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DNA Sequence and Characterization of GcvA, a LysR Family Regulatory Protein for the *Escherichia coli* Glycine Cleavage Enzyme System

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The gene encoding GcvA, the *trans*-acting regulatory protein for the *Escherichia coli* glycine cleavage enzyme system, has been sequenced. The *gcvA* locus contains an open reading frame of 930 nucleotides that could encode a protein with a molecular mass of 34.4 kDa, consistent with the results of minicell analysis indicating that GcvA is a polypeptide of approximately 33 kDa. The deduced amino acid sequence of GcvA revealed that this protein shares similarity with the LysR family of activator proteins. The transcription start site was found to be 72 bp upstream of the presumed translation start site. A chromosomal deletion of *gcvA* resulted in the inability of cells to activate the expression of a *gcvT-lacZ* gene fusion when grown in the presence of glycine and an inability to repress *gcvT-lacZ* expression when grown in the presence of inosine. The regulation of *gcvA* was examined by constructing a *gcvA-lacZ* gene fusion in which β-galactosidase synthesis is under the control of the *gcvA* regulatory region. Although *gcvA* expression appears to be autogenously regulated over a two- to threefold range, it is neither induced by glycine nor repressed by inosine.

The glycine cleavage (GCV) enzyme system catalyzes the oxidative cleavage of glycine into CO_2 and NH_3 and transfers the one-carbon (C_1) methylene unit to tetrahydrofolate (12). This C_1-containing molecule, 5,10-methylenetetrahydrofolate, can then be used as the C_1 donor in the biosynthesis of purines, methionine, thymine, and other cellular components (19).

Although a GCV enzyme system has been demonstrated in *Escherichia coli* (16, 24), it has not been well characterized. The GCV enzyme systems described for chicken liver mitochondria and *Arthrobacter globiformis* are composed of four proteins (P-protein, H-protein, L-protein, and T-protein) (12). P-protein catalyzes the decarboxylation of glycine to CO_2 and an aminomethyl group (11), while the lipoic acid-containing H-protein (6) serves both as an electron sink and as a carrier of the aminomethyl group derived from glycine (11). T-protein catalyzes the transfer of the C_1 unit from H-protein to tetrahydrofolate (7), and the reduced lipoic acid of H-protein is reoxidized to the disulfide form by L-protein (18).

In *E. coli*, three of the proteins of the GCV enzyme complex (T-protein, H-protein, and P-protein) are encoded by the *gcv* operon, which maps at min 62.5 on the *E. coli* chromosome (24, 31). The fourth protein, encoded by the *lpd* gene (L-protein), is not a component of the *gcv* operon; the *lpd* gene maps at min 2.5 (33).

The regulation of *gcv* is not well understood, but at least three regulatory proteins are involved. The first protein shown to be involved in the regulation of *gcv* is Lrp, or leucine-responsive regulatory protein. This protein is a global regulator involved in either activation or repression of many genes involved in amino acid biosynthesis, degradation, or transport (20). A mutation in *lrp* results in low and noninducible β-galactosidase synthesis in strains carrying a λ*plac*Mu phage inserted into *gcv* (14). We are currently examining the mechanism by which Lrp exerts its effects on *gcv*.

The PurR protein also has an effect on *gcv* expression. PurR is a negative regulator of many genes involved in nucleotide metabolism (9). When wild-type *E. coli* cells are lysogenized with a λ*gcvT-lacZ* phage, in which β-galactosidase synthesis is under the control of the *gcv* regulatory region (32), and are grown in the presence of glycine and inosine, a twofold reduction from the fully induced level of β-galactosidase occurs. This twofold repression is dependent upon a functional *purR* gene (36). Gel mobility shift and DNase I footprint assays have indicated that the purified PurR protein binds to the *gcv* control region near the *gcvT* transcription start site (36).

We previously showed that the activation of *gcv* in the presence of glycine requires a protein encoded by the *gcvA* gene at min 60.3 (37). Further studies indicated that the *gcvA* locus is also necessary for an additional fivefold, PurR-independent repression of *gcv* when cells are grown in the presence of exogenous purines but not glycine (36). To gain insight into the apparent role of *gcvA* as both an activator and a repressor of *gcv*, the sequence of *gcvA* has been determined and an initial characterization of the protein has been performed.

MATERIALS AND METHODS

Bacterial strains and plasmids. The genotypes of the *E. coli* K-12 strains and plasmids used in this study are listed in Table 1. The intermediate plasmids used for strain constructions are described below. Plasmid pGS255 carries the *gcvA* gene and was generated by cloning the 11.5-kb *Eco*RI-*Eco*RI fragment from pGS254 (37) into the *Eco*RI site of the low-copy-number vector pACYC184.

To construct plasmid pGS262, plasmid pGS255 was digested with *Ase*I, the 5′ overhangs were filled in with T4 DNA polymerase, and *Eco*RI linkers were added with T4 DNA ligase. The 1.8-kb DNA fragment containing *gcvA* and *orf2* was gel purified and ligated into the *Eco*RI site of the single-copy plasmid pGS225 (37) with T4 DNA ligase.

The 1.8-kb *Eco*RI-*Eco*RI DNA fragment from plasmid pGS262 was gel purified and cloned into the *Eco*RI site of pACYC184 to form plasmid pGS267. Plasmid pGS268 was constructed by digestion of pGS267 at a unique *Mlu*I restriction site (see Fig. 2) in *gcvA*, the ends were made blunt with T4

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype or description	Source or reference
Strains[a]		
GS162	Wild type	This laboratory
GS970	*gcvA1 serA*	This laboratory
GS973	*gcvA1* λ*gcvT-lacZ*	This laboratory
GS998	*gcvA1*	This study
GS1029	Δ(*gcvA orf2*) Σ*aadA* λ*gcvT-lacZ*	This study
ORN103	*minB*	21
GS727	*sbcB15 recB21 recC22*	This study
Plasmids		
pGS254	11.5-kb *Eco*RI-*Eco*RI fragment from Kohara phage 9A12 in pGS225; Kn^r	This laboratory
pGS255	11.5-kb *Eco*RI-*Eco*RI fragment from pGS254 in pACYC184; Tc^r	This laboratory
pGS262	1.8-kb *Eco*RI-*Eco*RI *gcvA orf2* fragment in pGS225; Kn^r	This study
pGS266	1.5-kb *Eco*RI-*Eco*RI *gcvA* fragment in pGS225; Kn^r	This laboratory
pGS267	1.8-kb *Eco*RI-*Eco*RI *gcvA orf2* fragment in pACYC184; Tc^r	This study
pGS268	pGS267 with a 16-bp translation terminator at the unique *Mlu*I site	This study

[a] These strains, with the exception of ORN103 and GS727, also carry *thi*, *pheA905*, Δ*lacU169*, *araD129*, and *rpsL150* mutations. GS727 also carries *thr-1*, *leuB6*, Δ(*gpt-proA*), *hisG4*, *argE3*, *thi-1*, *ara-14*, *lacY1*, *tsx-33*, *supE44*, *rac*, *rfbD1*, *rpsL31*, *kdgK51*, *xyl-5*, and *mtl-1* mutations. ORN103 also carries *thr-1*, *leu-6*, *thi-1*, *lacY1*, *xyl-7*, *ara-13*, *mtl-2*, *gal-6*, *rpsL*, *tonA2*, Δ*pil*, Δ(*argR-lac*)*U169*, and *recA13* mutations.

DNA polymerase, and a 16-bp universal translation terminator (Pharmacia LKB Biotechnology, Piscataway, N.J.) was ligated into this site.

To construct plasmid pGS266, plasmid pGS267 was digested with *Bss*HII, the ends were filled in with T4 DNA polymerase, and *Eco*RI linkers were added with T4 DNA ligase. The resultant plasmid was digested with *Eco*RI, and a 1.5-kb *Eco*RI-*Eco*RI DNA fragment containing *gcvA* was gel purified and ligated into the *Eco*RI site of plasmid pGS225.

To construct plasmid pGS271, plasmid pGS255 was digested with *Sma*I, *Eco*RI linkers were added, the products were digested with *Eco*RI, and an 8.3-kb *Eco*RI-*Eco*RI fragment was isolated and ligated into the *Eco*RI site of pACYC184.

To construct strain GS1029, plasmid pGS271 was digested at its unique *Mlu*I site and Bal31 exonuclease was used to make sequential deletions. *Sma*I linkers were ligated into the Bal31-digested plasmids, and restriction enzyme analysis was used initially to evaluate the extent of the deletions. The sequence of one of the plasmids (pGS278) containing an appropriately sized deletion was determined. The deletion extended from 91 nucleotides upstream of *gcvA* to the last 49 nucleotides within *orf2* (see Fig. 1). A DNA fragment carrying the *aadA* gene, encoding spectinomycin resistance (Sp^r), was isolated from plasmid pNN388 (4) as an 1,800-bp *Pvu*II-*Hin*dIII fragment in which the *Hin*dIII site had been filled in and to which *Sma*I linkers had been attached. The 1,800-bp *Sma*I-*Pvu*II fragment containing the Sp^r marker was cloned into the *Sma*I site of plasmid pGS278. The resultant plasmid, pGS279, was digested with *Eco*RI, and the linear 8.2-kb fragment containing the DNA flanking the regions of *gcvA* and the Sp^r marker was gel purified and used to transform an *E. coli recB recC sbcB* strain (GS727) to Sp^r. P1 phage were grown on an isolated Sp^r colony, and the lysate was used to transduce strain GS162λ *gcvT-lacZ* (see Results) to Sp^r, forming strain GS1029λ*gcvT-lacZ* (see Results). Since *gcvA* is 66% linked to *argA* (37), the chromosomal location of the Sp^r marker was confirmed by transducing a strain carrying an *argA81*::Tn*10* marker to Arg$^+$ with P1 phage grown on GS1029λ*gcvT-lacZ*. Approximately half of the Arg$^+$ transductants also acquired the Sp^r marker that had replaced *gcvA* and *orf2* on the chromosome.

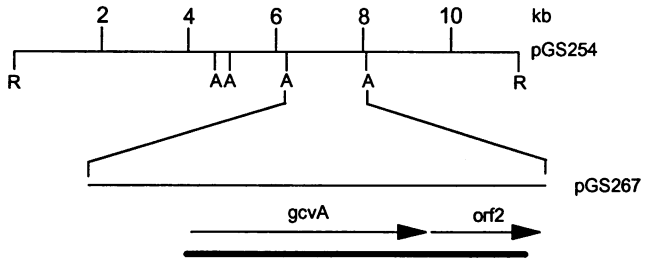

FIG. 1. Diagram of the 11.5-kb *Eco*RI-*Eco*RI fragment of plasmid pGS254 and subclone pGS267. The location and direction of transcription of *gcvA* and *orf2* are indicated by arrows. The limits of the chromosomal deletion constructed in GS1029 are indicated by a heavy line. A, *Ase*I; R, *Eco*RI.

Construction of a *gcvA-lacZ* gene fusion. To make a λ*gcvA-lacZ* gene fusion, plasmid pGS267 was digested with *Eco*RI and *Eco*RV, and a 519-bp fragment containing the 303-bp region upstream of *gcvA* and the first 72 codons of *gcvA* was isolated and ligated into the *Eco*RI-*Sma*I sites of plasmid pMC1403 (2), forming an in-frame translational fusion to the *lacZYA* genes. The resulting plasmid was designated pGS265. DNA sequencing (28) across the fusion junction confirmed that the correct reading frame was maintained. pGS265 was cut at a unique *Sal*I site at the end of the *lacZYA* segment, the ends were filled in with T4 DNA polymerase, and *Eco*RI linkers were added with T4 DNA ligase. A 6,686-bp *Eco*RI-*Eco*RI fragment containing the *gcvA* control region, the first 72 codons of *gcvA*, and the *lacZYA* genes was isolated and cloned into the *Eco*RI site of λgt2 (23). The resulting recombinant phage, designated λ*gcvA-lacZ*, was used to lysogenize *gcvA*$^+$ strain GS162 or *gcvA1* mutant GS998 as described previously (34). Lysogens were tested for the presence of a single copy of the λ phage by infection with λ*c*I90*c*17 (30).

Media. The minimal medium used was Vogel-Bonner minimal salts (35) supplemented with 0.4% glucose (GM). The complex medium used was Luria broth (LB) (17). Supplements were added at the following concentrations: phenylalanine and inosine, 50 μg/ml; glycine, 300 μg/ml; vitamin B_1, 1 μg/ml; kanamycin, 20 μg/ml; spectinomycin, 50 μg/ml; and tetracycline, 10 μg/ml.

P1 transductions. P1 *cml clr-100* phage were used for transductions as previously described (17).

Enzyme assays. β-Galactosidase assays were performed as described by Miller (17) by use of the chloroform-sodium dodecyl sulfate lysis procedure. All results are averages of two or more assays, with each sample being tested in triplicate.

Primer extension analysis. The transcription initiation site for *gcvA* was determined with a primer extension system from Promega, Madison, Wis. RNA was isolated as previously described (1) from strain GS970(pGS267) grown to log phase (5×10^8 cells per ml) in 40 ml of LB plus tetracycline. A synthetic primer complementary to the mRNA at nucleotides 319 to 340 was prepared (5′-CTCGTAAGGCATTTAGCG GTGG-3′), 10 pmol of ^{32}P-labeled primer was incubated with 19 μg of total RNA, and primer extension reactions were carried out according to the Promega protocol. Reaction

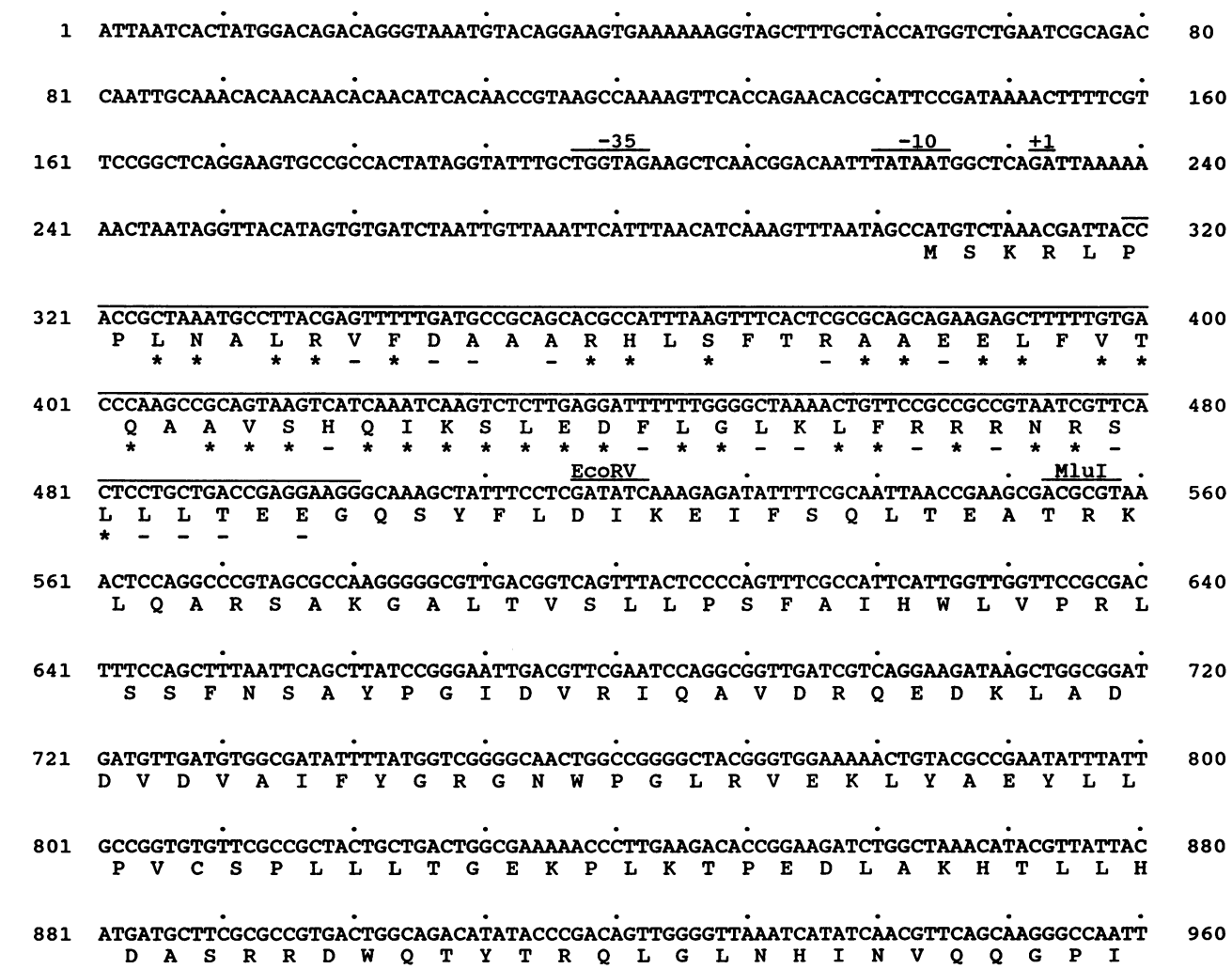

FIG. 2. Nucleotide and deduced amino acid sequences for the *E. coli gcvA* and *orf2* genes. Both strands of plasmid pGS267 DNA were sequenced by use of primers hybridized to vector sequences and then primers synthesized from the previously determined sequences. The transcription start sites are overlined and marked by +1, and the presumed −10 and −35 regions are indicated. The *gcvA* coding region spans nucleotides 304 to 1218, and *orf2*, which codes for a protein of unknown function, spans nucleotides 1240 to 1632. The region of *gcvA* showing similarity to the N-terminal sequence of the LysR family of regulators is overlined; amino acids that are identical matches to those in the LysR family consensus sequence are indicated by an asterisk; a dash indicates that amino acids at these positions can be any amino acid in the LysR consensus sequence. The N-terminal LysR consensus amino acid sequence is hpLR/NpLRxFxxhxpppphSxAApxLphS/TQP/SA/ThS/Tx/RQhp-pLEpxLGxxLFxRxpRxhxxxTxA, where p = T, S, N, Q, D, E, K, R, or H; h = V, I, L, or M; and x = any residue (29).

products were electrophoresed on a 5% polyacrylamide gel next to a dideoxynucleotide sequencing ladder (28) generated from 3 μg of plasmid pGS267 DNA with the same primer as that used for the primer extension reactions.

Minicell analysis. Minicell-forming strain ORN103 (21) was transformed with plasmid pGS267 or pGS268. Minicells were grown in 250 ml of LB plus tetracycline and isolated as described previously (26), except that M9 medium (27) was substituted for Rm salts. Cells were labeled at 37°C with 0.5 μl of 50-μCi/ml [^{35}S]methionine (for 20 min) as described previously (8) and 4 μl of 50-μCi/ml ^{14}C-amino acids (for 2.5 h), since GcvA contains only two internal methionines.

DNA sequencing. Synthetic primers were prepared by use of an Applied Biosystems DNA synthesizer, and sequence analysis was performed on CsCl-purified DNA by use of the automated DNA sequencer at the DNA Core Facility, University of Iowa. The Genetics Computer Group sequence program (3) was used for sequence analysis.

Nucleotide sequence accession number. The nucleotide sequence data reported here will appear in the GenBank nucleotide sequence data base under accession number U01030.

RESULTS

Localization of *gcvA* on plasmid pGS254. *gcvA* was originally isolated on an 11.5-kb *Eco*RI-*Eco*RI fragment from phage 9A12 of the Kohara λ library (13, 37). When a *gcvA1* mutant was transformed with a plasmid containing this DNA fragment (pGS254), both glycine-inducible GCV enzyme activity and β-galactosidase activity from a λ*gcvT-lacZ* gene fusion were restored (37). This plasmid also restored purine-dependent repression of the λ*gcvA-lacZ* fusion in the *gcvA1* mutant. To

```
961  TTTAGCCATAGCGCCATGGTGCTGCAAGCGGCTATCCACGGGCAGGGAGTGGCGCTGGCAAATAACGTGATGGCGCAATC  1040
     F  S  H  S  A  M  V  L  Q  A  A  I  H  G  Q  G  V  A  L  A  N  N  V  M  A  Q  S

1041 TGAAATCGAGGCCGGACGTCTTGTTTGCCCGTTTAATGATGTTCTGGTCAGTAAAAATGCTTTTTATCTGGTTTGTCATG  1120
      E  I  E  A  G  R  L  V  C  P  F  N  D  V  L  V  S  K  N  A  F  Y  L  V  C  H  D

1121 ACAGTCAGGCAGAACTGGGTAAAATAGCCGCCTTTCGCCAATGGATCCTGGCGAAAGCCGCTGCTGAACAAGAAAAATTC  1200
       S  Q  A  E  L  G  K  I  A  A  F  R  Q  W  I  L  A  K  A  A  A  E  Q  E  K  F

1201 CGCTTTCGTTATGAACAATAATTTACGTAGGGTACGACCATGACCAGCCGTTTTATGCTGATTTTCGCCGCCATTAGCGG  1280
     R  F  R  Y  E  Q  *  F  T  *  G  T  T  M  T  S  R  F  M  L  I  F  A  A  I  S  G

                        BssHII
1281 CTTCATTTTTGTGGCTCTGGGCGCTTTTGGCGCGCATGTGTTAAGTAAAACCATGGGGGGCGTTGAGATGGGCTGGATCC  1360
      F  I  F  V  A  L  G  A  F  G  A  H  V  L  S  K  T  M  G  G  V  E  M  G  W  I  Q

1361 AGACCGGCCTCGAATACCAGGCGTTTCATACGCTGGCGATCTTAGGTCTGGCGGTGGCAATGCAGCGTCGCATCAGTATC  1440
       T  G  L  E  Y  Q  A  F  H  T  L  A  I  L  G  L  A  V  A  M  Q  R  R  I  S  I

1441 TGGTTTTACTGGAGTAGCGTTTTCCTCGCGTTAGGCACGGTGTTGTTCAGCGGCAGCCTTTATTGCCTGGCGCTGTCCCA  1520
     W  F  Y  W  S  S  V  F  L  A  L  G  T  V  L  F  S  G  S  L  Y  C  L  A  L  S  H

1521 TCTGCGTTTGTGGGCGTTTGTCACTCCGGTTGGCGGCGTGAGCTTCCTCGCGGGCTGGGCGTTAATGTTAGTTGGTGCTA  1600
      L  R  L  W  A  F  V  T  P  V  G  G  V  S  F  L  A  G  W  A  L  M  L  V  G  A  I

1601 TCCGTTTAAAGCGCAAGGGCGTAAGTCATGAATAAGGTTGTATTGCTGTGCCGTCCGGGCTTTGAAAAAGAGTGCGCCGC  1680
       R  L  K  R  K  G  V  S  H  E  *

1681 AGAAATTACCG  1691
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FIG. 2—*Continued.*

identify the region of DNA on pGS254 encoding *gcvA*, different restriction fragments were subcloned and tested for their ability to activate a λ*gcvA-lacZ* fusion in response to glycine (data not shown). In this way, *gcvA* was localized to the central region of pGS254, and a 1.8-kb *Ase*I fragment containing this region of DNA (Fig. 1) was cloned into plasmid pACYC184 (see Materials and Methods). The resultant plasmid was designated pGS267.

DNA sequence of *gcvA*. The DNA sequence of both strands of most of the 1.8-kb *Eco*RI fragment of pGS267 was determined (Fig. 2). When all six possible reading frames were examined, two open reading frames were found. The larger open reading frame, from nucleotides 304 to 1218, was later identified as *gcvA* (see below) and could encode a protein of approximately 34.4 kDa. The smaller open reading frame, from nucleotides 1240 to 1632, potentially encoded a polypeptide of 14.6 kDa and was designated *orf2*. With the exception of 1 nucleotide at position 1340, the DNA sequence shown in Fig. 2 was identical to that determined for this region by Everett et al. (5).

Minicell analysis with pGS267. To examine whether the open reading frames identified in the DNA sequence were translated, minicell analysis was performed. Minicells form as a result of abnormal cell division and contain no chromosomal DNA but do contain plasmid DNA if the parent strain carries a multicopy plasmid. The major products of in vivo labeling of minicells are plasmid-encoded proteins.

Minicell-forming strain ORN103 was transformed with plasmids pGS267 and pGS268. pGS268 contains a translation terminator cloned into the unique *Mlu*I site of pGS267 (see Materials and Methods). A *gcvA1* mutant containing plasmid pGS268 could no longer activate the λ*gcvA-lacZ* fusion (data not shown). Two polypeptides were encoded by pGS267, one of approximately 33 kDa and another of approximately 14 kDa (Fig. 3). Expression of the larger, 33-kDa, protein was not seen when the polypeptides encoded by pGS268 were examined, and that of the 14-kDa protein was greatly reduced, suggesting that the *gcvA* and *orf2* genes might form an operon (Fig. 3).

Transcription start site for *gcvA*. The 5′ end of the *gcvA* mRNA transcript was identified by primer extension mapping. A 22-bp synthetic primer that hybridized to the RNA downstream of the *gcvA* ATG start codon was constructed. Total cellular RNA was isolated from strain GS970(pGS267) and used to direct the synthesis of DNA complementary to *gcvA* mRNA by use of reverse transcriptase. A comparison of the lengths of the products of the primer extension assay with the DNA sequencing ladder constructed with the same primer indicated that transcription of *gcvA* begins 71 and 72 bp upstream of the GcvA start codon at either an A or a G nucleotide (Fig. 4). A perfect match to the consensus sequence of the −10 region (5′-TATAAT) exists upstream of the transcription start site, but the −35 region shows only a 2-of-6-bp match to the consensus sequence (5′-TTGACA).

Construction of a *gcvA-orf2* deletion in the *E. coli* chromosome. Since two polypeptides encoded by pGS267 were identified by minicell analysis, it was not clear whether one or both of these proteins were necessary for glycine-induced activation and purine-mediated repression of a λ*gcvT-lacZ* fusion. To

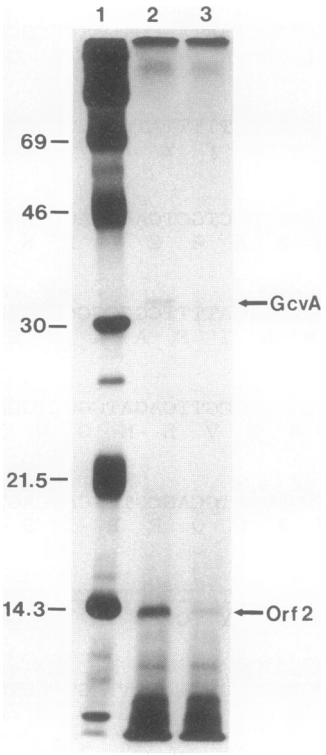

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of ^{35}S- and ^{14}C-labeled polypeptides produced in minicells. Lanes: 1, molecular mass markers, in kilodaltons; 2, proteins encoded by pGS267; 3, proteins encoded by pGS268.

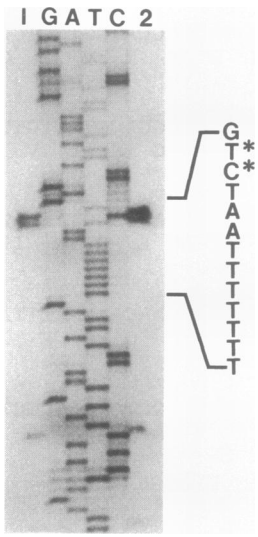

FIG. 4. Primer extension analysis of *gcvA* transcripts. Transcription start sites for RNAs isolated from strain GS970(pGS267) were determined with a ^{32}P-labeled oligonucleotide primer as described in Materials and Methods. DNA sequencing ladders (G, A, T, and C) were generated by the dideoxynucleotide method with the same primer as that used for the primer extension assay. Lanes: 1, 2 μl of primer extension products; 2, 4 μl of primer extension products. The nucleotide sequence of the noncoding strand of the *gcvA* promoter region is indicated to the right. The two start sites are indicated by asterisks.

answer this question, a chromosomal deletion of both of the genes encoding these proteins was constructed.

DNA from 91 nucleotides upstream of *gcvA* to the last 49 nucleotides within *orf2* was deleted from plasmid pGS271 and replaced with a spectinomycin resistance marker (see Materials and Methods). This deletion was introduced into the chromosome by linear transformation of a *recBC sbcB* strain and was shown to have recombined with and been inserted at the normal chromosomal *gcvA* locus (see Materials and Methods). P1 phage were grown on an isolated Sp^r transformant, and the lysate was used to transduce wild-type strain GS162 carrying a λ*gcvT-lacZ* fusion. In this strain (GS162λ*gcvT-lacZ*), β-galactosidase synthesis is under the control of the *gcv* regulatory region. Transductants were selected on LB agar plus spectinomycin, and one isolate was purified and designated GS1029λ*gcvT-lacZ*. To assess the phenotype associated with the deletion of the *gcvA* and *orf2* loci in GS1029λ*gcvT-lacZ*, the lysogen was transformed with a single-copy plasmid carrying both *gcvA* and *orf2* (pGS262), *gcvA* alone (pGS266), or no insert DNA as a control (pGS225).

The transformed lysogens were grown in GM medium containing either glycine or inosine or neither, and β-galactosidase activities were measured (Table 2). When the deletion mutant GS1029λ*gcvT-lacZ* was transformed with plasmid pGS225, no induction of β-galactosidase synthesis by glycine was seen. Furthermore, the addition of inosine resulted in only a twofold repression of β-galactosidase levels, attributable to the PurR repressor (36). When plasmid pGS262 was introduced into GS1029λ*gcvT-lacZ*, both wild-type induction by glycine and repression by inosine were restored. Essentially identical results were obtained when the lysogen carried plasmid pGS266, which encodes only the *gcvA* gene. These results indicate that *gcvA* alone is both necessary and sufficient for activation of *gcv* by glycine and for repression of *gcv* by purines.

Comparison of GcvA with the LysR family of regulators. A FASTA (3) search of the predicted amino acid sequences of the GcvA and Orf2 proteins indicated that GcvA was a member of the LysR family of bacterial activator proteins that bind DNA through a helix-turn-helix motif (10). The predicted α-helical region spans amino acids 23 to 53 (Fig. 2). GcvA shows the most similarity to the AmpR subfamily of regulatory proteins found in *Citrobacter freundii* and other gram-negative bacteria (29). In addition, GcvA has been shown to activate the *C. freundii ampC* β-lactamase gene, presumably because of its homology with the AmpR subfamily of proteins (5).

A comparison of the deduced amino acid sequence of Orf2 revealed no similarities to other proteins in the data bank. However, a hydrophilicity plot indicated that this polypeptide

TABLE 2. Complementation in *trans* of the *gcvA-orf2* double mutant by *gcvA*

Strain	β-Galactosidase activity (Miller units [6]) with the following addition[a]:		
	None	Glycine	Inosine
GS162λ*gcvT-lacZ*	146	799	8
GS1029λ*gcvT-lacZ*(pGS225) [Δ(*gcvA-orf2*)]	77	79	38
GS1029λ*gcvT-lacZ*(pGS262) [Δ(*gcvA-orf2*)/*gcvA*$^+$ *orf2*$^+$]	166	864	9
GS1029λ*gcvT-lacZ*(pGS266) [Δ(*gcvA-orf2*)/*gcvA*$^+$]	163	923	9

[a] Cells were grown in GM medium containing phenylalanine and vitamin B_1 along with the indicated supplement.

TABLE 3. Autorepression of a λ*gcvA-lacZ* fusion by GcvA

Strain	β-Galactosidase activity (Miller units [6] ± 1 SD) with the following addition[a]:		
	None	Glycine	Inosine
GS162λ*gcvA-lacZ* (*gcvA*$^+$)	3.0 ± 0.3	3.0 ± 0.3	3.0 ± 0.3
GS998λ*gcvA-lacZ* (*gcvA1*)	8.0 ± 0.9	7.8 ± 0.9	7.9 ± 0.6
GS998λ*gcvA-lacZ*(pGS267) (*gcvA1*/*gcvA*$^+$)	1.0 ± 0.3	1.1 ± 0.1	1.0 ± 0.2

[a] Cells were grown in GM medium containing phenylalanine along with the indicated supplement.

is hydrophobic throughout most of its length, indicating that it may be a membrane-associated protein (data not shown).

Regulation of *gcvA*. One possible explanation for the two different effects of GcvA on *gcv* expression (i.e., glycine induction and inosine repression) is that *gcvA* itself is regulated in response to glycine or inosine. To examine this possibility, a λ*gcvA-lacZ* translational fusion was constructed. A 519-bp *Eco*RI-*Eco*RV DNA fragment from pGS267 containing the region upstream of *gcvA* and the first 72 codons of *gcvA* was fused to the *lacZYA* genes of plasmid pMC1403 (see Materials and Methods). A DNA fragment from this plasmid that contained the sequences upstream of *gcvA* and the *lacZYA* genes was isolated and cloned into phage λgt2 (23). The resulting phage was used to lysogenize either a *gcvA*$^+$ strain (GS162) or a *gcvA1* mutant (GS998), and the lysogens were grown in GM medium containing no additions, inosine, or glycine and assayed for β-galactosidase activity.

As shown in Table 3, β-galactosidase synthesis from the λ*gcvA-lacZ* fusion did not appear to be regulated in response to either glycine or inosine. Although the levels of β-galactosidase synthesized from the fusion were low, the *gcvA1* mutant GS998λ*gcvA-lacZ* consistently showed about a two- to threefold increase in β-galactosidase synthesis, suggesting that GcvA might negatively autoregulate itself. To examine this possibility, the *gcvA1* lysogen was transformed with plasmid pGS267, which carries the wild-type *gcvA* gene. The transformed lysogen was grown as described above and assayed for β-galactosidase activity. The presence of the wild-type *gcvA* gene on the low-copy-number plasmid decreased β-galactosidase activity to a level below that in the wild-type lysogen. These data suggest that GcvA is capable of negative autoregulation over a small, i.e., two- to threefold, range.

DISCUSSION

The *gcvA* gene has been sequenced and shown to encode a single protein functioning as both a positive regulator and a negative regulator of the *gcv* operon. GcvA is a member of the LysR family of transcriptional regulators (29) and shows the most similarity to the AmpR subfamily of activator proteins. Minicell analysis and DNA sequence analysis indicated that *gcvA* encodes a polypeptide of approximately 34 kDa, a size consistent with those of most members of the LysR family (29).

Another common feature of this protein family is the ability of the proteins to negatively regulate their own expression (29). We showed that β-galactosidase synthesis encoded by a λ*gcvA-lacZ* fusion was increased two- to threefold in a *gcvA1* mutant (Table 3). Furthermore, the introduction of a plasmid containing *gcvA* to the *gcvA1* mutant resulted in superrepression of the λ*gcvA-lacZ* fusion (Table 3). These results suggest that GcvA also negatively autoregulates its own synthesis.

Target genes for the LysR family of proteins are often divergently transcribed from the regulator gene itself (29). *gcvA* appears to form an operon with at least one gene downstream (*orf2*), as determined by minicell analysis, but examination of the region up to 250 bp upstream of *gcvA* revealed no open reading frames of notable length. The known target for GcvA, the *gcv* operon, maps at min 62.5 on the *E. coli* chromosome (24), while *gcvA* maps at min 60.3 (37). Current experiments directed towards identifying promoters in the region upstream of *gcvA* should clarify whether *gcvA* is similar to the *E. coli cysB* (22) and *nhaR* (15, 25) genes for LysR-type regulators, in which no divergent promoter structure has been found, or whether there exists another, unidentified target for GcvA.

We do not know the function of the protein encoded by *orf2*. However, it is not involved in either activation or repression of the *gcv* operon under the conditions used in this study. A strain carrying a chromosomal deletion of both *gcvA* and *orf2* (GS1029) showed no detectable mutant phenotype with regard to growth rate, colony morphology, or auxotrophy. Further studies will be needed to determine the relationship of *orf2* to *gcvA*.

Although *gcvA* has been shown to be necessary for the activation of *gcv* in the presence of glycine (37) and in the repression of *gcv* when exogenous purines but not glycine are present (36), the expression of *gcvA* itself does not appear to respond directly to these metabolites. These data suggest that the opposite effects of GcvA on *gcv* expression are mediated through the GcvA protein itself, perhaps by the binding of different cofactors to specific sites. The central and C-terminal domains of many members of the LysR family of proteins have been shown to be involved in coinducer recognition and responses (29). To identify potential binding sites for a coinducer, we have devised a genetic screen for *gcvA* mutants that are capable of the activation but not the repression of *gcv* and, conversely, the repression but not the activation of *gcv*. If it is possible to separate these two functions of GcvA, mapping of the mutations should provide insight into the mechanism by which GcvA accomplishes these different tasks and contribute to our knowledge of the widespread LysR family of bacterial regulators.

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