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Glutamate Dehydrogenase: Genetic Mapping and Isolation of Regulatory Mutants of *Klebsiella aerogenes*

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The gene for glutamate dehydrogenase (*gdhD*) has been mapped in *Klebsiella aerogenes* by P1 transduction. It is linked to *pyrF* and *trp* with the order *pyrF-trp-gdh*. Complementation analysis using F' episomes from *Escherichia coli* suggests an analogous location in *E. coli*. Two mutants able to produce glutamate dehydrogenase in the presence of high levels of glutamine synthetase have been isolated. One, tightly linked to *gdhD*, shows normal repression control by glutamine synthetase but produces four times as much glutamate dehydrogenase activity as does the wild type under all conditions tested. The other revertant is not linked to *gdhD* or *glnA*.

Glutamate dehydrogenase (EC 1.4.1.4) (GDH) was once thought to be the principal means of assimilating free ammonia in enteric bacteria. The regulation of the levels of GDH in *Klebsiella aerogenes* led Tempest (13) to his discovery of a second pathway for ammonia assimilation (22) via the pair of enzymes glutamine synthetase and glutamate synthase. Brenchley and Magasanik (2) have shown that mutants of *K. aerogenes* lacking GDH have no observable phenotype whatsoever, confirming the use of the second pathway for ammonia assimilation. Although strains carrying a *gdhD* mutation (lacking GDH) have no selectable phenotype, introduction of an ancillary lesion such as *asm* (lacking glutamate synthase) leads to a requirement for glutamate not present when the strain is gdh^+. This has allowed us to map the *gdhD* gene in *K. aerogenes*.

Brenchley et al. (3) have established that glutamine synthetase plays a role in the control of GDH levels. In mutants in which glutamine synthetase is absent ($GlnA^-$), GDH levels are high whether ammonia is limiting or in excess; in mutants in which glutamine synthetase is produced at high levels under all conditions ($GlnC^-$), GDH levels are severely depressed and the strain is phenotypically Gdh^-. Thus, a $GlnC^-$ strain carrying an *asm* mutation requires glutamate for growth on glucose-ammonia minimal medium, even though it is genetically gdh^+. We have used this strain to select glutamate-independent revertants in an attempt to isolate mutants affected in the regulatory system controlling GDH levels. Two such mutants are described here.

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MATERIALS AND METHODS

Bacterial strains. Bacterial strains used in this study are listed in Table 1. *K. aerogenes* strains used were P1-sensitive derivatives (6) of *K. aerogenes* W-70. *Escherichia coli* strains used were derivatives of *E. coli* K-12 and were kindly provided by Barbara Bachmann.

Media and genetic procedures. Minimal medium (W), complex medium (LB), and all genetic procedures used in these experiments have been described previously (15). In general, 0.2% L-glutamine was used as the source of glutamate supplementation. Glutamate-requiring mutants of *K. aerogenes* form much smaller colonies than do wild type, even when glutamate is provided at 0.5%. When these mutants are supplied with glutamine, there is less of a difference. This presumably reflects a difficulty in transporting glutamate and, to a lesser extent, glutamine.

Enzyme assays. Glutamine synthetase was assayed by the γ-glutamyl transferase reaction described previously (14), except that the pH was adjusted to 7.55 where the adenylylated and nonadenylylated forms of the glutamine synthetase of *K. aerogenes* are isoactive (5). Glutamate synthase was assayed as described previously (3), and the values reported include the correction for interference from glutamate dehydrogenase in the sample described by Brenchley et al. (3). Histidase and glutamate dehydrogenase were assayed as described previously (3). Cells were grown in 150- to 200-ml cultures in 1-liter Fernbach flasks on a reciprocating shaker at 30 C (except for the experiments reported in Table 6 where the temperature was 37 C). Cultures were harvested during exponential growth (5×10^8 to 6×10^8 cells/ml) by centrifugation; the cells were washed once in 10 ml of 1% KCl and resuspended in 1.5 ml of buffer containing 10 mM imidazole-HCl, 10 mM $MnCl_2$, and 2 mM mercaptoethanol adjusted to pH 7.15. Crude extracts were prepared by sonic oscillation at 0 C (four bursts of 15 s with 30-s pauses in between), followed by centrifugation at $3,000 \times g$

TABLE 1. *Strain list*

Strain	Relevant characteristics	Source or reference
K. aerogenes W-70		
MK9000	Wild type	(15)
MK9011	*ilvA1 glnA6*[a]	(15)
MK9201	*asm-200 rha-6 glnA50*[a]	This laboratory
MK9204	*asm-200 glnA45*[a]	P1[s] of MK204 (3)
MK9514	*glnA45*[a]	This laboratory
MK9663	*asm-200 gdhD1*	P1[s] of MK261 (3)
MK9669	*asm-200 gdhD1 pyrF1*	EMS[b] of MK9663
MK9672	*asm-200 gdhD1 trp-1*	EMS of MK9663
MK9678	*asm-200 pyrF1*	P1·MK9204 × MK9669[c]
MK9681	*asm-200*	P1·MK9204 × MK9663[c]
MK9682	*asm-200 glnA45*[a]	P1[s] of MK204 (3)
MK9683	As MK9682 but Rev-5[d]	This paper
MK9684	As MK9682 but Rev-2[d]	This paper
MK9685	As MK9682 but Rev-3	This paper
MK9686	As MK9682 but Rev-4	This paper
MK9698	*asm-200*	P1·MK9000 × MK9672[c]
MK9701	*asm-200* Rev-2	P1·MK9684 × MK9672[c]
MK9708	*asm-200 glnA45*[a] *trp-1*	This laboratory
MK9715	Prototroph	P1·MK9000 × MK9698[c]
MK9716	Rev-2	P1·MK9000 × MK9701[c]
E. coli K-12		
KLF23	F' 123	B. Bachman (8)
KLF26	F' 126	B. Bachman (8)
KLF47	F' 147	B. Bachman (8)
KLF52	F' 152	B. Bachman (8)

[a] Mutants in *glnA* used here have two very different phenotypes: *glnA45* is GlnC⁻ (producing high levels of enzymatically active glutamine synthetase, even in the presence of excess ammonia), but *glnA50* and *glnA6* are GlnA⁻ (producing no enzymatically active glutamine synthetase under any condition and hence glutamine auxotrophs).

[b] EMS, Ethyl methane sulfonate.

[c] P1-mediated transduction.

[d] The mutations leading to the Rev-5 and Rev-2 phenotypes have been tentatively assigned the genetic designation *gdh-5* and *gdh-2*, respectively. For simplicity, the former designations are used throughout this communication.

for 20 min to remove the debris. Protein content of the crude extracts was determined by the method of Lowry et al. (9) using bovine serum albumin as the standard.

RESULTS

Strain MK9663 is a derivative of strain MK261 (3) selected for sensitivity to phage P1 (6). Since this strain carries the *asm-200* and *gdhD1* alleles, it requires glutamate for growth in glucose-ammonia minimal medium. Berberich (1) has indicated that the *gdh* locus of *E. coli* lies somewhere between 15 and 30 min on the Taylor and Trotter map (16) (see Fig. 1). We confirmed this observation by using F' episomes from *E. coli* to complement the *gdhD* mutation of strain MK9663. Figure 2 shows the *E. coli* map from 14 to 30 min and the regions covered by each of the episomes used here (8). Of the four episomes tested, only F' 126 gave growth on minimal medium without glutamate supplementation, suggesting that the *gdh* locus between *pyrD* (21.5 min) and *trp* (27 min). Since the *E. coli* and *K. aerogenes* genetic maps appear to be largely analogous (7, 15), we began to select markers in this region to establish linkage with P1 transduction.

After ethyl methane sulfonate mutagenesis and penicillin enrichment, several auxotrophic derivatives of strain MK9663 were isolated that could be supplemented by cytidine (Pyr⁻) and several that could be supplemented by tryptophan (Trp⁻). All of the Pyr⁻ strains and one stable Trp⁻ strain were transduced to wild type selecting for growth on minimal medium with a glutamate supplement. The Pyr⁺ and Trp⁺ transductants were then scored for growth on minimal medium to establish linkage to *gdh*. The *trp* mutation (*trp-1*) and one *pyr* mutation (*pyr-1*) showed cotransducibility with *gdh* and were used in the rest of the study.

To eliminate the possiblilty that we had cotransduced *asm* rather than *gdh*, we tested the cotransductants for growth on minimal me-

dium with serine or histidine as the only source of nitrogen. As expected for Asm^-, Gdh^+ strains (3), they did not grow on these media but did grow when these media contained, in addition, 0.2% ammonium sulfate. This established the linkage of *gdh* to *trp-1* and *pyr-1*.

This region of the map (Fig. 2) is rich in *pyr* markers, but episome complementation resolves them. F′ 147 carries only *pyrD*, F′ 123 carries only *pyrF*, and F′ 126 carries *pyrD*, *pyrF*, and also *pyrC*. The *pyr-1* marker was complemented by F′ 123 and F′ 126 but not by F′ 147, indicating that *pyr-1* is a mutation in the *K. aerogenes* gene analogous to *pyrF* of *E. coli*. We, therefore, refer to *pyr-1* as *pyrF1*. The *trp-1* marker is complemented by the same two episomes and is presumed to be a mutation in the *trp* operon.

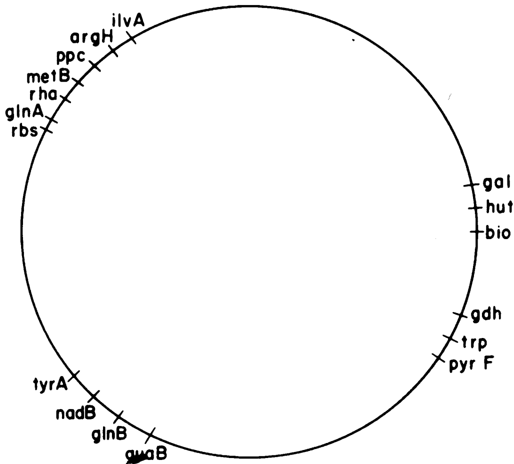

FIG. 1. *Schematic map of the K. aerogenes chromosome patterned after Taylor and Trotter (16) showing the markers discussed in this paper and others (7, 15). The relative positions of the four linkage groups shown have not been established and are assumed by analogy to the map of E. coli.*

Although this episome complementation helps us to define which genes are mutant, it does not give us any information about their position on the *K. aerogenes* chromosome. In *K. aerogenes* this can be established only by P1 cotransduction data. Table 2 shows the results of a transduction experiment which establishes the order of *pyrF-trp-gdhD*. If we consider the markers two at a time, crosses 1 and 2 give the *pyr-trp* linkage as 51/104 and 65/138, respectively, or about 48%. Crosses 1 and 3 give the *pyr-gdh* linkage as 1/104 and 11/96, respectively. This discrepancy, though not inordinate, deserves comment. In reconstruction experiments we find that Asm^- Gdh^- cells form smaller colonies than do Asm^- Gdh^+ cells, even

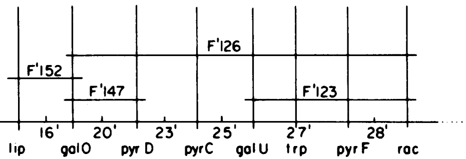

FIG. 2. *Map of the E. coli chromosome from 15 to 30 min (16) showing the regions covered by the four F′ episomes described in the text (not drawn to scale).*

TABLE 2. *Mapping of gdh*[a]

Cross no.	Donor (relevant genotype)	Recipient (relevant genotype)	Selected phenotype (no. analyzed)	Unselected phenotypes	Frequencies of unselected phenotypes
1	MK9672	MK9678	Pyr^+ (104)	Trp^+ Gdh^+	53
	(gdhD1 trp-1)	*(pyrF1)*		Trp^+ Gdh^-	0
				Trp^- Gdh^+	50
				Trp^- Gdh^-	1
2	MK9678	MK9672	Trp^+ (138)	Pyr^+ Gdh^+	53
	(pyrF1)	*(gdhD1 trp-1)*		Pyr^+ Gdh^-	20
				Pyr^- Gdh^+	17
				Pyr^- Gdh^-	48
3	MK9678	MK9672	Gdh^+ (96)	Pyr^+ Trp^+	24
	(pyrF1)	*(gdhD1 trp-1)*		Pyr^+ Trp^-	61
				Pyr^- Trp^+	11
				Pyr^- Trp^-	0

[a] Recipients were transduced with P1 grown on the donors (see text). Pyr^+ selection was done on GN Gln Trp plates; Trp^+ selection, on GN Gln Cyt plates; and Gdh^+ selection, on GN Trp Pyr plates. [G = 0.4% glucose, N = 0.2% $(NH_4)_2SO_4$, Gln = 0.2% glutamine, Cyt = 0.01% cytidine, Trp = 0.01% tryptophan]. Transductants were purified by streaking for single colonies before unselected phenotypes were tested.

when glutamine is the source of glutamate supplementation; when glutamate itself is used, the difference is still greater. This is presumed to reflect the poor transport of these substances by *K. aerogenes*. When *gdh* is an unselected marker in these crosses, it will introduce a bias favoring high cotransduction when the donor carries gdh^+ and favoring lower cotransduction when the donor carries *gdh*. Therefore, we cite linkages involving *gdh* only from data where *gdh* is the selected marker and where the reciprocal (control) cross gives a bias in the expected direction. The *pyr-gdh* linkage is then about 1%. Finally, crosses 2 and 3 give us the *trp-gdh* linkage as 70/138 and 35/96 or (by the argument above) about 36%. These data require that *trp* lie between *pyrF* and *gdh*.

The data in Table 2 can also be analyzed as a three-factor cross where the frequency of double crossover events should be much higher than quadruple crossover events. Of the three possible orders, cross 1 eliminates *pyrF-gdh-trp* because, if this were the order, then nearly half (50/104) of the $pyrF^+$ transductants would have received the distal *(trp)* marker from the donor without receiving the proximal *(gdh)* marker. Fifty percent is too high for such a quadruple crossover and we can eliminate this order. The data are, however, consistent with the order *pyrF-trp-gdh* or *trp-pyrF-gdh*. Cross 3 eliminates the latter possibility since a large percentage (24/96) of the gdh^+ transductants would have received the distal marker *(trp)* from the donor without receiving the proximal marker *(pyrF)*. As illustrated in Fig. 3, the data in cross 2 are consistent only with the order *pyrF-trp-gdh* (Fig. 3a). If the order were *pyrF-gdh-trp* (Fig. 3b), then 35% (48/138) of the trp^+ transductants would have undergone the quadruple event as shown to give *pyrFgdh*. If the order were *trp-pyrF-gdh* (Fig. 3c), then 38% (53/138) would have undergone the quadruple event as shown to give trp^+pyrF^+. Thus, on the basis of cotransduction frequencies and on the basis of a three-factor analysis, we propose the order *pyrF-trp-gdhD*.

Isolation and characterization of regulatory mutants affecting GDH levels. Previous work (3) has shown that the levels of GDH are regulated in response to the levels of glutamine synthetase in the cell. In mutants where glutamine synthetase is produced at high levels under all conditions ($GlnC^-$), GDH levels are severely depressed and the strain is phenotypically Gdh^-. Strain MK9204 *(glnA45,asm-200)* is a glutamate auxotroph because the *asm-200* mutation eliminates glutamate synthase activity and the *glnA45* mutation makes the strain

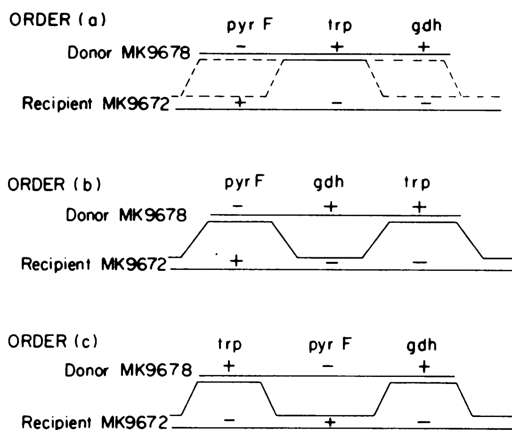

FIG. 3. *Three possible orders for the markers pyrF, trp, and gdhD. (a) The order deduced from analysis of data in Table 2. The dotted lines indicate the recombination events that would be required to generate the four classes of recombinants found in cross 2 of Table 2. (b) An order rejected because of the high frequency of $pyrF^-$,$gdhD^-$ among the trp^+ recombinants. The solid line shows the quadruple crossover event required to generate this class assuming this order. (c) An order rejected because of the high frequency of $pyrF^+$,$gdhD^+$ among the trp^+ recombinants, requiring a quadruple crossover event as shown.*

$GlnC^-$ and thus Gdh^-. Using this strain, we looked for regulatory mutants where GDH had escaped from the repression mediated by glutamine synthetase.

Strain MK9204 was plated on glucose-ammonia minimal medium without glutamate supplementation, and revertants able to grow were isolated at a frequency of about 10^{-7}. At least three classes of revertants would be predicted: (i) by reversion at *asm*, (ii) by reversion at *glnA*, and (iii) by relief of the Gdh^- phenotype. Certain growth characteristics associated with each of the mutations allow a preliminary screen. Growth characteristics of representative revertants and reference strains are shown in Table 3. Strains carrying the *asm-200* mutation cannot grow on a poor nitrogen source like serine because of a lack of glutamate synthase, which is required for the "low-ammonia" pathway for ammonia assimilation (MK9681). Thus, strain MK9685 appears to be a simple Asm^+ revertant. $GlnC^-$ strains (MK9514) produce a brown color when plated on media containing 0.2% ammonium sulfate and 0.2% tryptophan (14). Thus, strain MK9686 appears to be a $glnA^+$ revertant. Growth on glucose-histidine minimal medium also supports these interpretation: $GlnC^-$ strains grow on glucose-histidine

TABLE 3. *Growth tests of revertants and reference strains*[a]

Strain	Relevant characteristics	Growth tests			CN^R color test (14)
		GSer	GH	GN	
MK9000	Wild type	+	+	+	White
MK9681	Asm^-	–	–	+	White
MK9682	Asm^- $GlnC^-$	–	+	–	Brown
MK9683	Rev-5	–	+	+	Brown
MK9684	Rev-2	–	+	+	White
MK9685	Rev-3	+	–/+	+	Brown
MK9686	Rev-4	–	–	+	White

[a] Growth of strain was tested by streaking for single colonies on GSer, GH, and GN [minimal medium containing 0.4% glucose and 0.2% L-serine, L-histidine, or $(NH_4)_2SO_4$, respectively]. For the color test, the plates contained 0.4% sodium citrate and 0.2% each of $(NH_4)_2SO_4$, L-glutamine, and L-tryptophan. Plates were incubated 24 to 48 h, and plates were scored for presence of a brown pigment. Of six revertants analyzed, Rev-5, Rev-2, and Rev-3 are unique and there were two additional revertants with the same growth pattern as Rev-4.

whether they are Asm^+ or Asm^-, but Asm^- strains grow on glucose-histidine only if they are $GlnC^-$ (3). Strains MK9683 and MK9684 both grow on glucose-histidine and both fail to grow on glucose-serine. This suggests that they are still *asm-200glnA45* and that the reversion has abolished the Gdh^- phenotype. Strain MK9684 scores as a $GlnC^+$ on the tryptophan-brown color test despite the other growth indications. Assays, however, show that the strain is in fact still $GlnC^-$ (data not shown).

We next attempted to map the two revertants. Linkage to *trp* was tested to check possible location near *gdhD*. The *asm-200* and *glnA45* mutations were included in the recipient to allow phenotypic expression of the reversion marker. As shown in Table 4, the reversion (Rev-2) in strain MK9684 is about [illegible]% linked to *trp*, similar to the 36% *trp-gdh* linkage reported above. These data do not allow us to determine conclusively on which side of the *trp* the reversion site lies. Since the order of markers in the region is *pyrF-trp-gdhD* and the *pyrF-trp* linkage is nearly 50%, if the reversion site were not on the *gdh* side of trp, it would have to be farther from *trp* (and hence from *gdhD*) than *pyrF*. Since *pyrF* and *gdhD* are only about 1% linked, it is unlikely that the reversion site would be linked to *gdhD* at all. Since strain MK 9716 was cotransduced for the *gdh* region and the reversion site of the revertant strain MK 9684 (see below and Table 5), the reversion site must be tightly linked to *gdh*.

The reversion site in strain MK 9683 (Rev-5), however, showed no linkage to *trp*. Since strain MK 9683 might have an altered glutamine syn-

TABLE 4. *Linkage of the Rev-2 site to trp*[a]

Donor	Recipient	Selected marker	No. of transductants tested	No. able to grow on GN
9683 (Rev-5)	9708 (*asm-200 glnA45 trp-1)*	Trp^+	92	0
9684 (Rev-2)	9708 (*asm-200 glnA45 trp-1)*	Trp^+	98	32

[a] P1 transductants of MK9708 were selected for Trp^+ on plates containing 0.4% glucose, 0.2% $(NH_4)_2SO_4$, and 0.2% glutamine. Transductants were purified by streaking for single colonies on selective medium and were then tested for ability to grow on GN plates [containing 0.4% glucose and 0.2% $(NH_4)_2SO_4$ but no source of glutamate].

TABLE 5. *Levels of GDH in revertants and reference strains*[a]

Strain and relevant characteristics	Growth medium	Sp act of GDH
MK9000 (wild type)	H	105
	GH	8
	GNH	265
MK9682 ($GlnC^-$, Gdh^-) *(asm-200, glnA45)*	H	37
	GH	22
	GNH	25
MK9011 ($GlnA^-$, Gdh^+) *(glnA6)*	Hgln	130
	GHgln	330
MK9683 ($GlnC^-$, Gdh^+)	GH	145
	GNH	130
MK9684 ($GlnC^-$, Gdh^+)	GH	97
	GNH	110

[a] Crude lysates were prepared and assayed as described in the text. Growth media were H)0.4% histidine), GH (0.4% glucose and 0.2% histidine), and GNH [0.4% glucose, 0.2% $(NH_4)_2SO_4$, and 0.2% histidine]. Growth media for MK9011 were supplemented with 0.2% L-glutamine. Specific activities are given in nanomoles per minute per milligram of protein.

thetase allowing escape of GDH synthesis, we looked directly for linkage to *glnA*. Strain MK 9201 *(asm-200,rha-6,glnA50)*, auxotrophic for glutamine, was transduced to glutamine independence using phage grown on the revertant strain MK 9683. One such transductant, which also has been cotransduced to Rha+ (to insure against reversion of *glnA50*), was selected on glucose-histidine. Here either the original *glnA45* (GlnC) or the putative revertant would allow growth (see Table 2) but not *glnA50* (GlnA-). This transductant still required glutamate (but not glutamine) supplementation for growth on glucose-ammonia medium. This showed that only the *glnA45* mutation and not the reversion site had been transduced into the strain to replace the *glnA50* mutation. Thus, the reversion in strain MK 9683 is not an alteration in the structural gene for glutamine synthetase, *glnA* (4).

Analysis of GDH levels in regulatory mutants. To further characterize the revertants MK 9683 (Rev-5 – reversion site unknown) and MK 9684 (Rev-2 – reversion site linked to *trp*), the glutamate dehydrogenase from these strains was assayed and compared with that of the wild-type strain and of strain MK 9682 *(asm-200,glnA45)*, the parent of the revertants. The data in Table 5 show that the glutamate dehydrogenase levels in the wild-type strain MK 9000 change drastically in response to the nitrogen source. In strain MK 9682 the glutamine synthetase levels are high under all conditions (3) and the GDH levels are quite low. In both revertants the GDH levels are like those of the parent (MK 9682) in that they do not change in response to the nitrogen source, but in both cases the level of GDH is four- to fivefold higher than in the parent. Since the site of the reversion in strain MK 9683 has not yet been found, we could not move this marker into a strain where the effects of the *glnA*+ allele on GDH levels could be studied. The reversion site in strain MK 9684 is linked to *trp*, and it was therefore possible to construct isogenic *glnA*+ strains carrying the *gdh* region from wild type and the *gdh* region from the revertant strain MK 9684. Phage grown on the wild type and on the revertant MK 9684 were used to transduce strain MK 9672 *(asm-200,trp-1,gdhD)* to Trp+. The transductants were then scored for cotransduction of *gdhD* by their ability to grow on glucose ammonia minimal medium. One such cotransductant derived from each cross was saved and purified. To remove the *asm-200* marker, phage grown on wild type were used to transduce these two *asm-200*-carrying strains to Asm+ by selecting for growth on glucose-serine minimal medium. This set of crosses yielded two strains: MK 9715 (prototroph) and MK 9716 (isogenic with MK 9715, except that the *trp-gdh* region is derived from the revertant MK 9684). These strains were grown under various conditions and assayed for the enzymes, as shown in Table 6.

In both strains the glutamine synthetase levels respond normally to ammonia, showing a five- to sixfold repression on glucose-ammonia-histidine as opposed to glucose-histidine medium and a still further two- to threefold repression when histidine is the sole source of carbon and nitrogen. Histidase, an enzyme known to be controlled by glutamine synthetase (17), responds to ammonia identically in the two strains, showing that there is no defect in strain MK 9716 affecting the ability of glutamine synthetase to regulate enzyme synthesis. Likewise, glutamate synthase levels, whose control of *K. aerogenes* is poorly understood, are identical in both strains.

The levels of GDH, however, are three- to fourfold higher in strain MK 9716 than in strain MK 9715 under all three conditions. This agrees with the fourfold effect comparing the parent strain MK 9682 and revertant strain MK 9684 in Table 5. It is important to note that the control of GDH in response to glutamine synthetase in strains MK 9716 and MK 9684 is identical to the control of the wild-type *gdh*+ lacking the reversion site (MK 9715 and MK 9682), except that the levels are elevated fourfold.

GDH is somewhat repressed when cultures are grown on histidine rather than on glucose-ammonia histidine, even though the levels of glutamine synthetase are very low in the histi-

TABLE 6. *Enzyme levels in an isogenic pair of strains with and without the Rev-2 reversion*[a]

Strain	Growth medium	Enzyme activities			
		GS[b]	Histidase[c]	Glutamate synthase[c,d]	GDH[c]
MK9715	GH	2.4	730	140	50
(wild	GNH	0.54	60	240	325
type)	H	0.2	1,200	20	190
MK9716	GH	2.3	800	130	135
(Rev-2)	GNH	0.34	80	240	1,160
	H	0.13	1,350	20	690

[a] Crude lysates were prepared and assays were performed as described in the text. Growth supplements were GH (0.4% glucose and 0.2% ammonium sulfate), GNH (0.4% glucose, 0.2% ammonium sulfate, and 0.2% L-histidine), and H (0.4% L-histidine).

[b] Specific activity expressed as micromoles of product formed per minute per milligram of protein.

[c] Specific activity expressed as nanomoles of product formed per minute per milligram of protein.

[d] Glutamate synthase activities have been corrected for interference by GDH as suggested by Brenchley et al. (3).

dine medium. The nature of this repression is not yet understood in *K. aerogenes*, but the data in Table 5 show that the effect is seen whether the glutamine synthetase is repressible (MK 9000) or absent due to a small deletion in *glnA* (MK 9011). Since the fourfold elevation in GDH levels is seen even in histidine medium where this other, non-glutamine synthetase-mediated control is operative, we suggest that the regulatory sites affecting GDH levels are intact and the apparent reversion results in fourfold high levels of GDH under all conditions.

DISCUSSION

Glutamine synthetase has been implicated in the control of a large number of enzymes of nitrogen metabolism (11); so far the molecular mechanism of this control has been demonstrated only in the case of *hut* operons (17). Studies using mutants that produce glutamine synthetase at high levels under all conditions (GlnC$^-$) and mutants that lack glutamine synthetase (GlnA$^-$) suggest that, in contrast to the *hut* operons, *gdh* might be repressed by high levels of active glutamine synthetase in *K. aerogenes*. Independent of this regulation by glutamine synthetase, another system, as yet undefined, acts to regulate GDH levels. This other control was first observed by Magasanik et al. in another strain of *K. aerogenes* (10). Growth on histidine as sole source of carbon and nitrogen leads to a two- or threefold reduction in GDH levels, even when the glutamine synthetase levels are very low (in wild type) or completely absent (in GlnA$^-$ mutants). Whether each of these independent regulatory systems acts directly on *gdh* expression or whether they act through a common intermediate (e.g., by activating production of a specific *gdh* repressor) is not known. Further study of mutants such as the revertant unlinked to *gdh* described here may begin to cast some light on this question.

The Rev-2 reversion in strain MK 9684 (tightly linked to *gdh*) leads to elevated levels of GDH, relative to those in the isogenic parent or wild type, under all conditions tested. However, both the glutamine synthetase control and the other control appear to be operating to the same extent as in the wild type. It is, therefore, unlikely that this reversion affects an operator site for *gdh*. It is likely that the reversion represents a mutation in the promoter for *gdh*, leading to increased transcription but subject to the same controls. Our data, however, do not exclude the possibility that the reversion represents an amplification of the number of copies of *gdh* in the cell though the stability of the reversion makes such amplification unlikely. Nor can we exclude the possibility that it represents a structural mutation in GDH, leading to a fourfold increase in the specific activity of the enzyme. Measures (12) has reported a substantial stimulation of GDH activity by potassium ions. We observed this effect equally in the wild-type and revertant strains.

The Rev-5 reversion in strain MK 9683 (not linked to *glnA* or *gdh*) is more difficult to assess. Since the effect of the glutamine synthetase control is more pronounced than the other control, the GlnC$^-$ phenotype of MK 9683 would mask the non-glutamine synthetase control; therefore, we cannot say whether this relief from repression is specific for the glutamine synthetase control only or whether the other control would be relieved as well. If glutamine synthetase acts indirectly through or directly in concert with some other regulatory element, then this mutant might help us understand the mechanism of this control. We plan to study this mutant further.

Although the regulation of GDH synthesis in *E. coli* differs from that in *K. aerogenes* (S. L. Streicher and B. Magasanik, manuscript in preparation), the map position of the *gdhD* genes is probably the same in both organisms. P1 transductions in *K. aerogenes* suggests that *gdhD* lies near min 26 (using the *E. coli* designations). Episome complementation using F' episomes from *E. coli* suggests that the analogous *E. coli* gene lies between 21.5 and 27 min. Although final determination of the map position in *E. coli* awaits mapping by P1 transduction, this result both confirms and refines the earlier report of Berberich (1) and suggests that the *E. coli gdhD* is likely to lie near min 26 of the *E. coli* map. The *K. aerogenes* mapping data presented here provide a fourth region of map with strong similarity to the *E. coli* map. Except for one transposition in the region near min 77 (15), the *K. aerogenes* map matches *E. coli* in the regions near min 77 (*glaA* region), 49 (*nadB* region), 17 (*gal-bio* region), and 27 (*trp* region).

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