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Growth Inhibition Caused by Overexpression of the Structural Gene for Glutamate Dehydrogenase (*gdhA*) from *Klebsiella aerogenes*

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Two linked mutations affecting glutamate dehydrogenase (GDH) formation (*gdh-1* and *rev-2*) had been isolated at a locus near the *trp* cluster in *Klebsiella aerogenes*. The properties of these two mutations were consistent with those of a locus containing either a regulatory gene or a structural gene. The *gdhA* gene from *K. aerogenes* was cloned and sequenced, and an insertion mutation was generated and shown to be linked to *trp*. A region of *gdhA* from a strain bearing *gdh-1* was sequenced and shown to have a single-base-pair change, confirming that the locus defined by *gdh-1* is the structural gene for GDH. Mutants with the same phenotype as *rev-2* were isolated, and their sequences showed that the mutations were located in the promoter region of the *gdhA* gene. The linkage of *gdhA* to *trp* in *K. aerogenes* was explained by postulating an inversion of the genetic map relative to other enteric bacteria. Strains that bore high-copy-number clones of *gdhA* displayed an auxotrophy that was interpreted as a limitation for α-ketoglutarate and consequently for succinyl-coenzyme A (CoA). Three lines of evidence supported this interpretation: high-copy-number clones of the enzymatically inactive *gdhA1* allele showed no auxotrophy, repression of GDH expression by the nitrogen assimilation control protein (NAC) relieved the auxotrophy, and addition of compounds that could increase the α-ketoglutarate supply or reduce the succinyl-CoA requirement relieved the auxotrophy.

In the enteric bacterium *Klebsiella aerogenes*, glutamate dehydrogenase (GDH) carries out the NADPH-dependent synthesis of glutamate from α-ketoglutarate and ammonia. It is one of only two enzymes in the cell capable of net assimilation of ammonia into glutamate (for a review, see reference 29). The other enzyme, glutamate synthase (GOGAT), uses glutamine in place of ammonia and thus functions in conjunction with glutamine synthetase, the enzyme responsible for assimilating ammonia into glutamine. GDH is a hexameric protein composed of six identical subunits (30) and should be coded for by a single structural gene, *gdhA*. Glutamate plays two different roles in cellular metabolism: it is the source of 85% of the nitrogen in cellular material, and it plays a role in osmoprotection (16, 34). Thus, it seemed reasonable that multiple regulatory loci might exist. *nac* (which codes for the nitrogen assimilation control protein [NAC]) is one such locus. When *K. aerogenes* is grown in nitrogen-deficient medium, *gdhA* is strongly repressed by NAC, which is itself under the control of the global Ntr system (20, 31).

In 1974, Brenchley and Magasanik (6) described a mutant of *K. aerogenes* that had reduced levels of GDH activity, but they were not able to determine if the mutation responsible, *gdh-1* (then called *gdhD1*), was in the structural gene for GDH. In 1976, Bender et al. (5) showed that the *gdh-1* mutation was linked to the *trp* operon, in contrast to the *Escherichia coli* *gdhA* gene, which is not linked to *trp* (14). They further identified a class of GDH-overproducing mutants (e.g., *rev-2*) that led to a fourfold increase in the total GDH activity but was still responsive to regulation by NAC (although not so strongly as was the wild type). The *rev-2* mutation was tightly linked to the locus defined by *gdh-1*. The fact that the genetic maps of *E. coli* and *K. aerogenes* are similar, coupled with the fact that regulatory mutations were isolated at this site, led us to question whether *gdh-1* did in fact lie in *gdhA*, the structural gene for GDH.

Cloning of *gdhA*$^+$. *K. aerogenes* strain KB2560 (*gltB200 gdh-1* and lysogenic for Mu *cts62 h*P1#1) lacks both GOGAT and GDH activities and cannot grow without exogenous glutamate (7). An in vivo cloning procedure (13) was used to generate plasmids that enabled KB2560 to grow in the absence of glutamate (the strains and plasmids used in this work are listed in Table 1). Roughly half of these clones contained apparent *gltB* (GOGAT) clones and the others contained apparent *gdh* clones. A 2.4-kb *Pst*I restriction fragment from one of the GDH clones was subcloned into pUC19 and tested for complementation. This plasmid, pGDH4, restored GDH activity to a *gdh-1* strain. The DNA sequence of the *Pst*I fragment was determined and found to contain an open reading frame (ORF) with near identity (99% at the nucleotide level, 100% at the amino acid level) to the partial *gdhA* sequence previously reported for *K. aerogenes* (22, 35). In addition, this ORF was 81% identical at the nucleotide level (90% at the amino acid level) to the *gdhA* sequence from *E. coli* (21, 33). Thus, the *gdhA*$^+$ gene was able to restore GDH activity to a strain carrying the *gdh-1* mutation.

***gdhA* is linked to *trp*.** Since the nature of the *gdh-1* mutation was unknown, it was necessary to construct an authentic *gdhA* mutation for genetic mapping. The streptomycin- and spectinomycin-resistant (Sm Sp) Omega (Ω) cartridge (26) was cloned into a unique *Hpa*I site within the *gdhA* gene of pGDH4. This inactivated *gdhA* gene (*gdhA2*::Ω) was crossed onto the *K. aerogenes* chromosome and replaced the resident wild-type

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TABLE 1. Strains and plasmids used in this work

Strain or plasmid	Genotype or relevant characteristics[a]	Source or reference
KC895[b]	*gltB200 ntr-45*	20
KC1043	Wild type	2
KB2560[b]	*gltB200 gdhA1* Mu *cts h*P1#1	Mu lysogen of KB630[c]
KC2637	*gdhA1*	This laboratory
KC2668	Δ[*bla*]-*2*	17
KC2863	*gdh-3*	This work
KB2907[b]	*gltB200 gdhA1* Mu *cts h*P1#1 *nac-306*::Tn*5*tac1	This work
KC3183	*gdhA2*::Ω	This work
KC3228	KC2668/pGDH4	This work
KC3902	KC2668/pCB515	This work
KC4356	KC2668/pCB513	This work
KC4358	KC2637/pCB644	This work
KC5100	*gdhA12*[d]	This work
pUC19	High-copy-number cloning vector	Gibco-BRL
pACYC184	Medium-copy-number cloning vector	8
pGB2	Low-copy-number cloning vector	9
pGDH4	*gdhA*$^+$ cloned into pUC19	This work
pGDH5	Ω cloned into *Hpa*I site (in *gdhA*) of pGDH4	This work
pS4BC	Mu clone of *gdhA*$^+$	This work
pCB513	*gdhA*$^+$ cloned into pACYC184	This work
pCB515	*gdhA*$^+$ cloned into pGB2	This work
pCB644	*gdhA1* cloned into pGDH4 context	This work
pCB725	*gdhAp-lacZ* fusion in pRJ800	This work
pCB1205	*gdh-3-lacZ* fusion in pRJ800	This work

[a] Unless otherwise noted, all of the strains were derived from MK53 and carry the *hutC515* and *dadA1* alleles (17, 27). KC strains differ from KB strains in that they have been cured of the plasmid pPN100, which encodes (among other things) Sm^r.

[b] Not derived from MK53, therefore *hutC*$^+$ and *dad*$^+$.

[c] KB630 (*gltB200 gdhA1*) is a P1-sensitive version of MK261 (6).

[d] Generated with a one-step gene inactivation method using PCR products (10). This allele replaces the promoter of *gdhA* from position −116 to +73 (which includes the first four nucleotides of the ORF) with a kanamycin resistance cassette.

gene. The resulting strain had low levels of GDH, comparable to those of strains carrying *gdh-1* (Table 2). Another mutant, *gdhA12* (in which the promoter and first four nucleotides of *gdhA* were replaced with a kanamycin resistance cassette) also displayed low but nonzero levels of GDH. It thus appeared that the residual activity observed resulted from an enzyme other than GDH, and the low levels of GDH observed in a *gdh-1* mutant are not inconsistent with this mutation mapping to *gdhA*. The loss of GDH activity in strains carrying *gdhA2*::Ω was cotransducible with the Sm Sp resistance and was tightly linked to *gdh-1*. In addition, *gdhA2*::Ω, like *gdh-1*, was linked to *trp* but not to *nas* (data not shown). The linkage of *gdhA* to *trp* in *K. aerogenes* can be explained by an inversion of a chromosomal region relative to the same region in *Salmonella enterica* serovar Typhimurium LT-2. This inversion is similar to the inversions found in this area of *E. coli* and *S. enterica* serovar Enteritidis (4, 19). However, the *K. aerogenes* inversion appears to be smaller: *gdhA, nar* (or *nas*), and *dad* remain outside the boundaries of the inversion, but *trp* and *pyrF* remain inside.

The *gdh-1* mutation lies within *gdhA*. To confirm that *gdh-1* was an allele of *gdhA*, we tested for complementation between *gdh-1* and *gdhA2*::Ω. The cloned *gdhA2*::Ω (pGDH5) failed to complement *gdh-1*, but prototrophic recombinants arose at significant frequencies when this plasmid was present in a *gltB200 gdh-1* strain. All the plasmids tested in this manner that carried the region from bp 226 to 303 of the structural gene (as well as flanking DNA) yielded recombinants at a frequency similar to that of pGDH5. The plasmid pCB584, which contained only this region and no additional flanking DNA, also yielded recombinants, but at a lower frequency. This was presumably due to the small amount of homologous DNA contained in the fragment. Thus, the only sequence information needed to correct the deficiency in GDH caused by *gdh-1* lies within this region (which corresponds to amino acids 76 to 101 of the polypeptide).

A 700-bp fragment of *gdhA* that includes the region with *gdh-1* was cloned twice from independent PCR experiments, and the DNA sequences were determined. In both cases, a single nucleotide change (G to A at position 281 with respect to the ORF) was the only change detected. This would result in a glycine-to-glutamate change at position 94 in the amino acid sequence of GDH. Thus, the original *gdh* mutation, *gdh-1*, defines the structural gene in *K. aerogenes* and can be renamed *gdhA1*.

A regulatory mutation affecting *gdhA* expression. Another mutation affecting GDH formation (*rev-2*) that had been isolated previously had higher (but still regulated) levels of GDH under all conditions tested and was also linked to *trp* (5). The simplest explanation for *rev-2* was that it was an up-promoter mutation at *gdhA* or a structural mutation in *gdhA* that increased the specific activity of the enzyme. However, *rev-2* might have defined a regulatory gene near *gdhA*. The original *rev-2* isolate had been lost, so we used the same selection to isolate seven independent mutants with the same phenotype as the original *rev-2* strain. This mutant was isolated as a glutamate-independent revertant of an Ntr-constitutive *gltB* strain (KC895, *ntr-45 gltB200*). The parent is a glutamate auxotroph due to the lack of GOGAT activity and the repression of *gdhA* by the Ntr system (via NAC). Most glutamate prototrophs resulted from mutations that lay in either *ntrC* or *nac* and affected the nitrogen regulation of many operons. In contrast, *rev-2*-like mutants were specific for GDH expression. The *gdh-3* mutation was typical of the seven mutations isolated in this study in that it was linked to *trp* and resulted in increased levels of GDH that were still regulated by nitrogen (Table 2).

TABLE 2. Effects of *gdh* mutations on GDH activities

Strain	Genotype	Enzyme[a]	Sp act (nmol/min/mg) in[b]:	
			−N	+N
KC1043	*gdhA*$^+$	GDH	45	379
KC2637	*gdhA1*	GDH	ND	14
KC3183	*gdhA2*::Ω	GDH	ND	8
KC5100	*gdhA12*	GDH	ND	12
KC2863	*gdh-3*	GDH	579	1,420
KC2668/pCB725	*gdhAp-lacZ*	LacZ	ND	860
KC2668/pCB1205	*gdh-3p-lacZ*	LacZ	ND	2,710

[a] LacZ, β-galactosidase. Assays were performed on whole cells as described previously (20, 25).

[b] Specific activities are averages of at least two independent experiments. +N, nitrogen-rich medium (glucose minimal medium supplemented with both ammonium sulfate and glutamine); −N, nitrogen-limited medium (glucose minimal medium supplemented with only glutamine (0.2%) as the sole nitrogen source). Cells were grown and prepared for enzyme assays as described previously (31). ND, not determined. β-Galactosidase activity is reported for promoter-*lacZ* fusions of the particular *gdhA* allele cloned into the plasmid pRJ800.

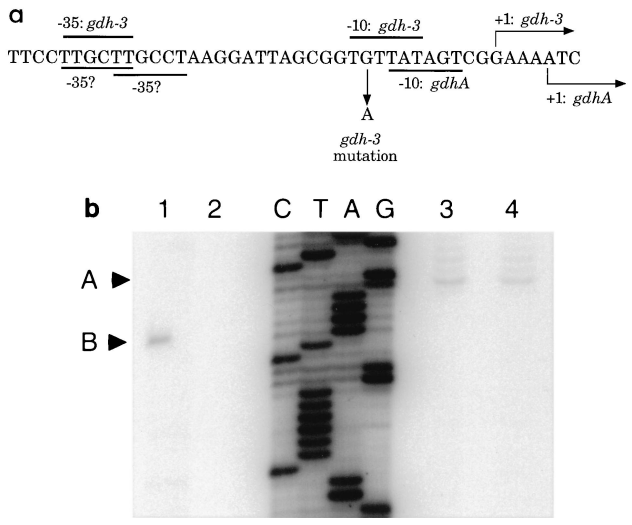

FIG. 1. Mapping the start of transcription of *gdhA* and *gdh-3*. (a) Nucleotide sequence of the *gdhA* promoter region. Putative −10 and −35 regions and the start of transcription, as shown by primer extension analysis, are indicated. (b) Primer extension analysis (24) of *gdhA* and *gdh-3*. A, major start site for *gdh-3*; B, start site for *gdhA*. Total RNA was isolated from cells grown under nitrogen excess conditions (GN supplemented with 0.2% glutamine) or nitrogen limitation conditions (glucose minimal medium supplemented with 0.2% glutamine). Lane 1, KC1043 (wild type) with nitrogen excess; lane 2, KC1043 with nitrogen limitation; lane 3, KC2863 (*gdh-3*) with nitrogen excess; lane 4, KC2863 with nitrogen limitation.

Our attempts to clone the *gdh-3* allele by multicopy complementation of *gdhA1* were unsuccessful. Therefore, we tested directly whether the *gdh-3* mutation was a promoter mutation affecting *gdhA*. The 5′ region of the *gdhA* gene from the *gdh-3* strain was amplified by PCR and cloned in front of a promoterless *lacZ* (pCB1205). This construct showed an increased level of β-galactosidase expression compared with the wild-type *gdhA* promoter *lacZ* fusion (Table 2). The DNA sequence of this region was determined and contained a single nucleotide change of G to A at position −14 relative to the start of transcription (Fig. 1a) of wild-type *gdhA* (as determined below). An identical analysis of the other six independently isolated mutants revealed the same nucleotide change.

Given the location of the single nucleotide change in the *gdh-3* promoter region, it seemed likely that the mutation created a novel promoter with greater strength than that of wild-type *gdhA*. Primer extension analysis was performed on both a wild-type strain and a *gdh-3* strain under nitrogen excess and nitrogen-limiting conditions (Fig. 1b). This analysis showed that the start of transcription had changed, with the primary site 4 nucleotides upstream of the wild-type initiation point but with weaker possible initiation at adjacent sites as well (Fig. 1b, lane 1 versus lane 3). The wild-type start of transcription appeared to be abolished in this mutant. The experiment also reflected the strong nitrogen regulation of *gdhA* transcription; no transcript was detected from the wild-type promoter under nitrogen-limiting conditions (Fig. 1b, lane 1 versus lane 2). The detection of a transcript from the *gdh-3* promoter under nitrogen-limiting conditions was similar to the enzyme assay data; the *gdh-3* allele responds to nitrogen limitation, but the effect is significantly less than that seen for the wild type (Table 2).

The phenotype of the *gdh-3* allele can be explained by the creation of a new −10 region for the *gdhA* promoter. The change created a close match to a −10 recognition region that is spaced 16 nucleotides away from a possible −35 binding region of the wild-type promoter (Fig. 1a). The proposed −10 region in the wild-type promoter is spaced 19 nucleotides from this −35 region and 15 nucleotides from another possible −35 region. Thus, the proposed novel −10 site would result in a promoter that has more favorable spacing between the consensus hexamers than does the wild-type promoter (28).

We were surprised to discover that all seven of our independent isolates bore the same mutational change. Perhaps this is a hot spot for spontaneous mutation, or it may reflect the fact that the selection depends on relief of NAC-mediated repression of *gdhA*. Clearly, GDH formation in the *gdh-3* strain under nitrogen limitation was much higher than in the wild type. Moreover, the relative strength of the NAC-mediated repression was much weaker in this strain (ca. 2.5-fold) than in the wild type (at least eightfold). A simple explanation for the reduced repression by NAC is that a stronger binding site for RNA polymerase made the polymerase a better competitor for the site. But repression of *gdhA* by NAC is complex, and other explanations remain possible.

Overproduction of GDH activity leads to auxotrophy. Our inability to isolate *gdh-3* clones via the in vivo cloning protocol (which uses a high-copy-number origin of replication) led us to suspect that overproduction of GDH activity might prevent growth in minimal medium. However, our original *gdhA*$^+$ clone was present in high copy numbers. Closer analysis of strains freshly transformed with either pS4BC (high-copy-number *gdhA*$^+$ Mu plasmid) or pGDH4 (high-copy-number *gdhA*$^+$ pUC plasmid) revealed that these strains could not grow on glucose ammonia minimal medium (GN) (Table 3) and that secondary mutations which suppressed this growth defect occurred at a high frequency (5×10^{-4} for a wild-type strain carrying pGDH4). The growth defect was relieved by the addition of glutamate to the medium. This auxotrophy was not seen when the (inactive) *gdhA1* allele replaced *gdhA*$^+$ on the high-copy-number plasmid pCB644. In 1976, Struhl and Magasanik described a mutant of *K. aerogenes* that they explained

TABLE 3. Effects of overexpression of GDH on the ability of strains to use different nitrogen sources

Strain and plasmid[b]	Growth observed on indicated media[a]					
	GN		GN Glt		G Ser	
	No IPTG	1 mM IPTG[c]	No IPTG	1 mM IPTG	No IPTG	1 mM IPTG
KC2668, pCB644	++	++	++	++	++	++
KB2907, pCB644	−	−	++	++	−	−
KB2907, pGDH4	−[d]	++	++	++	+	−
KB2907, pS4BC	−	++	++	++	+	−

[a] Growth phenotypes were scored after 24 h at 30°C on solid media (G, glucose; N, ammonium sulfate; Glt, glutamate; Ser, serine) that did or did not include IPTG as indicated. −, no growth; +, slow growth; ++, full growth.

[b] KC2668 is wild type, while KB2907 contains the *gltB200 gdh-1 nac-306*::Tn*5*tac1 alleles. pCB644 contains *gdhA1* cloned in high copy numbers, while pGDH4 and pS4BC contain *gdhA*$^+$ cloned in high copy numbers.

[c] IPTG was used as an inducer of *nac* expression from the *nac-306*::Tn*5*tac1 (*tacp*::*nac* fusion) allele present in KB2907.

[d] Suppression of the phenotype occurred at a high frequency.

TABLE 4. Doubling times and GDH activities of *Klebsiella* strains containing multicopy levels of *gdhA*$^+$

Strain	Relevant genotype	Medium[a]	GDH[b] sp act (nmol/min/mg)	Doubling time (min)
KC2668	Wild type	GN	492	57
		GN Glt	569	55
		G Gln	76	72
		G Ser	43	202
		CN	212	64
		Gly N	266	72
		GN Lys	249	64
		GN Met	663	56
		GN Lys, Met	ND	61
		GN Glt, Lys, Met	120	51
KC3228	pGDH4 (high-copy-number *gdhA*$^+$)	GN	30,500[c]	NA
		GN Glt	30,700	102[d]
		G Gln	8,950	108
		G Ser	6,920	213
		CN	9,420	70
		Gly N	NA	No growth
		GN Glt, Lys	19,100	67
		GN Glt, Met	34,900	76
		GN Lys, Met	ND	165
		GN Glt, Lys, Met	12,100	56
KC4356	pCB513 (medium-copy-number *gdhA*$^+$)	GN	12,000	235
		GN Glt	11,300	83
KC3902	pCB515 (low-copy-number *gdhA*$^+$)	GN	2,120	66
		GN Glt	2,360	59
KC4358	pCB644 (high-copy-number *gdhA1*)	GN	14	65
		GN Glt	14	65

[a] Cells were grown as described previously (31) in W4 minimal media supplemented as follows: G, 0.4% glucose; N, 0.2% ammonium sulfate; Glt, 0.4% L-glutamate; Gln, 0.2% L-glutamine; Ser, 0.2% L-serine; C, 0.4% citrate; Gly, 0.4% glycerol; Lys, 0.01% L-lysine; Met, 0.01% L-methionine.

[b] GDH specific activities are averages of at least two independent experiments. Assays were performed on whole cells or extracts as described previously (20). ND, value not determined; NA, value not applicable.

[c] KC3228 fails to grow in glucose ammonia minimal media. This GDH value was obtained by washing cells that had been grown in the presence of glutamate and inoculating them at a high cell density in GN medium. The culture was allowed to incubate for several hours before cells were harvested and GDH levels were determined.

[d] Doubling times for the strain in GN Glt medium were variable but averaged 102 min.

had a reduced ability to form succinyl-coenzyme A (CoA) from α-ketoglutarate (32). The similarity between the phenotypes of that mutant and our GDH overproducers was striking and led us to the hypothesis that the overproducers converted most of the available α-ketoglutarate to glutamate. This in turn would lead to a limitation for succinyl-CoA. Three sets of observations supported this hypothesis. First, enzymatically active GDH was required for the phenotype. Second, conditions that reduced GDH production reduced the severity of the phenotype. Third, conditions that reduced the demand for succinyl-CoA and/or α-ketoglutarate also reduced the severity of the phenotype.

The severity of the auxotrophy reflected the copy number of *gdhA*$^+$ in the cell. A wild-type strain that contained no additional copies of *gdhA*$^+$ (KC2668) had a doubling time of 57 min (Table 4). The presence of the low- or medium-copy-number clones increased the doubling time to 66 or 235 min, respectively, and the presence of the high-copy-number clone (pGDH4) prevented growth entirely. An equivalent high-copy-number plasmid which contained the nonfunctional *gdhA1* allele had little impact on growth (Table 4; compare results for pCB644 and pGDH4). In order to confirm that pCB644 produced the same amount of polypeptide (albeit inactive) as pGDH4, the protein profiles of strains bearing the wild-type and mutant clones were compared. Both strains contained large amounts of a 45-kDa protein (which probably corresponds to a GDH monomer), and their amounts of polypeptide appeared to be identical (data not shown). Thus, the auxotrophy induced by the overproduction of GDH was linked to the enzyme's activity, not to overproduction of polypeptide.

Many of the tested growth conditions that allowed KC3228 (high-copy-number *gdhA*$^+$) to grow in minimal medium also reduced the amount of GDH activity present in the cell. Repression of *gdhA* transcription was the most straightforward explanation for the reduction of GDH activity. The transcriptional regulator NAC has been shown to repress *gdhA* from *K. aerogenes* (20, 31). In KB2907, where the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *tac* promoter controls *nac* expression, the addition of IPTG was enough to allow the strain to grow on GN (Table 3). In addition, when KC3228 was grown with glutamine or serine as the limiting nitrogen source, GDH levels were reduced roughly fourfold (Table 4) and the strain was able to grow. The addition of lysine to the medium also reduced GDH levels approximately twofold. The mechanism of this repression was unclear but was apparently linked

to transcription, since *gdhA* promoter fusions to *lacZ* also reflected this lysine-dependent repression (data not shown).

Our hypothesis was that the large amounts of GDH in the cell depleted the α-ketoglutarate levels in the cell, but a direct test of this hypothesis was complicated by the fact that *K. aerogenes* does not transport α-ketoglutarate. However, the replacement of glucose with citrate or succinate as the sole carbon and energy source circumvented this complication. These compounds feed into the tricarboxylic acid cycle and increase the flux into α-ketoglutarate and succinyl-CoA (1, 11). Under these conditions, overexpression of GDH did not affect the growth rate. Furthermore, addition of lysine and methionine, which need succinyl-CoA for synthesis (12, 23), reduced the demand for succinyl-CoA and the severity of the phenotype. However, the addition of both lysine and methionine was not sufficient to restore the full growth rate to a GDH overproducer. This is consistent with the observation that the addition of these two amino acids did not fully restore wild-type growth to α-ketoglutarate dehydrogenase mutants of *E. coli* (15).

By comparing GDH activities and growth rates in strains KC3228 and KC4356, it was possible to show that lysine and methionine reduced the severity of the phenotype independent of repression effects. This was most clearly shown by comparing three cultures (Table 4) that each had about 12,000 U of GDH activity per mg: KC3228 (high-copy-number *gdhA*$^+$) grown in GN supplemented with glutamate, lysine, and methionine and KC4356 (medium-copy-number *gdhA*$^+$) grown in either GN or GN supplemented with glutamate. While all three conditions provided roughly the same amount of GDH, the doubling time of KC4356 decreased from 235 min to 83 min with the addition of glutamate, and KC3228 with all three supplements grew faster still, doubling every 56 min. The effect of methionine alone is easily seen by examining the results for strain KC3228. In GN supplemented with glutamate, the strain had 30,700 U of GDH activity per mg and a doubling time of 102 min. The addition of methionine to the medium maintained high levels of GDH (34,900 U/mg), but the strain doubled faster (a doubling time of 76 min). The effect of lysine was harder to isolate because of the twofold repression caused by the addition of lysine to the medium. Nevertheless, strain KC4356 grown without lysine had high levels of GDH (11,300 U/mg) and had a doubling time of 83 min, while strain KC3228 grown in the presence of lysine had even higher levels of GDH (19,100 U/mg) yet grew faster, doubling every 67 min. Thus the addition of lysine, methionine, or both appeared to reduce the requirement for succinyl-CoA and allow faster growth.

Other growth conditions relieved the auxotrophy, but these conditions reduced both the total GDH activity and the demand for succinyl-CoA and α-ketoglutarate. For example, growth with serine as the sole nitrogen source severely limits the rate at which ammonia is supplied to the cell. This in turn limits the amount of α-ketoglutarate that GDH can convert to glutamate, thus slowing the drain on the α-ketoglutarate supply. However, when ammonia is limiting for *K. aerogenes*, GDH formation is severely repressed. Nevertheless, it is clear that when strain KC3228 was grown with serine as the sole nitrogen source, it grew as well as wild-type *K. aerogenes*, despite the fact that it had 160 times as much GDH as the wild type. Thus, restricting ammonia, a substrate of the GDH reaction, had an effect independent of repression.

Finally, it is not surprising that the *gdhA1* mutation of *K. aerogenes* is enzymatically inactive. The *gdhA1* allele of *E. coli* affects a lysine critical for catalytic activity (K92); this mutant GDH can still form hexamers but does not have enzymatic activity (18). The *gdhA1* allele of *K. aerogenes* changes the glycine at position 94 to a glutamate; such a severe change close to an active-site residue would be expected to have an effect on enzymatic activity.

Nucleotide sequence accession number. The DNA sequence of a 2.4-kb *Pst*I restriction fragment from a *gdh* strain cloned in this study has been deposited in the GenBank nucleotide sequence database under accession no. AF332586.

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