in the presence of 60 mM Mg^{2+}

specific activities were obtained with allantoin as carbon and nitrogen source. Under these circumstances high amounts of ammonia are produced by the cells. NADP-GDH was 10–60-fold lower in nitrogen-limited cultures (Table 2). Batch culture experiments revealed that the enzyme was repressed by glutamate (Table 3).

NAD-GDH content was about equal under nitrogen-limited and carbon-limited conditions but the enzyme level was elevated in cultures with glutamate as nitrogen source (Table 2). Also in batch cultures glutamate derepressed NAD-GDH (Table 3). The enzyme seems to be induced by its substrate. Ammonia or citrate did not severely repress NAD-GDH.

The inducible enzyme allantoinase was high in cells grown under carbon limitation with allantoin as carbon, nitrogen and energy source. A 10-fold lower activity was observed in allantoin-nitrogen limited chemostat cultures (Table 2).

Urease levels paralleled GS content of cells. Nitrogen limitation resulted in a strong derepression, especially at high dilution rates. In the presence of excess ammonia- or allantoin-nitrogen, urease became repressed (Table 3).

Biochemical Parameters of Ammonia Assimilation Enzymes

NAD-GDH and NADP-GDH could be separated by DEAE-cellulose chromatography. The elution profile (Fig. 2) shows that NADP-GDH was only active with NADPH and that NAD-GDH exclusively could use NADH as coenzyme, although a small peak of NADP-activity eluted together with NAD-GDH. However, after further purification by Sephadex G-200 gelfiltration a preparation of NAD-GDH containing no NADP-dependent activity was obtained.

Table 4. Parameters from ammonia assimilatory enzymes in *Pseudomonas aeruginosa*

	Enzymes			
	NADP-GDH	NAD-GDH	GOGAT	GS
pH optimum biosynthetic reaction	7.7	7.7	7.5	7.5
pH optimum catabolic reaction	8.5	8.2	–	–
K_m for ammonia (mM)	2	15	–	0.2
K_m for 2-oxoglutarate (mM)	0.2	1.6	2.0	–
K_m for glutamate (mM)	–	–	–	1.6
K_m for glutamine (mM)	–	–	1.7	–

GS = glutamine synthetase; GOGAT = glutamate synthase; NADP-GDH = NADP-glutamate dehydrogenase; NAD-GDH = NAD-glutamate dehydrogenase

This two-step purification procedure resulted in a 60-fold enhancement in specific activity of NADP-GDH and the resulting material was used for the determination of the kinetic parameters shown in Table 4. Calibration of the Sephadex G-200 column revealed a molecular weight between 100,000 and 120,000 for NADP-GDH.

NAD-GDH was purified 60-fold and had a molecular weight between 200,000 and 250,000. The K_m values of NAD-GDH for its substrates are considerably higher than the corresponding values of NADP-GDH. This implies that NAD-GDH is only active at higher substrate concentrations, reflecting the catabolic function of this enzyme.

For partial purification of GOGAT, crude extract from cells grown on 35 mM citrate and 75 mM NH_4Cl was fractionated on a DEAE-cellulose column. GOGAT eluted between NAD- and NADP-GDH from the column and the purified preparation was used for the determination of the kinetic data (Table 4). GOGAT was only active with NADPH as coenzyme and glutamine could not be replaced by ammonia as amino donor.

Kinetic parameters for glutamine synthetase were determined with crude extract obtained from cells grown in 35 mM citrate and 1 mM KNO_3. In the biosynthetic assay, the observed K_m value for ammonia was about 10-fold lower than the K_m of NADP-GDH for ammonia and therefore, GS must be more efficient in ammonia incorporation at lower substrate levels.

Discussion

In this paper we describe the regulation and properties of ammonia assimilatory enzymes in *Pseudomonas aeruginosa*. Glutamine synthetase (GS), one of the key enzymes in this process, is regulated by repression/derepression of enzyme synthesis and by adenylylation/deadenylylation control. High levels of deadenylylated, biosynthetically active GS were observed in nitrogen-limited chemostat cultures and in batch cultures containing a low amount of nitrate as nitrogen source. GS was repressed and adenylylated in chemostat cultures growing with an excess of ammonia, glutamate or allantoin nitrogen.

Although Tronick et al. (1973) provided evidence for adenylylation/deadenylylation control of *P. aeruginosa* GS, its physiological significance remained unclear. Nyberg and Clarke (1978) did not observe a strong elevation of GS in nitrogen-limited chemostat cultures and could not conclude about the adenylylation state of the enzyme. We treated cells with cetyltrimethylammoniumbromide (CTAB) before harvesting to fix the adenylylation state of GS (Bender et al., 1977) and included Mg^{2+} in the transferase assay mixture to stimulate the activity of deadenylylated GS and to allow a determination of the isoactivity point to be made. A strong Mg^{2+}-dependence of the transferase activity of deadenylylated GS was recently also described by Siedel and Shelton (1979) for *Azotobacter vinelandii*.

After establishment of the appropriate assay conditions, it appeared that the regulation of the synthesis and inactivation of GS in *P. aeruginosa* is very similar to its control in enteric bacteria (Stadtman et al., 1970; Bender et al., 1977; Brenchley et al., 1975) and other gram-negatives (Tronick et al., 1973; Kleinschmidt and Kleiner, 1978). As already stated by Tronick et al. (1973) adenylylation/deadenylylation control of GS seems to be a general phenomenon in gram-negative bacteria.

Although in chemostat cultures at low dilution rate and in the presence of excess ammonia some repression of glutamate synthase (GOGAT) synthesis was observed, GOGAT levels were found not to be directly correlated to the limitation applied. However, GOGAT formation is controlled as shown by its repression by glutamate. So the enzyme is regulated similar to GOGAT from enteric bacteria (Miller and Stadtman, 1972; Senior, 1975; Brenchley et al., 1973, 1975).

P. aeruginosa contains two GDH's (Brown et al., 1973) that could be separated by DEAE-cellulose chromatography and gelfiltration. NADP-GDH and NAD-GDH showed molecular weights of about 110,000 and 220,000, respectively. NADP-GDH was repressed under nitrogen-limited conditions and by glutamate while NAD-GDH was induced by glutamate. This, together with the observed kinetic parameters, is in good agreement with the proposed catabolic and biosynthetic function for NAD-GDH and NADP-GDH, respectively. The presence of two functionally and structurally different GDH's has also been demonstrated in *Thiobacillus novellus* (LéJohn and McCrea, 1968) and *Hydrogenomonas* H 16 (Krämer, 1970).

In *Klebsiella aerogenes*, biosynthetic GDH is regulated by GS (Brenchley et al., 1973). Although NADP-GDH activities were inversely correlated with GS levels, it is not yet possible to draw conclusions regarding the involvement of GS in the repression of NADP-GDH in *P. aeruginosa*. The same holds true for the regulation of urease synthesis, which was highest under nitrogen-limited conditions, as in *K. aerogenes* (Friedrich and Magasanik, 1977). Some indication for the involvement of GS in the regulation of the hut genes of *P. aeruginosa* came from the observed escape from catabolite repression of histidase under nitrogen-limited conditions (Potts and Clarke, 1976). Further conclusions about the regulation of histidase, NADP-GDH, urease and possibly also enzymes of the allantoin metabolism by GS can only be drawn after studies with mutants that contain no or an altered GS. In preliminary experiments, however, glutamine auxotrophs of our strain invariably showed high reversion rates and the same was found with *P. aeruginosa* strain PAO. The development of mutagenesis and enrichment procedures suitable for the isolation of GS-mutants is under study now.

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