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Cloning, Mapping, and Sequencing of the Gene Encoding *Escherichia coli* Quinoprotein Glucose Dehydrogenase

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***Escherichia coli* contains pyrroloquinoline quinone-dependent glucose dehydrogenase. We cloned and sequenced the gene (*gcd*) encoding this enzyme and showed that the derived amino acid sequence is highly homologous to that of the *gdhA* gene product of *Acinetobacter calcoaceticus*. Stretches of homology also exist between the amino acid sequence of *E. coli* glucose dehydrogenase and other pyrroloquinoline quinone-depenent dehydrogenases from several bacterial species. The position of *gcd* on the chromosomal map of *E. coli* was determined to be at 3.1 min.**

The bacterial enzyme glucose dehydrogenase (GDH; EC 1.1.99.17) can convert glucose to gluconic acid (21) and is dependent on the presence of the cofactor pyrroloquinoline-quinone (PQQ) (15). The GDH is located on the outside of the periplasmic membrane, and therefore the reaction products are formed not in the periplasm but in the periplasmic space. PQQ-dependent GDH is present in a wide variety of bacterial species, some of which, such as *Acinetobacter calcoaceticus* (21) and *Klebsiella aerogenes* (32), produce the cofactor PQQ themselves. Other species, such as *Escherichia coli* (22), *Klebsiella pneumoniae* (32), and *Acinetobacter lwoffi* (35), can produce PQQ-dependent GDH but are able to perform the reaction only when PQQ is present in the culture medium. They apparently lack the pathway for PQQ biosynthesis. It has been suggested that PQQ can be considered a vitamin (35).

Despite the lack of PQQ, *E. coli* is able to use glucose as the sole carbon source. The phosphotransferase system (PTS) in *E. coli* provides the cell with a transport system for the uptake and metabolism of glucose. Therefore, the in vivo activity of GDH is preferably studied in *E. coli* strains that are deficient in the latter pathway. An *E. coli* mutant lacking enzyme I of the PTS is unable to grow on glucose; however, when PQQ is added to the culture medium, glucose is suitable as the sole carbon source (22).

Recently we reported the presence of two different PQQ-dependent GDH enzymes, designated GDH-A and GDH-B, in *A. calcoaceticus* (9). GDH-A is a monomer of M_r 86,956 and is probably firmly incorporated into the cytoplasmic membrane with its hydrophobic N-terminal domain (8). The second enzyme, GDH-B, is a dimer consisting of two identical subunits of M_r 50,231. It lacks a hydrophobic terminus but contains a signal peptide which is removed from the protein during transport across the cytoplasmic membrane (9). The activity of GDH-B can be shown in vitro only in cell extracts from *A. calcoaceticus* in the presence of an artificial electron acceptor. We suggested that *A. calcoaceticus* lacks a component in the electron transport chain of the GDH-B reaction. The in vitro activities of GDH-A and GDH-B can be distinguished by their substrate specificities. Both enzymes oxidize D-glucose, but disaccharides such as lactose and maltose are converted to their corresponding acids only by GDH-B, whereas 2-deoxyglucose is a specific GDH-A substrate.

The DNA and derived amino acid sequences of *gdhA* and *gdhB* have been determined (8, 9). There appears to be no obvious homology between the two enzymes.

This report describes the cloning of the gene encoding the PQQ-dependent GDH from *E. coli*. The aim of this study was to determine whether the PQQ-dependent GDH in *E. coli* is a GDH-A-like or GDH-B-like enzyme and to construct insertion mutants in which the effect of the total lack of GDH in *E. coli* could be investigated. The *E. coli* gene was designated *gcd* to avoid confusion with the glutamate dehydrogenase gene. The DNA sequence was determined, and the derived amino acid sequence was compared with the sequences of both *A. calcoaceticus* GDH enzymes and with those of other known PQQ-dependent dehydrogenases. These comparisons suggest the existence of a putative PQQ-binding domain in all quinoproteins. Finally, the *gcd* gene was mapped on the *E. coli* chromosome.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. *E. coli* PP1795 (*ptsI thi galP gcd*) (10) was used for selection of the *gcd* clone. *A. calcoaceticus* PP1367 (*gdhA*) (10) was tested for complementation by the *gcd* clone. Bacterial matings between *E. coli* and *A. calcoaceticus* were performed as described previously (19), using *E. coli* S17-1 as the donor (34). Mapping of *gcd* on the *E. coli* chromosome was performed with strain LN681 (F^- *dnaC28 thyA deo gyrA thi supE42 trp::Mu*$^+$) (5). For P1 cotransduction experiments, we used strains Hfr3000 YA139 (*panB6 thi-1 relA spoT1*) (11) and AT986 (*dapD8 thi-1 relA spoT1*) (6). Insertion mutants of the *gcd* gene were constructed by using PPA42 (*ptsI thi galP*) (obtained from P. Postma, Amsterdam, The Netherlands) and JM101 [*lac pro supE thi*(F *traD36 proAB lacI*^{q} *lacΔ15*)] (30).

Plasmid vector pBR322 (4) was used for construction of a genomic library from *E. coli*. The broad-host-range vector pRK293 served for transfer of the *gcd* gene to *A. calcoaceticus*. The M13 vector used in insertion mutagenesis was M13mp9, which carries UAG nonsense codons in genes I and II.

E. coli and *A. calcoaceticus* were cultured in L broth, on

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L-agar plates, or in defined minimal medium (31). Tetracycline was used at a concentration of 20 μg/ml, ampicillin was used at 40 μg/ml, and chloramphenicol was used at 25 μg/ml.

GDH activity in *A. calcoaceticus* was determined by growth on minimal medium plates containing L-arabinose as the sole carbon source (19) and on MacConkey indicator agar plates containing 1% D-glucose. GDH-positive strains show red colonies on this medium, and GDH negatives are white (19).

Chemicals and reagents. All restriction enzymes, T4 DNA ligase, the Klenow fragment of DNA polymerase I, and calf intestine phosphatase were obtained commercially. Purified PQQ was a gift of M. van Kleef and was used at a concentration of 12 μM.

Analysis of plasmid DNA. Plasmid DNA from *E. coli* was isolated as described previously (27). Electrophoresis of DNA fragments was carried out on 1% agarose gels in 40 mM Tris acetate–2 mM EDTA (pH 8).

Construction of a genomic library from *E. coli*. Total chromosomal DNA from *E. coli* PPA42 was obtained from a 250-ml culture at a concentration of 8×10^8 cells per ml and prepared as described previously (19). About 100 μg of DNA was partially digested with *Sau*3AI. Fragments of 10 to 15 kb in size were isolated by electrophoresis on a 1% low-melting-point agarose gel and cutting out of the DNA bands of the appropriate size. The DNA was recovered from the agarose slice by remelting of the agarose for 5 min at 65°C and purification of the DNA by phenol-chloroform treatment. After ethanol precipitation, the DNA was ligated in the *Bam*HI site of plasmid vector pBR322. After *Bam*HI digestion, this vector was treated with calf intestine phosphatase (27) to avoid self-ligation.

The hybrid plasmids were transformed to the *gcd* mutant strain PP1795 and plated on L-agar plates containing ampicillin. Transformants were subsequently tested for complementation of the *gcd* mutation by streaking the colonies on minimal medium containing glucose and PQQ.

DNA sequencing. Sequencing was performed by the dideoxy method (33). Sequences were compiled and analyzed by using the sequence analysis software package of the University of Wisconsin Genetics Computer Group (13).

An *Eco*RI-*Sal*I DNA restriction fragment containing the *gcd* gene was cloned in the polylinker of M13mp18 and M13mp19. Deletion derivatives were isolated as described previously (18).

Mapping of *gcd* on the *E. coli* chromosome. The *gcd* gene was localized on the chromosome by using the DnaC synchronization method (16) and by hybridization with DNA of several lambda bacteriophages from the fully characterized complete library from the *E. coli* chromosome reported by Kohara et al. (24). Dot blots were prepared by spotting 5 μl of lambda stocks at 10^9 phages per ml on a strip of Hybond CE membrane (Amersham). The filter was then treated with denaturing and neutralizing solutions according to Masson and Williams (28) and baked at 80°C for 2 h. Prehybridization and hybridization were carried out as described by de Massy et al. (12). The *gcd* probe used (pGP478) was ^{32}P labeled with a nick translation kit (Amersham).

Southern blotting was performed by digestion of *E. coli* DNA with several restriction endonucleases, electrophoresis on a 0.7% agarose gel containing 1 μg of DNA per lane, and subsequent transfer to GeneScreen membranes (New England Nuclear) by the alkaline transfer method (7). DNA restriction fragments carrying either a part of or the complete *gcd* sequence were labeled by the hexanucleotide primer extension method (17). Hybridization was performed as described previously (9).

Construction of insertion mutants. The cloned *gcd* gene from *E. coli* was used to make an insertion in the *E. coli* chromosomal *gcd* gene. A 1.8-kb fragment from Tn*9* carrying the gene encoding chloramphenicol resistance (*cat*) was cloned in the *Sma*I site of the *gcd* gene. The *Eco*RI-*Sal*I fragment containing the *gcd* gene with the *cat* insertion was cloned in M13mp9 (29). This construct was used as described by Blum et al. (3) to recombine the *cat* insertion into the chromosomal *gcd* gene of *E. coli* PPA42 (*ptsI*).

Determination of growth rates. Strains were grown overnight in batch culture in 20 ml of rich medium containing 1% D-glucose or in mineral medium with 1% D-glucose. No antibiotics were added. The precultures were diluted 1:100 in 20 ml of the same medium either with or without PQQ. Growth of the culture was followed by determining the optical density at 600 nm.

RESULTS AND DISCUSSION

Isolation of the *gcd* gene. Partially digested chromosomal DNA fragments of *E. coli* PPA42 (*ptsI*) varying in size between 10 and 15 kb were ligated in the *Bam*HI site of vector plasmid pBR322 and used to transform *E. coli* PP1795 (*ptsI gcd*). Since the chromosomal library was derived from a *ptsI* mutant, complementation for growth on glucose and PQQ will be obtained only if the transformant contains the cloned *gcd* gene.

Complementation for growth on glucose, however, could not be selected directly since individual $PtsI^-$ Gcd^+ cells are not able to form colonies on minimal medium with glucose and PQQ. Growth is observed only when the cells are plated at a high concentration or in a streak in which many cells are close to each other. This phenomenon is probably due to a high diffusion rate of the gluconic acid that is produced on the outside of the cytoplasmic membrane by the oxidation of glucose by GDH. The diffusion of gluconic acid was shown by streaking PPA42 (*ptsI*) and PP1795 (*gcd ptsI*) next to each other on the same agar plate containing glucose and PQQ. Because of the presence of gluconic acid-producing cells (PPA42), PP1795 is able to grow. Apparently, the high diffusion rate interferes with efficient uptake of gluconic acid by the cells. To overcome this problem, the PP1795 cells transformed with the genomic library were first enabled to form colonies on L agar containing ampicillin. These colonies were subsequently tested for complementation of the *gcd* mutation by being streaked on mineral medium containing glucose and PQQ.

One of 1,000 transformants in PP1795 tested in this way was able to restore growth on glucose and PQQ. To show that the complementation was not due to reversion of the

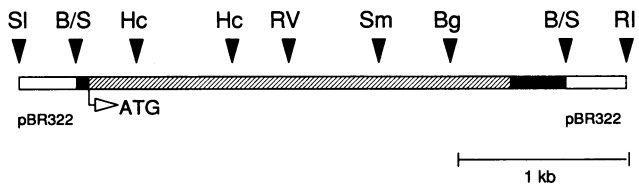

FIG. 1. Restriction map of the *Eco*RI-*Sal*I fragment from pGP478 containing the *gcd* gene. Symbols: □, pBR322 sequences; ■, inserted but noncoding regions; ▨, the *gcd* open reading frame. The start codon ATG is indicated. Restriction sites: Bg, *Bgl*I; B/S, *Bam*HI site ligated with a *Sau*3AI site; RI, *Eco*RI; RV, *Eco*RV; SI, *Sal*I; Sm, *Sma*I.

FIG. 2. Nucleotide and derived amino acid sequences of the *gcd* open reading frame. At the end of the open reading frame, the three stop codons TAG, TGA, and TAA are indicated (*). The putative −10 region of the *gcd* promoter is indicated by bold letters, and the putative membrane-spanning protein domains are underlined.

chromosomal mutation, the plasmid was isolated from this transformant, retransformed to PP1795, and shown to be able to complement the *gcd* mutation. The clone contains a 10-kb insert in the pBR322 *Bam*HI site and was designated pGP475. A subclone containing the complete *gcd* gene was constructed by partial digestion of pGP475 DNA with *Sau*3AI and isolation of fragments 2.5 to 3 kb in size. These DNA restriction fragments were ligated with *Bam*HI-digested, phosphatase-treated pBR322 DNA, and PP1795 was transformed with this ligation mixture. Complementing clones were selected as described above. In this way, a subclone (pGP478) was isolated which contained an insert of 3.0 kb and was able to complement the *gcd* mutation. Figure 1 shows a restriction map of the pGP478 insert.

To test whether the isolated *E. coli gcd* clone was able to complement the *gdhA* mutation in *A. calcoaceticus* PP1367, we inserted a fragment containing the cloned gene in the broad-host-range vector pRK293 (14). First, the *Eco*RI site from pGP478 was converted to a *Sal*I site with *Sal*I linkers, and the resulting 3.7-kb *Sal*I fragment was ligated into the *Xho*II site from pRK293 in both orientations. The *Xho*II site is within the coding region of the *kan* gene of this plasmid. Since the *kan* gene is functional in *A. calcoaceticus*, one of these constructs is expected to have the *gcd* gene expressed under control of the *kan* promoter. The two clones were designated pGP487 (containing the former *Eco*RI site nearest the *kan* promoter) and pGP488 (with the *gcd* fragment in the opposite orientation). Both plasmids complemented the *gcd* mutation in PP1795. They also complemented the *gdhA* mutation in *A. calcoaceticus* PP1367, as shown on MacConkey indicator agar containing 1% D-glucose and on mineral medium containing L-arabinose, since it has been shown that arabonic acid, which is the product of the *gdhA* reaction when L-arabinose is the substrate, can be used as a carbon

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1440 CAGACCGTTCACCACGACCTGTGGGATATGGATCTTCCGGCACAGCCGACGCTGGCGGACATCACCGTTAATGGTCAGAAAGTGCCAGTT
     GlnThrValHisHisAspLeuTrpAspMetAspLeuProAlaGlnProThrLeuAlaAspIleThrValAsnGlyGlnLysValProVal

1530 ATTTACGCTCCGGCGAAAACCGGCAACATTTTTGTGCTCGATCGTCGTAATGGCGAACTGGTGGTTCCGGCACCGGAAAAACCGGTTCCC
     IleTyrAlaProAlaLysThrGlyAsnIlePheValLeuAspArgArgAsnGlyGluLeuValValProAlaProGluLysProValPro

1620 CAAGGTGCTGCCAAAGGCGATTACGTTACCCCTACTCAACCATTCTCGGAACTGAGCTTCCGTCCGACGAAAGATTTGAGCGGTGCGGAT
     GlnGlyAlaAlaLysGlyAspTyrValThrProThrGlnProPheSerGluLeuSerPheArgProThrLysAspLeuSerGlyAlaAsp

1710 ATGTGGGGAGCCACCATGTTTGACCAACTGGTGTGCCGCGTGATGTTCCACCAGATGCGCTATGAAGGCATTTTCACCCCGCCATCTGAA
     MetTrpGlyAlaThrMetPheAspGlnLeuValCysArgValMetPheHisGlnMetArgTyrGluGlyIlePheThrProProSerGlu

1800 CAGGGTACGCTGGTCTTCCCGGGTAACCTGGGGATGTTCGAATGGGGCGGGATTTCCGTTGATCCAAATCGTGAAGTGGCGATTGCCAAC
     GlnGlyThrLeuValPheProGlyAsnLeuGlyMetPheGluTrpGlyGlyIleSerValAspProAsnArgGluValAlaIleAlaAsn

1890 CCAATGGCACTGCCGTTTGTTTCGAAACTGATCCCGCGTGGTCCTGGCAACCCGATGGAGCAGCCGAAAGATGCCAAAGGCACGGGTACG
     ProMetAlaLeuProPheValSerLysLeuIleProArgGlyProGlyAsnProMetGluGlnProLysAspAlaLysGlyThrGlyThr

1980 GAATCCGGCATTCAGCCACAGTACGGTGTACCGTATGGTGTCACGCTCAACCCGTTCCTCTCACCGTTTGGTCTGCCATGTAAACAGCCA
     GluSerGlyIleGlnProGlnTyrGlyValProTyrGlyValThrLeuAsnProPheLeuSerProPheGlyLeuProCysLysGlnPro

2070 GCATGGGGTTATATCTCGGCGCTGGATCTGAAAACTAATGAAGTGGTGTGGAAGAAACGTATTGGTACGCCGCAGGACAGTATGCCGTTC
     AlaTrpGlyTyrIleSerAlaLeuAspLeuLysThrAsnGluValValTrpLysLysArgIleGlyThrProGlnAspSerMetProPhe

2160 CCGATGCCGGTTCCGGTGCCGTTCAATATGGGTATGCCGATGCTGGGCGGGCCAATCTCCACGGCGGGTAACGTGCTGTTTATCGCCGCT
     ProMetProValProValProPheAsnMetGlyMetProMetLeuGlyGlyProIleSerThrAlaGlyAsnValLeuPheIleAlaAla

2250 ACGGCAGATAACTACCTGCGCGCTTACAACATGAGCAACGGTGAAAAACTGTGGCAGGGTCGTTTACCAGCGGGTGGTCAGGCTACGCCA
     ThrAlaAspAsnTyrLeuArgAlaTyrAsnMetSerAsnGlyGluLysLeuTrpGlnGlyArgLeuProAlaGlyGlyGlnAlaThrPro

2340 ATGACCTATGAAGTGAATGGTAAGCAGTATGTGGTGATCTCCGCAGGCGGTCACGGTTCATTTGGTACGAAGATGGGCGACTATATTGTG
     MetThrTyrGluValAsnGlyLysGlnTyrValValIleSerAlaGlyGlyHisGlySerPheGlyThrLysMetGlyAspTyrIleVal

2430 GCTTATGCGCTGCCGGATGATGTGAAGTAAGACTTGCTCAGATTGCTGACAACGTGCGCGTTGTTCATGCCGGATGCGGCGTGAACGCCT
     AlaTyrAlaLeuProAspAspValLysEndAspLeuLeuArgLeuLeuThrThrCysAlaLeuPheMetProAspAlaAlaEndThrPro

2520 TATCCAGCCTACAAAATTGTGCAAAATCAATGGATTGCACAGCTAGCGTAGGCCTGATAAGCGTAGCGCATCAGGCAGATTTGCGTTTGT
     TyrProAlaTyrLysIleValGlnAsnGlnTrpIleAlaGlnLeuAla *     * *

2610 CATCAGTTTAATGCCCGGAGAGATC
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FIG. 2—*Continued.*

source by *A. calcoaceticus* (19). The conclusion from these results is that the cloned *gcd* gene from *E. coli* encodes a GDH-A-like enzyme. The fact that in *A. calcoaceticus* the insert of pRK293 expresses an active GDH in both orientations suggests that the *E. coli* promoter that controls *gcd* expression is also active in *A. calcoaceticus*. However, we cannot exclude the presence of an unidentified promoter within the *kan* gene of pRK293 or the presence of promoter activity in the pBR322 part of the insert.

DNA sequence of the *gcd* gene. The sequence of the *gcd* gene (Fig. 2) was determined as described in Materials and Methods. It contains an open reading frame of 2,634 bp that encodes a putative protein of 796 amino acid residues with an M_r of 87,064. Analogous to the GDH-A from *A. calcoaceticus* (8), the *E. coli* GDH as predicted from the derived amino acid sequence contains five hydrophobic domains in the N-terminal part which might serve as membrane-spanning segments to ensure strong anchorage of the protein in the cytoplasmic membrane.

In a search for promoterlike sequences upstream of the ATG start codon in pGP478, we could find five possible −10 sequences but no appropriate matching −35 promoter region. Since the insert of pGP478 contains only 68 bp upstream of the *gcd* start codon, it is possible that the transcription of *gcd* in pGP478 is initiated in the *tet* gene of pBR322. The direction of transcription of *gcd* in pGP478 is, however, opposite that of the *tet* gene. Furthermore, of the seven promoterlike regions known in pBR322 (reviewed in reference 2), none can be responsible for *gcd* expression. Therefore, we consider it more likely that at least the −10 region of the *gcd* promoter is present in the 68-bp upstream region. The −35 sequence either is also present in this region (which would probably mean that the promoter is relatively weak) or is provided by a fusion of a −35-like sequence within the *tet* gene. The putative −10 sequence is underlined in Fig. 2.

The amino acid sequence of the *E. coli* GDH deduced from the nucleotide sequence was compared with the predicted amino acid sequences of the two PQQ-dependent GDHs from *A. calcoaceticus* (GDH-A and GDH-B), using the University of Wisconsin computer program (13). A graphic dot plot of a comparison between the derived amino acid sequences from the *A. calcoaceticus gdhA* gene and from *E. coli gcd* (Fig. 3A) shows that these proteins are very homologous at the amino acid level. The DNA homology is less obvious (not shown). When a similar comparison was made between *E. coli* GDH and *A. calcoaceticus* GDH-B, no significant homologies were found except for a small region shown in Fig. 4A. Comparing the *E. coli* GDH with another type of PQQ-dependent enzyme, methanol dehydro-

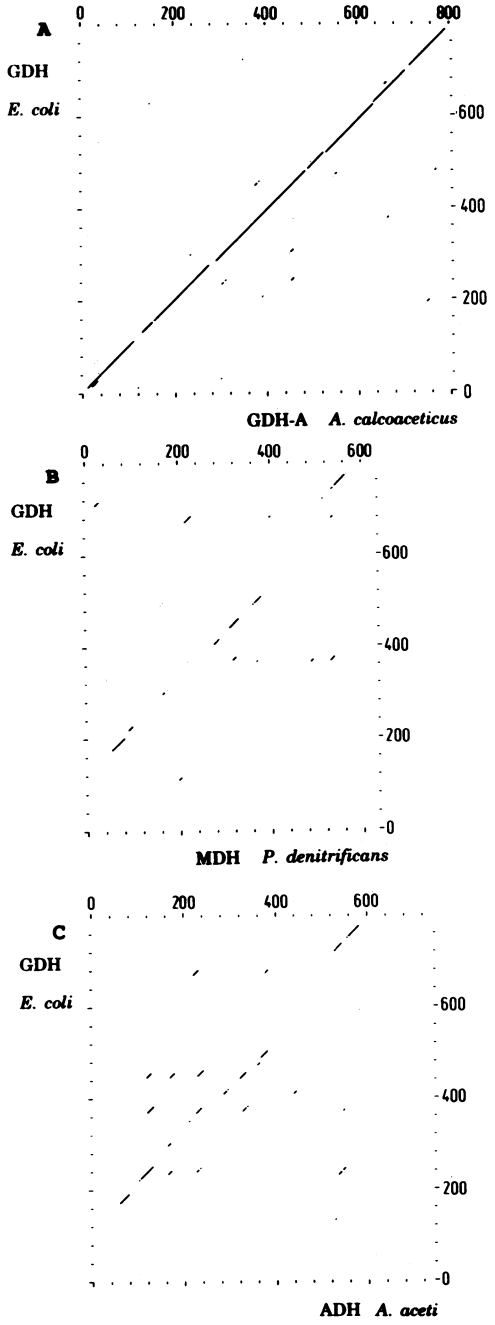

FIG. 3. Dot matrix analysis of the derived amino acid sequences from four PQQ-dependent dehydrogenases. Comparison of the *E. coli* GDH with the GDH-A from *A. calcoaceticus* (A), MDH from *P. denitrificans* (B), and ADH from *A. aceti* (C) was made by using the University of Wisconsin Genetics Computer Group COMPARE program (13) with a stringency of 14 of 21 homologous amino acids. The output was obtained by using the DOTPLOT program (13). The spacing and number of dots indicate the homology between the two compared quinoproteins.

genase (MDH), from either *Paracoccus denitrificans* (20) or *Methylobacterium organophilum* (26), we could detect several small regions of homology at the amino acid level. Figure 3B shows these regions in a dot plot made for *E. coli* GDH and *P. denitrificans* MDH. When GDH was compared with MDH from *M. organophilum*, an almost identical dot plot was found since the two MDH enzymes are highly homologous (26). Figure 3C shows the homologous regions between GDH and alcohol dehydrogenase (ADH) from *Acetobacter aceti* (23), which is also a PQQ-dependent enzyme. It is striking that enzymes using a common coenzyme but very different substrates show more homology than do two enzymes, GDH-A and GDH-B, that use both the same coenzyme and largely the same substrates. We suggest that one or more of the homology regions in ADH, MDH, GDH-A, and the *E. coli* GDH are involved in PQQ binding. The largest region of homology that is found in all five quinoproteins, which is the C-terminal domain, is shown in detail in Fig. 4B. In this same region, also a weaker but significant homology can be shown between *E. coli* GDH and *A. calcoaceticus* GDH-B (Fig. 4A). However, most of the residues that are identical in *E. coli* GDH and GDH-B from *A. calcoaceticus* differ from those conserved in *E. coli* GDH and the other quinoproteins in Fig. 4B. Only four amino acids in this 24-residue stretch are identical in all PQQ-dependent dehydrogenases for which the DNA sequence is known. The relevance of this more distantly related region of GDH-B is uncertain, but it could also be involved in the interaction with the coenzyme PQQ.

Mapping of the *gcd* gene on the *E. coli* chromosome. As a first approximation of the position of the *gcd* gene on the chromosomal map of *E. coli*, we used the method described by Fayet and Prère (16). In this technique, a cloned gene can be mapped by DNA-DNA hybridization of this gene with pulse-labeled DNA of a synchronized culture. Because of the bidirectional nature of replication, this method gives two possible positions, in the clockwise and counterclockwise arms. A culture of LN681 (*dnaC28*) was synchronized and pulse-labeled at 90-s intervals as described previously (16, 25). Since LN681 contains a Mu prophage in the *trp* gene, the total length of the chromosome in this strain was taken as 4,756 kb, with *oriC* at coordinate 4038 (25). To determine the velocity of replication in both arms, the half-replication times ($t_{1/2}$) of two reference markers were determined. For the clockwise replication arm, the $t_{1/2}$ of the Mu phage in *trp*, which is located at 2,070 kb from *oriC*, was 29.2 min, which makes the velocity on the clockwise replication arm 70.89 kb/min. Likewise, the velocity on the counterclockwise replication arm was determined to be 79.69 kb/min. By using a marker located at 2,343 kb from *oriC* (plasmid pBS28 from J. P. Bouché), the $t_{1/2}$ of the *gcd* gene was found to be 13.6 min. From this result it can be calculated that *gcd* is located $13.6 \times 70.89 = 964.1$ kb from *oriC* on the clockwise arm (corresponding to a position around 4 min on the linkage map of Bachmann [1]) or at $13.6 \times 79.69 = 1{,}083.78$ kb from *oriC* on the counterclockwise arm (around 60 min on the linkage map). Next, to discriminate between these two possibilities, we screened the lambda library of Kohara et al. (24) for the presence of the *gcd* gene. This library is composed of overlapping lambda clones for which the map positions are defined. pGP478 DNA was hybridized with dot blots of lambda phages that carry DNA from either the 2- to 6-min or the 59- to 62-min region of the chromosome. DNAs of phages 4E11 and 17C11 (24) show a strong signal with this probe. These results show that the *gcd* gene is located at approximately 3 min on the *E. coli* chromosome.

To obtain a more detailed map position of *gcd*, we made a Southern blot of *E. coli* chromosomal DNA, digested with several restriction enzymes: *Bam*HI, *Bgl*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Pst*I, and *Pvu*II. *Bgl*I and *Eco*RV are of special interest because the cloned *gcd* gene contains an internal *Bgl*I site and an *Eco*RV site (Fig. 1). The blot was hybridized

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GCD     730 DNYLRAYNMSNGEKLWQGRLPAGGQATPMTYEVNGKQYVVISAGGHG 776
GDH-B   179 QKIYYTIGDQGRNQLAYLFLPNQAQHTPTQQELNGKDYHTYMGKVLR 225
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B

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GCD     730 DNYLRAYNMSNGEKLWQGRLPAGGQATPMTYEVNGKQYVVISAGGHG 776
GDH-A   736 DNYLRAFNVTNGKKLWEARLPAGGQATPMTYEINGKQYVVIMAGGHG 782
MDH-1   524 DGFIKARDSDTGDLLWKFKLPSGVIGHPMTYKHDGRQYVAIMYGVGG 570
MDH-2   519 DGYLKARDSDTGDLLWKFKIPSGAIGYPMTYTHKGTQYVAIYYGVGG 565
ADH     537 NGEFHAYDATNGSDLYKFDAQSGIIAPPMTYSVNGKQYVAVEVGWGG 583
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FIG. 4. (A) Comparison of the homologous domains of *E. coli* GDH (GCD) and *A. calcoaceticus* GDH-B. Identical and similar amino acid residues are indicated by underlined bold and bold symbols, respectively. (B) Amino acid sequence of the homologous domains in several PQQ-dependent dehydrogenases: GDH-A (*A. calcoaceticus*), MDH-1 (*P. denitrificans*), MDH-2 (*M. organophilum*), and ADH (*A. aceti*). The four amino acid residues that are identical in all six enzymes, including GDH-B, are indicated (^).

with either the *Sal*I-*Eco*RI fragment containing the complete *gcd* sequence (Fig. 5A), the *Sal*I-*Bgl*I fragment carrying the N-terminal encoding part (Fig. 5B), or the *Bgl*I-*Eco*RI fragment containing the C-terminal encoding part (Fig. 5C). Table 1 shows a comparison between the sizes of the restriction fragments that should contain the *gcd* gene according to the restriction map data presented by Kohara et al. (24) and the actual sizes of the fragments that hybridized with the *gcd* probe as shown in Fig. 5. The sizes of both *Bam*HI and *Bgl*I deviate from the restriction map, but the sizes of the remaining five endonucleases agree with it. However, the *Eco*RV restriction data for the 3-min region are lacking in the map of Kohara et al. (24). It is therefore not possible to draw any conclusions from this blot regarding the distribution of these *Eco*RV sites, since the size of the hybridizing fragments (two bands of 2.5 kb) is less than the size of the total unknown region. Of particular interest is the hybridization pattern from the *Bgl*I-digested DNA. According to Fig. 5A, which shows a blot probed with the complete *gcd* sequence, two *Bgl*I fragments hybridized. However, in Fig. 5B (hybridization with the N-terminal encoding part upstream of the *Bgl*I site) only one band appeared, whereas in Fig. 5C (hybridization with the C-terminal encoding part downstream of the *Bgl*I site) only the other band was detected. This finding indicates that the *Bgl*I site in the 3.1-min region of the map of Kohara et al. (24) is the internal *Bgl*I site in the *gcd* gene. Finally, the difference in hybridization data between the N- and C-terminal probes shows that the *gcd* gene is transcribed in the same direction as the replication fork.

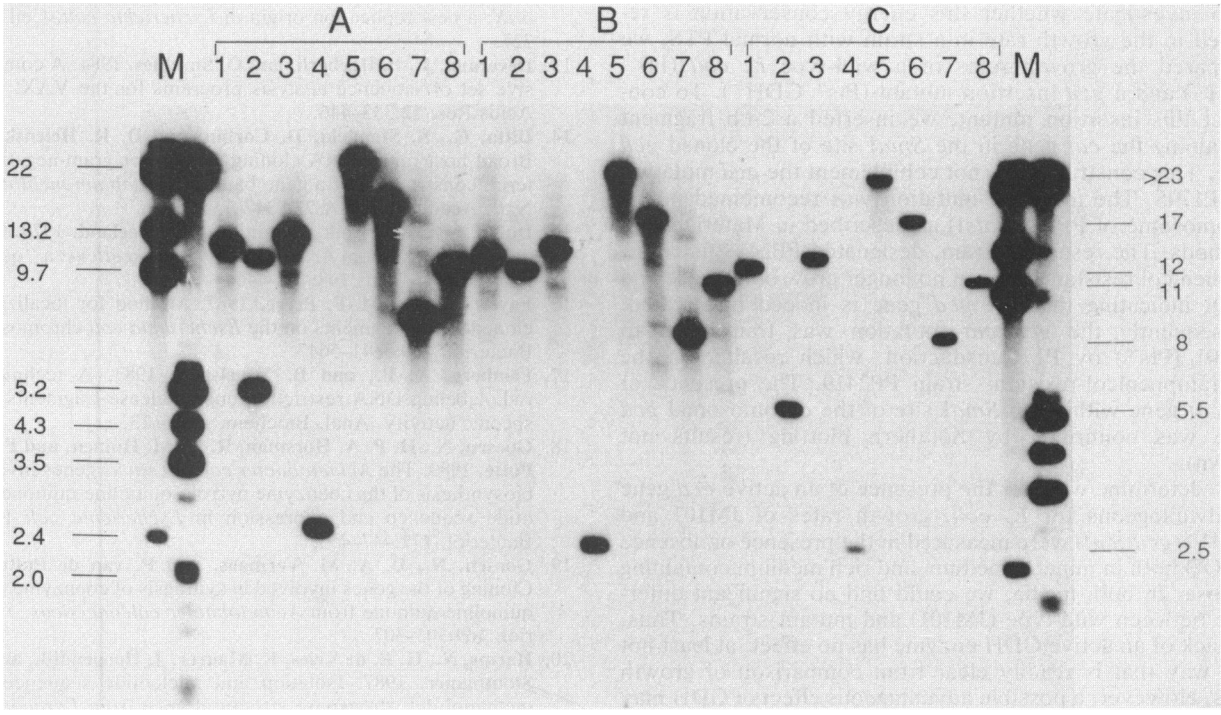

FIG. 5. Southern blots of *E. coli* chromosomal DNA, hybridized with the complete *gcd* gene: the 3.5-kb *Eco*RI-*Sal*I fragment (A), the N-terminal encoding part, the *Bgl*I-*Sal*I fragment (B), or the C-terminal part, the *Bgl*I-*Eco*RI fragment (C). Lanes: M, λ markers (λ DNA digested with *Bgl*II [left] or with *Eco*RI and *Hin*dIII [right]); 1, *Bam*HI; 2, *Bgl*I; 3, *Eco*RI; 4, *Eco*RV; 5, *Hin*dIII; 6, *Kpn*I; 7, *Pst*I; 8, *Pvu*II.

TABLE 1. Comparison of endonuclease sizes

Restriction endonuclease	Size (kb)	
	Expected	Actual
*Bam*HI	20	12
*Bgl*I	7.5; 5.5	11; 5.5
*Eco*RI	13	13
*Eco*RV		2.5; 2.5
*Hin*dIII	26	>26
*Kpn*I	17	17
*Pst*I	8	8
*Pvu*II	11	11

The map position as determined by Southern blotting predicts efficient cotransduction of *gcd* and two nearby loci: *panB* at 3.4 min (in strain YA139) and *dapD* at 3.8 min (in AT986). Indeed, cotransduction frequencies with these markers are 10 and 42%, respectively, which offers additional support of the map position of *gcd* at 3.1 min.

Effect of a *gcd* insertion mutation on growth rate. *E. coli* K-12 contains a gene encoding PQQ-dependent GDH but can use the enzyme only when PQQ is present in its biotope. In *A. calcoaceticus*, the gluconic acid that is formed by the GDH-A reaction is not further degraded, probably because of the absence of a transport system for this reaction product which is formed in the periplasmic space. However, it was shown that *A. calcoaceticus*, *E. coli*, and *Pseudomonas aeruginosa* can benefit from the GDH reaction because a proton motive force is generated by the oxidation of glucose, which supplies the driving force for the active uptake of lactose and amino acids (35). This finding indicates an involvement of GDH in energy conservation rather than in sugar metabolism.

To investigate whether this energy conservation is reflected in the growth rate in a strain with normal PTS, we compared the growth rates of a wild-type *E. coli* (Pts^+ GDH^+) and a *gcd* insertion mutant (Pts^+ GDH^-). To construct this insertion mutant, we inserted a 2-kb fragment containing the *cat* gene in the *Sma*I site of the cloned *gcd* gene. This construct does not complement the *gcd* mutation in PP1795. The insertion mutation was recombined in the chromosome of PPA42 (*pts*I) as described in Materials and Methods. The resulting strain, designated PP2418, is chloramphenicol resistant and can no longer grow on glucose and PQQ, indicating that the *gcd* gene is indeed inactivated. Subsequently the *gcd*::*cat* mutation was transferred to JM101 (Pts^+) by P1 transduction, which resulted in the chloramphenicol-resistant strain PP2419. The presence of the *cat* gene within the *Sma*I site of the chromosomal *gcd* gene was confirmed by Southern blotting (results not shown).

To determine whether the presence of an active *gcd* gene is advantageous for *E. coli*, growth rates of JM101 and PP2419 (*gcd*::*cat*) were measured in the presence or absence of PQQ both in mineral medium and rich medium containing glucose. In both media, we could find no significant difference between wild-type (JM101) and mutant strains. Thus, the lack of an active GDH enzyme has no effect, at least not in a way that is readily clear from comparison of growth rates. However, a possible advantageous effect of GDH may operate only in the natural surroundings of the bacteria, when growth conditions are difficult and energy must be released from the substrate in a short time.

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