Lack of a Regulatory Function for Glutamine Synthetase Protein in the Synthesis of Glutamate Dehydrogenase and Nitrite Reductase in *Escherichia coli* K12

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SUMMARY

Synthesis of glutamine synthetase (GS) in anaerobic batch cultures of *Escherichia coli* was repressed when excess NH_4^+ was available, but derepressed during growth with a poor nitrogen source. In wild-type bacteria there was only a weak inverse correlation between the activities of GS and glutamate dehydrogenase (GDH) during growth in various media. No positive correlations were found between the activities of GS and nitrite reductase, or between GS and cytochrome c_{552} : both of these proteins were synthesized normally by mutants that contained no active GS. Although activities of GS and GDH were low in two mutants that are unable to synthesize cytochrome c_{552} or reduce nitrite because of defects in the *nirA* gene, the *nirA* defect was separated from the GS and GDH defects by transduction with bacteriophage P1. Attempts to show that catabolite repression of proline oxidase synthesis could be relieved during NH_4^+ starvation also failed. It is, therefore, unlikely that nitrite reduction or proline oxidation by *E. coli* are under positive control by GS protein. The regulation of the synthesis of enzymes for the utilization of secondary nitrogen sources in *E. coli* is, therefore, different from that in *Klebsiella aerogenes*, but is similar to that in *Salmonella typhimurium*.

INTRODUCTION

We recently reported that continuous cultures of *Escherichia coli* strains OR75 and Hfr Cavalli can grow anaerobically with nitrite as the sole nitrogen source (Cole *et al.*, 1974). Yields of bacteria were similar when growth was limited by the concentration of either nitrite or ammonium in the feed, and under both conditions synthesis of nitrite reductase, cytochrome c_{552} , glutamate dehydrogenase (GDH) and glutamine synthetase (GS) were derepressed. The concomitant derepression or induction of GS and GDH in *E. coli* was unexpected because it was inconsistent with the proposed mechanism for regulation of GDH synthesis in the related bacterium, *Klebsiella aerogenes* (Brenchley, Prival & Magasanik, 1973). It was suggested that in the latter organism, GS is the repressor for GDH synthesis because the activity of GDH was inversely proportional to the activity of GS during growth in different media: furthermore, GS synthesis was regulated normally in mutants unable to synthesize GDH, but GDH synthesis was derepressed in mutants defective in GS (Brenchley *et al.*, 1973). Although GS synthesis in *E. coli* appeared to be regulated by NH_4^+ and by glutamine, as in *K. aerogenes*, we suggested that GDH synthesis might be regulated differently in the two organisms (Cole *et al.*, 1974).

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Table 1. *Origin of strains of E. coli*

Strain	Phenotype or genotype†	Source or method of isolation
Hfr Cavalli	Hfr <i>met</i>	NCIB10237; NCIB, Torrey Research Station, Aberdeen
OR56	Prototrophic Hfr	R. Curtiss III
OR75	Prototrophic Hfr	R. Curtiss III
OR75chl5	Chl ⁻ Gas ⁻ Nar ⁻	From OR75: spontaneously resistant to 10 mM-KClO ₃ during anaerobic growth on nutrient agar+0.8 % (w/v) glucose
FS323	<i>glnA his</i>	B. A. Tyler
*CB40	<i>nirA1</i>	See Cole & Ward (1973)
CB64	<i>cysB trp</i>	M. Jones-Mortimer
CB70	<i>glnA67</i>	S. B. Primrose
CB71	<i>glnA⁺ met⁺</i>	Conjugate CB70 × Hfr Cavalli for 60 min at 37 °C. Select for Gln ⁺ ; purify Gln ⁺ Met ⁺ recombinants
CB72	<i>glnA⁺</i>	Transduce CB70 with bacteriophage P1 propagated on OR75. Then as for CB71
CB98	<i>nirA1 cysB⁺ pro trp his</i>	<i>E. coli</i> F ⁻ <i>pro his trp cysB</i> × P1 propagated on CB40. Select Cys ⁺ transductants; purify Cys ⁺ Nir ⁻ clone
*CB207	<i>nirA2</i>	NTG mutagenesis of OR56. Select small colonies after anaerobic growth with 5 mM-NO ₃ ⁻ +0.1 mM-NH ₄ ⁺ as nitrogen source. The <i>nir</i> defect was cotransducible with <i>cysB⁺</i>
CB222	<i>nirA1 pro trp recA</i>	Conjugate CB98 with KL16-99. Select for His ⁺ ; purify Rec ⁻ Nir ⁻ clone
CB240	<i>glnA his Str⁻</i>	FS323 spontaneously resistant to 100 µg streptomycin sulphate ml ⁻¹
CB240 F' <i>glnA⁺</i>	<i>glnA Str⁻/glnA⁺</i>	Conjugate CB240 with JC1553/KLF133. Purify Gln ⁺ Str ⁻ clone
CB241	<i>glnA his⁺ recA Str⁻</i>	Conjugate CB240 with KL16-99. Select for His ⁺ ; purify Rec ⁻ Gln ⁻ clone
CB241 F' <i>glnA⁺</i>	<i>glnA recA Str⁻/glnA⁺</i>	Conjugate CB241 with JC1553/KLF133. Purify Gln ⁺ Str ⁻ clone
CB243 and CB244	<i>nirA1 cysB⁺ trp</i>	CB64 × P1 propagated on CB40. Purify Cys ⁺ Nir ⁻ clones
JC1553/KLF133	<i>glnA⁺/glnA⁺ recA</i>	B. Bachmann
KL16-99	Hfr <i>recA1 thi</i>	M. Jones-Mortimer

* CB40 and CB207 carry at least one other mutation in a gene which regulates GS and GDH synthesis.

† The symbols *nir* and Nir⁻ refer to the genotype and phenotype of mutants that are unable to reduce nitrite during anaerobic growth.

The growth conditions used by Magasanik and his colleagues differed from those of our earlier experiments, not only because *K. aerogenes* was grown aerobically instead of anaerobically, but also because bacteria were grown in batch rather than in continuous culture. We now report the results of experiments designed to show how GS and GDH synthesis in anaerobic batch cultures of *E. coli* are regulated by the composition of the growth medium, and whether GS regulates GDH synthesis in this organism.

In addition to its catalytic role and its postulated role as a repressor of GDH genes, unadenylylated GS protein activates transcription of *Klebsiella* genes that are involved in the metabolism of secondary nitrogen sources such as histidine and possibly elemental nitrogen (Tyler, Deleo & Magasanik, 1974; Tubb, 1974; Streicher *et al.*, 1974). Not only was there a strong correlation between the activities of GS and nitrite reductase during continuous culture experiments in which nitrite was the only nitrogen source for growth (Cole *et al.*, 1974), but also mutants have been isolated that appear to be defective in the regulation of both of these proteins (Kavanagh & Cole, 1976). We have, therefore, investigated whether GS protein is required for nitrite reduction and cytochrome *c*₅₅₂ synthesis in *E. coli*.

Fig. 1. Growth rate of *E. coli* OR75 in nitrogen-free salts plus 0.8 % (w/v) glucose supplemented with: 0.1 g Casamino acids l^{-1} (●); 1.0 g Casamino acids l^{-1} plus 20 mM- NH_4Cl (○); 20 mM- NH_4Cl (□); or 5 mM- $NaNO_2$ (■). Conical flasks (2 l capacity) containing 1.8 l medium equilibrated at 37 °C were inoculated with 200 ml of a stationary-phase culture of *E. coli* OR75 in similar medium. The arrows indicate the stages in the growth cycle at which bacteria were harvested for enzyme analyses.

METHODS

The origins of the various strains of *Escherichia coli* are presented in Table 1. Nitrogen-free (NF) salts contained (per l distilled water): 10.5 g K_2HPO_4 ; 4.5 g KH_2PO_4 ; 1 g trisodium citrate; 125 mg Na_2SO_4 ; and 5 ml sulphur-free trace metals (Cole *et al.*, 1974). The carbon and energy source was glucose at 27 mM, and nitrogen sources were as indicated in the text: these were added aseptically before inoculation from sterile stock solutions of 40 % (w/v) glucose, 20 % (w/v) Casamino acids, 2 M- NH_4Cl and 1 M- $NaNO_2$. Inocula for batch cultures were aerated at 37 °C for 6 to 8 h in 20 ml Lennox (1955) broth, and were transferred to 200 ml of prewarmed NF salts in a 250 ml conical flask. After 16 h at 37 °C without aeration, the whole culture was transferred into 1.8 l NF salts in a 2 l conical flask.

Bacteria were harvested and washed as described previously (Cole *et al.*, 1974) and were resuspended in two to five times their volume of 50 mM-Tris/HCl plus 5 mM-EDTA plus 5 mM-ascorbate, pH 8.0. They were broken by passage through a French pressure cell at 4 °C and 69 MPa (10 000 lbf in $^{-2}$). Cell extracts were prepared and enzymes were assayed as described previously (Cole *et al.*, 1974). Controls for glutamate synthase assays were performed as recommended by Brenchley (1973).

Lennox (1955) broth was used for conjugation experiments. Recipient and donor cultures in the exponential phase of growth were mixed in a ratio of between 5 and 20:1 with 10 ml broth which had been warmed to 37 °C. After 1 min of vigorous aeration, mating was allowed to proceed with extremely gentle agitation. Transduction procedures were those of Lennox (1955) with only minor modifications.

RESULTS

Growth rates on different nitrogen sources

The mean generation time for *Escherichia coli* OR75 during the exponential phase of anaerobic growth was 1.98 h with 20 mM- NH_4^+ , 1.2 h with 20 mM- NH_4^+ and 1 g Casamino acids l^{-1} ,

Table 2. *Effect of NH₄⁺ during growth on the synthesis of glutamine synthetase by batch cultures of E. coli*

GS activities are expressed in nmol min⁻¹ (mg protein)⁻¹. Induction ratios for GS are expressed as the ratio of means of the GS activities of bacteria grown without and with 20 mM-NH₄⁺ added to 1 g Casamino acids l⁻¹. The number of subunits adenylylated in the GS dodecamer ($\bar{n}$) is calculated from the expression $12(1-a/b)$, where a is the activity of GS assayed with 60 mM-Mg²⁺ and b is the activity without added Mg²⁺. Results of two independent experiments with each nitrogen source are shown so that the reproducibility of the data can be assessed.

Strain	Nitrogen source*	Glutamine synthetase (Mg ²⁺ absent)	Induction ratio	Glutamine synthetase (Mg ²⁺ present)	Induction ratio	$\bar{n}$
OR75	5 mM-NH ₄ ⁺	83	—	60	—	3.3
		98	—	90	—	1.0
	CA	1356	8.4	936	8.9	3.7
		1124		1092		0.3
	CA + 20 mM-NH ₄ ⁺	182		153		1.9
		113		74		4.1
OR75chl5	CA	1430	10.3	974	7.2	3.8
		1110		1161		-ve
	CA + 20 mM-NH ₄ ⁺	166		122		3.2
		188		83		6.7

* CA, 1 g Casamino acids l⁻¹.

1.33 h with Casamino acids alone, and 2.08 h with 5 mM-nitrite as the nitrogen source (Fig. 1). When the nitrogen source was a limited supply of Casamino acids, the growth rate gradually declined at extinctions greater than 0.35, which corresponds to a cell density of 150 mg dry weight l⁻¹. The arrows in Fig. 1 indicate the stages in the growth cycles at which bacteria were harvested for enzyme analyses: no nitrite remained in the culture supernatant when wild-type bacteria were grown with this nitrogen source.

Effect of NH₄⁺ on glutamine synthetase

GS was repressed during growth with 5 mM-NH₄Cl as the source of nitrogen, but de-repressed during growth with 1 g Casamino acids l⁻¹ (compare lines 1 to 4, Table 2). When Casamino acids were supplemented with an excess of NH₄Cl, GS synthesis was again repressed (Table 2, lines 5 and 6). In each of these experiments the activity of GS was only slightly inhibited by 60 mM-Mg²⁺; the enzyme was, therefore, predominantly unadenylylated. Essentially similar data were obtained from experiments with *E. coli* strain CB64 (data not shown) and with a spontaneous mutant of strain OR75 which is resistant to 10 mM-KClO₃ during anaerobic growth (Table 2, lines 7 to 10).

Effect of NH₄⁺ on glutamate dehydrogenase, glutamate synthase, nitrite reductase and cytochrome c₅₅₂ synthesis

The activities of GDH and glutamate synthase were higher when strains OR75 and OR75chl5 were grown with excess NH₄⁺ than during growth with Casamino acids alone, but the GDH activities were not inversely proportional to those of GS (Tables 2 and 3). During growth with excess NH₄⁺, GS was repressed 7- to 10-fold relative to the activity during growth with Casamino acids alone, but GDH activity was only derepressed by a factor of 1.7 to 2.8. Similar induction ratios can be calculated for glutamate synthase (see

Table 3. *Effect of NH₄⁺ during growth on the activities of glutamate dehydrogenase and glutamate synthase*

Enzyme activities are expressed in nmol min⁻¹ (mg protein)⁻¹. Induction ratios are the inverse of those in Table 2, that is, the ratio of the means of the enzyme activities in bacteria grown with and without 20 mM-NH₄⁺ added to the Casamino acids. Results of two independent experiments with each nitrogen source are shown so that the reproducibility of the data can be assessed.

Strain	Nitrogen source*	Glutamate dehydrogenase	Induction ratio	Glutamate synthase	Induction ratio
OR75	CA	168	1.7	126	1.8
		144		81	
	CA + 20 mM-NH ₄ ⁺	279		203	
		237		171	
OR75chl5	CA	181	2.8	141	2.5
		183		89	
	CA + 20 mM-NH ₄ ⁺	630		279	
		378		292	

* CA, 1 g Casamino acids l⁻¹.

Table 4. *Effect of the nitrogen source during growth on cytochrome c₅₅₂ synthesis and nitrite reductase activity*

Enzyme activities are expressed in nmol min⁻¹ (mg protein)⁻¹; cytochrome c₅₅₂ synthesis in pmol (mg protein)⁻¹. Each line of data is the average of at least two experiments.

Strain	Nitrogen source*	Glutamine synthetase (Mg ²⁺ absent)	Nitrite reductase	Cytochrome c ₅₅₂
OR75	CA	1092	63	< 20 to 40
	CA + 20 mM-NH ₄ ⁺	148	89	50
	20 mM-NH ₄ ⁺	171	78	32
	5 mM-NO ₂ ⁻	424	510	286
	20 mM-NH ₄ ⁺ + 5 mM-NO ₂ ⁻	91	437	242
OR75chl5	CA	1270	575	165
	CA + 20 mM-NH ₄ ⁺	177	629	265

* CA, 1 g Casamino acids l⁻¹.

Tables 2 and 3). The activity of GDH during growth with Casamino acids plus 20 mM-NH₄⁺ was far lower (<20 %) than that observed when the same strain was grown either with 5 mM-nitrite, or with 5 mM-nitrite plus 20 mM-NH₄⁺ (Kavanagh & Cole, 1976).

Nitrite reductase activity and cytochrome c₅₅₂ synthesis were partially derepressed or induced during anaerobic growth with 1 g Casamino acids l⁻¹ as the nitrogen source, and specific activities or concentrations of these proteins did not decrease when excess NH₄⁺ was added (Table 4, lines 1 and 2). The highest activities or concentrations of both of these proteins were found in bacteria which had been grown with 5 mM-nitrite as the sole nitrogen source, or with 5 mM-nitrite plus 20 mM-NH₄Cl (Table 4, lines 4 and 5). *Escherichia coli* will only tolerate low concentrations of nitrite, so it was impossible to grow bacteria with a large excess of this nitrogen source. The increased activity of GS in cultures grown with 5 mM-nitrite was not unexpected because bacteria were harvested after the last traces of nitrite had been metabolized: their growth-yield was therefore nitrogen-limiting. However, in contrast

Table 5. Nitrite reduction and ammonia assimilation in mutants defective in nitrite reductase and glutamine synthetase

Enzyme activities are expressed in $\text{nmol min}^{-1} (\text{mg protein})^{-1}$; cytochrome c_{552} synthesis in $\text{pmol} (\text{mg protein})^{-1}$. Data in lines 1 to 3 are the means of enzyme activities in two independent cultures: other data are for single growth experiments.

Strain and relevant genotype	Nitrogen source*	Glutamine synthetase	Glutamate dehydrogenase	Nitrite reductase	Cytochrome c_{552}
1. CB207 <i>nirA2</i>	CA	297	58	< 5	< 20
2. CB207 <i>nirA2</i>	CA + 20 mM- NH_4^+	69	69	< 5	< 20
3. CB222 <i>nirA1</i>	CA	23	94	< 5	< 20
4. CB244 <i>cysB</i> ⁺ <i>nirA1</i>	CA	1100	195	< 5	< 20
5. CB244 <i>cysB</i> ⁺ <i>nirA1</i>	2.5 mM- NO_2^- + 20 mM- NH_4^+	178	266	31	< 20
6. CB243 <i>cysB</i> ⁺ <i>nirA1</i>	CA	1248	185	< 5	< 20
7. CB243 <i>cysB</i> ⁺ <i>nirA1</i>	2.5 mM- NO_2^- + 20 mM- NH_4^+	176	311	28	< 20
8. CB70 <i>glnA67</i>	CA + 2 mg glutamine ml^{-1}	5	513	260	35
9. CB71 <i>glnA</i> ⁺	CA	473	238	145	58
10. CB72 <i>glnA</i> ⁺	CA + 2 mg glutamine ml^{-1}	100	610	96	36
11. CB240 <i>glnA</i>	CA + 2 mg glutamine ml^{-1}	12	77	60	< 20
12. CB240 <i>glnA</i> /F' <i>glnA</i> ⁺	CA	134	132	58	< 20
13. CB241 <i>glnA recA</i>	NB + 5 mM- NO_2^- + 2 mg glutamine ml^{-1}	13	98	178	91
14. CB241 <i>glnA recA</i> /F' <i>glnA</i> ⁺	NB + 5 mM- NO_2^-	60	150	230	68

* CA, 1 g Casamino acids l^{-1} ; NB, normal strength nutrient broth (Oxoid).

to the data from the earlier continuous culture experiments (Cole *et al.*, 1974), there were no correlations between the activities of GS and nitrite reductase, or between GS and the concentration of cytochrome c_{552} . Subsequent experiments were therefore designed to investigate whether GS protein is required for nitrite reduction to occur in these bacteria.

Effects of mutations in genes for glutamine synthetase and nitrite reductase

Mutants unable to reduce nitrite due to defects in the *nirA* gene were isolated from two strains of *E. coli*: CB222 is a derivative of CB40, the original *nirA1* mutant described by Cole & Ward (1973); CB207 was selected for further study from a group of new isolates with the *NirA*⁻ phenotype (Newman & Cole, unpublished). Both CB207 and CB222 lacked nitrite reductase activity and cytochrome c_{552} , and GS and GDH were at least partially repressed irrespective of whether the supply of NH_4^+ was limited or in excess (Table 5, lines 1 to 3). We were unable to demonstrate whether GS synthesis was regulated normally by NH_4^+ in CB40 because no growth occurred when this strain was transferred to media used to demonstrate derepression of GS in wild-type bacteria. The *nirA1* defect carried by strain CB222 was transduced by bacteriophage P1 from CB40 into CB64 by selecting *Cys*⁺ transductants that were unable to reduce nitrite. In four out of four of these transductants, synthesis of GS and GDH were regulated normally by NH_4^+ , but cytochrome c_{552} and nitrite reductase were absent: typical data are shown in lines 4 to 7 of Table 5. Six other *pyrF*⁺ *nirA* or *cysB*⁺ *nirA* transductants constructed with phage which had been propagated on three different *nirA* mutants grew normally in nitrogen-limited media. The original *nirA* isolates, therefore, carry multiple mutations, one in the *nirA* gene and at least one other that affects the rates of GS and GDH synthesis and growth in nitrogen-limited media. However, the loss of nitrite reductase activity and cytochrome c_{552} synthesis was due to the defective *nirA* gene rather than to the defective synthesis of GS protein.

GS was virtually absent from *E. coli* CB70 which carries the *glnA67* defect in the structural gene for GS (Berberich, 1972). Nitrite reductase and GDH were more active in this strain than in OR75, and cytochrome *c*₅₅₂ was also detected (Table 5, line 8). The *glnA*⁺ allele from *E. coli* Hfr Cavalli was introduced into strain CB70 by conjugation, and recombinants that were both Gln⁺ and Met⁺ were purified by repeated single colony isolations on nutrient agar. Activities of nitrite reductase and GDH in these strains were lower than in CB70, but synthesis of GS had been restored: data for one of these strains, CB71, are shown in line 9 of Table 5. Similar data were obtained with strain CB72, a derivative of CB70 in which the *glnA*⁺ allele from OR75 had been introduced by transduction with bacteriophage P1 (Table 5, line 10). Two other strains CB240 and CB241 were constructed which carried the *glnA* defect of *E. coli* strain FS323. CB241 was a His⁺ RecA⁻ derivative of CB240 which grew very poorly in minimal media: nutrient broth plus 5 mM-nitrite was therefore used to obtain sufficient bacteria in which nitrite reductase and cytochrome *c*₅₅₂ synthesis had been induced. Nitrite reductase activity was detected in both of these mutants, and although GS was virtually absent, GDH activity was also low (Table 5, lines 11 and 13). When the episome KLF133 which carried a *glnA*⁺ allele was transferred into these strains, GS activity was partially restored and the activity of GDH also increased: however, nitrite reductase activity and cytochrome *c*₅₅₂ synthesis were unaffected (Table 5, lines 12 and 14).

Growth with proline as the nitrogen source

Histidine can be used either as a carbon source or as a nitrogen source for the growth of *Klebsiella aerogenes*. In the absence of a carbon source that can be metabolized more readily than histidine, cyclic adenosine 3':5'-monophosphate (cyclic AMP) and the cyclic AMP-binding protein activate transcription of *hut* genes which specify the enzymes for histidine catabolism. These genes are also activated by unadenylylated GS when histidine is the only available nitrogen source (Tyler *et al.*, 1974). Thus, catabolite repression of the *hut* operon is effectively reversed by NH₄⁺-starvation, and NH₄⁺-repression due to the lack of unadenylylated GS is reversed by depriving these bacteria of alternative carbon sources. Proline metabolism by *K. aerogenes* is believed to be subject to similar dual controls (Prival, Brenchley & Magasanik, 1973), and, unlike histidine, proline is able to supply either carbon or nitrogen to support the growth of *E. coli*. If GS activates transcription of the genes for proline oxidase in *E. coli*, then wild-type bacteria should grow equally well with proline as the sole nitrogen source with a strong catabolite repressor (Epstein, Rothman-Denes & Hesse, 1975). *Escherichia coli* OR75 was, therefore, plated on to solid media in which either glucose or glycerol was the carbon source, and either 10 mM-proline or 10 mM-NH₄⁺ was the nitrogen source. After 48 h at 37 °C, very little growth had occurred on any of the proline plates, but large translucent colonies had formed on either type of NH₄⁺ plate. After 72 to 96 h a mixture of normal and mucoid colonies had formed on the proline plus glycerol plates, but little growth had occurred on proline plus glucose. Bacteria from both the mucoid and the translucent colonies produced both types of clone when subcultured on to similar media, and growth on proline plus glycerol was always more rapid than growth on proline plus glucose. It appeared, therefore, that proline catabolism in *E. coli* was subject to strong catabolite repression by glucose even when NH₄⁺ was absent and when GS was presumably derepressed.

DISCUSSION

Synthesis of GS in *E. coli* OR75 was repressed whenever NH_4^+ was in excess and was apparently induced when batch cultures were supplied with a poor nitrogen source. These data are consistent with those from the earlier continuous culture experiments (Cole *et al.*, 1974), and confirm that GS synthesis is regulated by NH_4^+ in this organism as in *Klebsiella aerogenes* (Brenchley *et al.*, 1973), *Salmonella typhimurium* (Brenchley, Baker & Patil, 1975; Betteridge & Ayling, 1976) and *Klebsiella pneumoniae* (Tubb, 1974).

A recently isolated *nirA* mutant, CB207, which lacked nitrite reductase activity and cytochrome c_{552} also had low activities of GS and GDH. Strains CB98 and its derivative, CB222, were constructed from the original *nirA* mutant by cotransducing the *nirA* defect with *cysB*⁺ into a multiple auxotroph: GS and GDH were also apparently repressed in both of these strains (Table 5, and unpublished). Although this suggested that either the *nirA* gene alone or the *nirA* gene and other closely-linked genes regulated the synthesis of all four proteins, GS and GDH were regulated normally when another recipient, CB64, was used to construct *cysB*⁺ *nirA* transductants (Table 5). Subsequent experiments (not described here) have confirmed that the GS and GDH defects of CB40 and CB207 are not closely linked to the *nirA* defect. We therefore conclude that the recipient used to construct CB98 and CB222 must also carry defects in the regulation of GS and GDH. In subsequent experiments, no correlations were found between the cellular concentrations of GS and cytochrome c_{552} , or between GS and nitrite reductase activity. Nitrite reductase and cytochrome c_{552} synthesis were induced by nitrite but they were not repressed by NH_4^+ (Table 4). Although these results are apparently in conflict with earlier data from continuous culture experiments, they are fully supported by studies with mutants defective in GS synthesis: these reduced nitrite normally (Table 5). Furthermore, OR75Ch15, which was resistant to chlorate because of a defect in an unidentified nitrate reductase gene, contained high activities or concentrations of nitrite reductase or cytochrome c_{552} during anaerobic growth without nitrite (Table 4; for the original description of this phenotype, see Venables, Wimpenny & Cole, 1968). This partial constitutivity for nitrite reductase was not due to the constitutive synthesis of GS, however, because GS in this strain was regulated normally by NH_4^+ (Table 2). It is, therefore, unlikely that nitrite reduction and nitrite assimilation by *E. coli* are under positive control by GS protein. Similarly, catabolite repression of proline oxidation by glucose was still apparent when proline was the only nitrogen source for growth. We were, therefore, unable to show that proline oxidase is regulated positively by GS as is histidase synthesis in *K. aerogenes*.

Although GS appears to be the repressor for GDH synthesis in *K. aerogenes*, other factors appear to be at least as important in regulating the activity of GDH in *E. coli*. We have previously suggested that GDH synthesis is stimulated by high intracellular concentrations of 2-oxoglutarate but inhibited by high concentrations of glutamate (Cole *et al.*, 1974). Senior (1975) subsequently demonstrated that there was a direct correlation during growth in continuous culture between the activity of GDH and the 2-oxoglutarate:glutamine ratio. We have also demonstrated that GDH synthesis is apparently induced during anaerobic growth in minimal medium with nitrite, but repressed when Casamino acids or nutrient broth is added to growth media (Kavanagh & Cole, 1976, and unpublished). Presumably Casamino acids and nutrient broth contain one or more corepressors of GDH synthesis, and these could include glutamate and glutamine. One cannot exclude the possibility that GS also repressed GDH synthesis, and data in Table 3 are consistent with this suggestion. It is apparent, however, that if such a regulatory mechanism is operative, it is weak compared

with other controlling factors. Similar conclusions were recently drawn concerning GDH synthesis in *Salmonella typhimurium* (Brenchley *et al.*, 1975).

In conclusion, we have been unable to demonstrate that GS is as important a factor in regulating nitrogen metabolism in *E. coli* as it is in *K. aerogenes*. This is possibly due to differences in the regulatory genes for GDH and proline oxidase in the two organisms rather than to differences in their GS proteins because genes for nitrogen fixation are expressed normally when the *glnA*⁺ gene from *E. coli* is introduced into a *glnA* strain of *K. aerogenes* (Shanmugam & Valentine, 1975).

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