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The *gntP* Gene of *Escherichia coli* Involved in Gluconate Uptake

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The *gntP* gene, located between the *fim* and *uxu* loci in *Escherichia coli* K-12, has been cloned and characterized. Nucleotide sequencing of a region encompassing the *gntP* gene revealed an open reading frame of 447 codons with significant homology to the *Bacillus subtilis* gluconate permease. Northern (RNA) blotting indicated that the *gntP* gene was monocistronic and was transcribed as an mRNA with an apparent molecular size of 1.54 kb. The transcriptional start point was determined by primer extension analysis. The *gntP* gene was found to be under catabolite repression and was not induced by gluconate. Also, expression seemed to be stringently controlled. Several observations indicated that the GntP protein is an inner membrane protein; it contains characteristic membrane-spanning regions and was isolated predominantly from the inner-membrane fraction of fractionated host cells. A topology analysis predicted a protein with 14 membrane-spanning segments. The inability of a mutant strain to grow on gluconate minimal medium could be relieved by introduction of a plasmid encoding the *gntP* gene. Finally, the kinetics of GntP-mediated gluconate uptake were investigated, indicating an apparent K_m for gluconate of 25 μM.

Gluconate is an important food source of *Escherichia coli* in its natural environment, the gut (26). In order to shunt gluconate into the main metabolic routes, the bacterium must carry out two initial operations. In the first, extracellular gluconate is imported into the cytoplasm, and in the second, the gluconate is phosphorylated to 6-phosphogluconate by a kinase. 6-Phosphogluconate can enter two central metabolic pathways; it is catabolized primarily through the Entner-Doudoroff pathway but also through the pentose phosphate pathway. Therefore, only two enzyme functions, a permease and a kinase, seem to be specifically required for gluconate catabolism. However, both of these functions are carried out by more than one enzyme. In fact, no less than three gluconate permeases and two kinases have been described hitherto. The 75-min region of *E. coli* K-12 contains the genes of the GntI system, i.e., *gntT* and *gntU*, encoding high- and low-affinity transport systems, respectively, and *gntK*, which encodes a gluconokinase. The GntII system, a subsidiary system for gluconate transport and phosphorylation, located in the 96-min region, includes the *gntS* gene, encoding another high-affinity transport system, and the *gntV* gene, encoding a thermosensitive glucokinase (15). Various observations have suggested that the genes of the GntI and the GntII systems are differentially regulated (1, 5, 19). The GntI system is specifically induced by gluconate and is regulated by the *gntR* gene (also located in the 75-min region) product, a repressor of the genes of the GntI system and the Entner-Doudoroff pathway but not of the genes of the GntII system (9, 19, 32). It is not known how the expression of the GntII system is controlled (15).

At present it is not clear why this multitude of genes with seemingly redundant functions exists in *E. coli*. Furthermore, the interplay among and roles of the various gluconate permeases and kinases during utilization of this food source by *E. coli* are at present inadequately understood and several, often conflicting, observations concerning the regulation of the various genes have been reported. One of the problems could be that so many different gene products with similar functions are involved. In this paper, we report on a new gene, *gntP*, located immediately downstream of the *fim* gene cluster at 98 min, encoding a fourth *E. coli* gluconate permease.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. The following *E. coli* K-12 strains were used for this study: HB101 [F^- Δ(*mcrC-mrr*) *leu supE ara galK lacY proA rpsL xyl mtl recA*] (4); W1485 (F^+ λ) (2); and M6 (*hfrC gntT gntU gntR gntK edd*), kindly provided by T. Istúriz, derived from *E. coli* M2 (*hfrC gntT*) (19), which was originally derived from *E. coli* M1 (*hfrC*, prototroph) (32). *E. coli* M6 is unable to grow on gluconate minimal medium because the mutations in *gntT* and *gntU*, the two transporters of the GntI system, eliminate its ability to transport gluconate in batch cultures with gluconate as the sole carbon and energy source. The gluconate transporter(s) of the GntII system (subsidiary system) are not induced under these growth conditions (1, 15). Likewise, the gluconate transporter encoded by the *gntP* gene described in this report is subject to strong catabolite repression and is repressed when growing on gluconate or other sugars. The *gntR* mutation renders expression of the Entner-Doudoroff pathway (*edd eda*) and gluconokinase (*gntK*) constitutive. Therefore, genetic complementation of the defects in gluconate transport activity in *E. coli* M6 is sufficient to allow growth on minimal gluconate medium. Cells were grown on solid or in liquid media supplemented with the appropriate antibiotics.

Plasmids. Plasmid pPIL38, containing a 26-kbp insert harboring the *fim* region and flanking sequences from *E. coli* K-12 strain PC31, has been described previously (17). Plasmid pPKL133 (*gntP*$^+$) and plasmid pPKL134 (*gntP*$^+$) were made by insertion of a 2.6-kbp *Kpn*I-*Eco*RI fragment or a 5-kbp *Pst*I-*Bam*HI fragment from plasmid pPIL38 into *Kpn*I-*Eco*RI- or *Pst*I-*Bam*HI-restricted plasmid pGEM3 (Promega), respectively. Plasmid pGP1-2 (27), containing the T7 polymerase gene under the control of the λP_L promoter, was used in connection with pGEM3-based plasmids in specific transcription and radiolabelling experiments.

DNA techniques. Isolation of plasmid DNA was carried out by the mini-lysate method of Del Sal et al. (8). Restriction endonucleases were used according to the manufacturer's specifications (Biolabs).

Nucleotide sequencing. The nucleotide sequence of the region containing the *gntP* gene was determined by the dideoxynucleotide chain termination method (24) with the Sequenase Kit, version 2 (U.S. Biochemical Corp., Cleveland, Ohio), with α-^{35}S-dATP, as described in the manufacturer's instructions. Alkali-denatured plasmid DNA of plasmid pPKL133 or pPKL134 was used as the template. Oligonucleotide primers were synthesized at the core facilities of the Department of Microbiology, Technical University of Denmark.

Northern (RNA) analysis. Total RNA was prepared from growing cultures by using a hot-phenol method described previously (6). An RNA hybridization probe specific for *gntP* was prepared by transcription of a 590-bp *Pvu*II fragment from pPKL133 that was subcloned into the *Sma*I site of pBluescriptIIKS. The

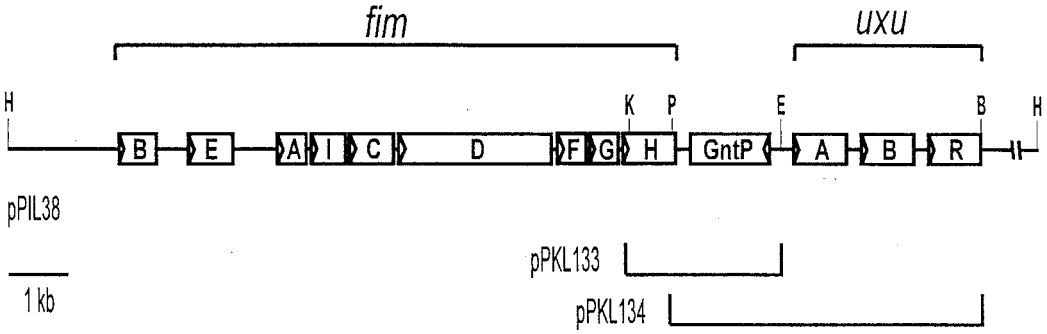

FIG. 1. Overview of the *gntP* gene and the *fim* and *uxu* gene clusters as present in plasmid pPIL38. Arrows indicate transcriptional orientation. The extents of subcloned fragments present in other recombinant plasmids are indicated by bars. Only DNA inserts are shown. Relevant restriction sites are indicated: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I.

probe was labelled by in vitro transcription in the presence of digoxinin-labeled UTP from the T3 promoter with T3 RNA polymerase. Northern hybridization and detection of the labelled RNA probe with alkaline phosphatase-linked antidigoxinin antibody were as described by the manufacturer (Boehringer Mannheim, Indianapolis, Ind.).

Primer extension analysis. RNA was prepared essentially as previously described (20). Primer extension reactions were performed by using a 5′-kinase-labelled oligonucleotide, 5′-CAGTACCAGCATCAGACC, complementary to a position 39 bases downstream from the potential start of the *gntP* gene. A reverse transcriptase kit, SuperScript II, from Gibco, BRL, was used in accordance with the manufacturer's instructions for synthesis of labelled cDNA. Samples were loaded on a standard 6% polyacrylamide DNA sequencing gel and run together with the corresponding DNA sequencing samples resulting from employing the same oligonucleotide primer and plasmid pPKL133 as the template.

Expression from T7 promoter systems. Exclusive expression and radiolabelling of the GntP protein were performed as described by Tabor and Richardson (27). Cell fractionation was carried out essentially as described by Osborn et al. (22).

Gluconate uptake assay. In order to measure gluconate transport, cells were grown in Luria broth and harvested at late log phase at an A_{550} of 0.8. Semiquantitative measurement of gluconate transport was conducted as described by Istúriz et al. (15). The method for kinetic measurements of GntP-mediated gluconate transport was a slight modification of the procedure of Walsh et al. (31), as described previously (23). For determination of the substrate specificity of GntP, uptake of 50 μM radioactive gluconate was assayed in the presence of a fourfold excess of unlabelled sugars. The assay was conducted as described previously (23). The data were analyzed by using Enzfitter software (Biosoft, Cambridge, United Kingdom). In the kinetic experiments, the velocity values of the negative control (*E. coli* M6, no plasmid) were subtracted from the experimental values obtained with *E. coli* M6(pPKL133). These control values were very low. The experiment was repeated three times with nearly identical kinetic values.

RESULTS

Characterization of the *gntP* gene. Plasmid pPIL38 contains a 26-kbp chromosomal insert from *E. coli* K-12 strain PC31 which straddles the 98.0-min region (Fig. 1). This plasmid harbors the well-characterized *fim* gene cluster of 9.5 kbp, required for biosynthesis of type 1 fimbriae, and additional flanking sequences, notably approximately 16 kbp of the region downstream of *fim* (17, 18). Two overlapping fragments from plasmid pPIL38, containing the region adjacent to and downstream of the *fim* genes, were cloned into the pGEM3 vector, resulting in plasmids pPKL133 and pPKL134 (Fig. 1). Nucleotide sequence analysis of a 2.0-kbp sector downstream of the *fimH* gene (EMBL database accession no. X91735) revealed an open reading frame of 447 codons, preceded by a potential ribosomal binding site, which we define as the *gntP* gene. In addition, the nucleotide sequence at the end of the sequenced sector distal to *fim* proved to be virtually identical to the published partial nucleotide sequence of the *uxuA* gene (3), thereby confirming the close linkage of these loci. Unlike the *fim* and *uxu* genes, the *gntP* gene is oriented counterclockwise on the *E. coli* K-12 map.

Northern analysis of *gntP* expression. Northern analysis revealed the pattern of *gntP* expression. An RNA hybridization probe specific to *gntP* was found to bind to a single 1.54-kb transcript in total RNA prepared from *E. coli* W1485, indicating that the gene is monocistronic (Fig. 2). The *gntP* mRNA was present at high levels in logarithmic-phase cultures grown on complex medium under either aerobic or anaerobic conditions but was present at low levels in stationary-phase cultures. Also, the *gntP* mRNA was present at very low levels when cells were grown on complex medium with added glucose or gluconate and the *gntP* transcript was virtually undetectable in cells grown aerobically on complex medium containing a mixture of glucose and gluconate. These results indicate that the

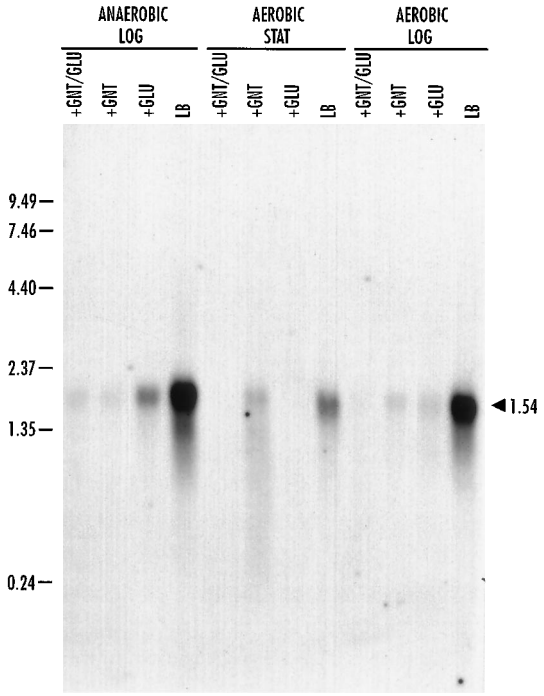

FIG. 2. Northern blot analysis of the *E. coli gntP* gene. Total RNA samples (5 μg per lane) were prepared from *E. coli* W1485 grown aerobically or anaerobically to log phase (LOG) or stationary phase (STAT) on LB medium without added carbohydrate (LB) or on LB medium containing (0.4%) glucose (GLU), gluconate (GNT), or both (GNT/GLU). RNA size markers (in kilobases) are indicated on the left, and the *gntP*-specific transcript is indicated by the arrowhead on the right.

gntP gene is subject to strong catabolite repression and is not induced by gluconate.

Mapping the 5′ terminus of the *gntP* transcript. The position of the 5′ end of the *gntP* transcript was investigated by primer extension analysis. RNA samples were isolated from both exponentially growing and stationary-phase cultures (in complex medium without added sugars) of bacterial host cells harboring either plasmid pPKL133 or pGEM3 (vector control) and used as templates. A 5′ terminus for the *gntP* transcript was detected at a position corresponding to the G residue 38 bases upstream of the initiation codon (Fig. 3). Also, two secondary signals were observed at positions corresponding to 6 and 7 bases upstream of the initiation codon, respectively. These latter signals were presumably due to processing of the primary transcript, since they are located after the potential ribosomal binding site. The sequence upstream of the *gntP* transcriptional initiation site shows significant homology to the consensus for the 18-bp spacing class of *E. coli* σ^{70} promoters as defined by O'Neill (21). The −10 region, has four of six conserved residues (TAACAT), while the −35 region has only three of six conserved residues (GCAACA). Interestingly, the −35 region overlaps a catabolite activator protein (CAP) binding site which is optimally located at position −41 with respect to the transcriptional initiation site (13). This result is in keeping with the low levels of *gntP* mRNA observed in cells grown on media containing catabolite-repressing sugars (described above). The CAP binding site upstream of *gntP*, TGTGA-N_6-TCGCA, exhibits almost perfect symmetry and shows excellent fit to the canonical CAP binding motif, TGTGA-N_6-TCACA (7). It is not uncommon for promoters under catabolite repression to have a particularly poor homology to the canonical σ^{70} −35 region (7). It is also noteworthy that the sequence between the −10 region and the transcriptional start shows homology to promoters known to be under stringent control (28). Furthermore, Northern analysis and primer extension experiments indicated very low levels of *gntP* mRNA in stationary-phase cultures (Fig. 2 and 3), indicative of a promoter that is subject to stringent control.

Transcriptional termination. Analysis of the 3′ region of the *gntP* gene revealed a region of dyad symmetry located 10 to 33 bases downstream of the translational stop codon. This sector could potentially constitute a rho-dependent transcriptional terminator (Fig. 3C). According to the position of the detected transcriptional start point and the position of the hypothetical terminator, the *gntP* transcript would constitute approximately 1,415 bases.

The *gntP* gene product. Analysis of radiolabelled GntP protein by the T7 polymerase-promoter system showed the protein to be produced as a polypeptide with an apparent molecular mass of 36.0 kDa (Fig. 3). The observed molecular weight of GntP on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis is considerably lower than the predicted molecular weight of 47,100, calculated on the basis of the deduced primary structure. However, underestimation of molecular weight is common for hydrophobic integral membrane proteins, and this class of proteins has also been frequently reported to cause smearing on protein gels, a feature readily apparent with GntP (see, for example, reference 25). Further support for this notion was provided by analysis of cell fractions, which indicated that the GntP protein was predominantly located in the inner-membrane fraction (Fig. 4).

Topology of the GntP protein. The GntP protein has a high proportion of hydrophobic amino acids (I, L, V, M, W, Y, and F, ~48%). The majority of these are clustered in a number of segments in the sequence and often interspersed with a few charged amino acid residues (Fig. 5). Furthermore, the protein appears to lack an N-terminal signal sequence. Taken together, such features could be indicative of an inner-membrane localization. This conclusion was further supported by prediction of the topology by using the TOPPRED program according to method of von Heijne and Manoil (29, 30). The hydrophobicity plot of the GntP protein is shown in Fig. 6. The method used identifies 14 transmembrane segments in the GntP sequence with peaks above the upper cutoff level. According to the positive-inside rule, which states that lysine and arginine residues are preferably located on the cytoplasmic side of the membrane (29), the N-terminal part of GntP should be located on the periplasmic side of the membrane.

The model with 14 transmembrane segments and with the N-terminal part of the protein located in the periplasm has 16 lysine and arginine residues positioned in the intracellular loops and only 3 in extracellular loops, i.e., a charge bias of 13, and is therefore in excellent agreement with the positive-inside rule of von Heijne (29). The predicted model with 14 transmembrane segments resulted from an algorithm employing a window length of 15 residues. If a window length of 21 was used instead, as in the original work of von Heijne, transmembrane segments 4 and 5 would fuse and result in a model with 13 membrane-spanning segments. However, such a model deviates more from the positive-inside rule, having a positive-charge bias of only 5, i.e., 12 lysine and arginine residues in the cytoplasmic loops and 7 in the extracellular loops.

The TOPPRED program predicts only the number and orientation of the transmembrane segments. The precise borders of the transmembrane segments and the loop regions have therefore been assigned manually by inspection of the sequence. The resultant model is shown in graphical form in Fig. 6, and the predicted transmembrane segments are marked in the sequence in Fig. 5. We are aware that some of the membrane-spanning segments in our proposed model are quite short, notably, segments 5, 10, and 11, with 14 or 15 residues. A model with fewer membrane spans cannot be excluded, but on the basis of the hydrophobicity analysis we regard the 14-membrane-span model as the most likely.

Gluconate uptake and substrate specificity of GntP. *E. coli* M6 is unable to grow on gluconate minimal medium. On the other hand, *E. coli* M6(pPKL133) is able to grow on gluconate medium and forms red colonies on gluconate-MacConkey plates. This result is indicative of genetic complementation of the lesion in gluconate transport in *E. coli* M6. Apparently, expression of *gntP* on plasmid pPKL133 is not repressed by gluconate. We went on to measure gluconate transport by *E. coli* M6(pPKL133) using the semiquantitative method of Istúriz et al. (15). *E. coli* M6(pPKL133) was clearly positive by comparison with the negative control, *E. coli* M6 (no plasmid), and the positive control, *E. coli* W1485 (wild type). We then studied the kinetics of gluconate transport by *E. coli* M6 (pPKL133). The apparent K_m of GntP for gluconate is 25 μM, and the apparent V_{max} is 43 nmol/min/mg of protein (Fig. 7). The data in Table 1 give an indication of the substrate range of GntP. Transport of radioactive gluconate was modestly inhibited by fourfold excesses of nonradioactive glucuronic acid, maltose, lactose, sucrose, fructose, and galactose but not by manitol or glucose. These data suggest that GntP has a fairly broad specificity.

DISCUSSION

The *E. coli gntP* gene was cloned on a 26-kb genomic DNA fragment (pPIL38) by virtue of its presence in the region between the *fim* and *uxu* genes. The gene was identified as a putative gluconate permease gene on the basis of sequence

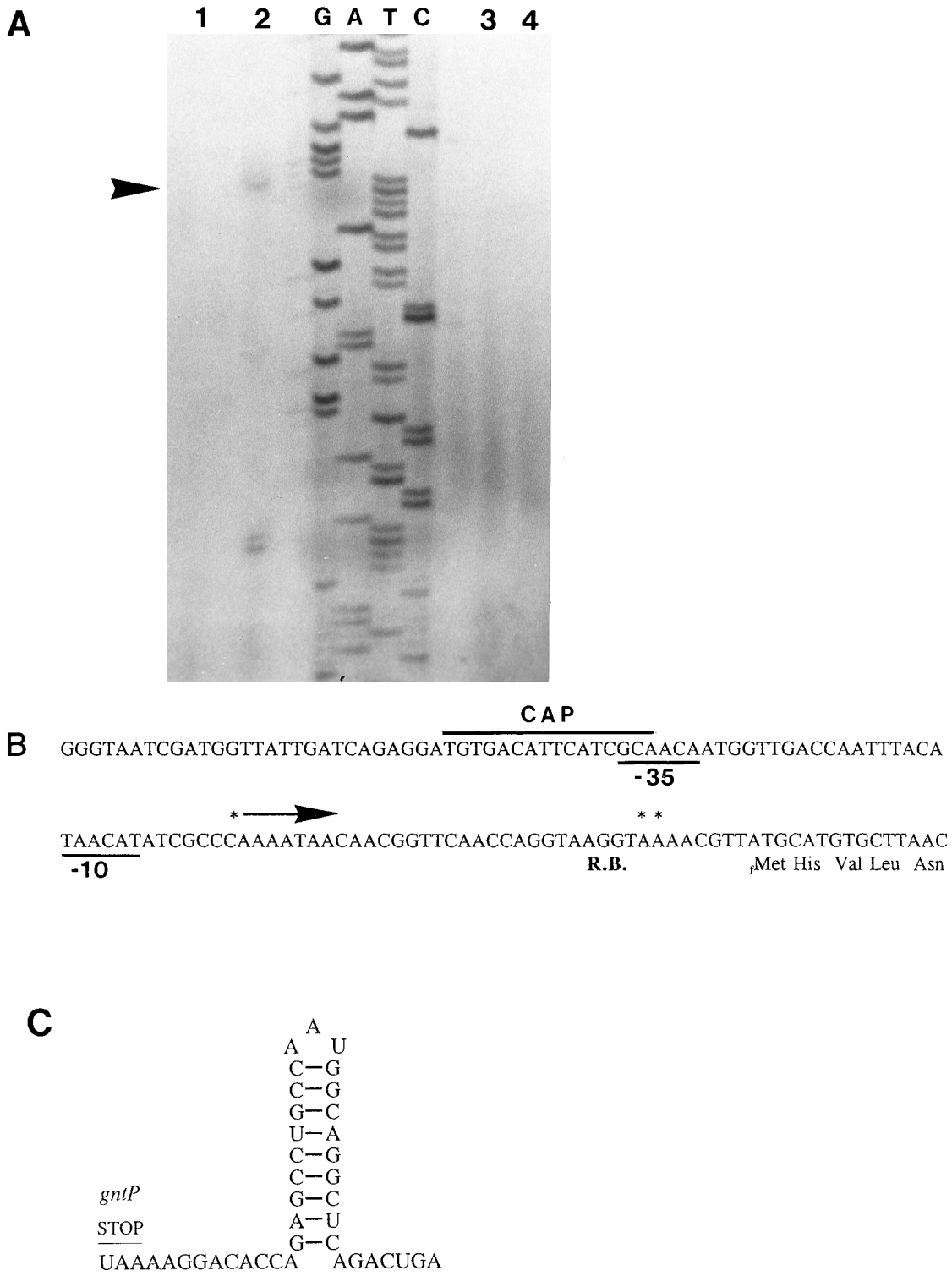

FIG. 3. Determination of the 5′ end of the *gntP* transcript by primer extension analysis. (A) Lanes 1 and 2 show reverse transcriptase reactions from exponentially growing cells containing plasmids pGEM3 (control) and pPKL133, respectively. The arrowhead indicates the start position of the *gntP* transcript. Lanes 3 and 4 show reverse transcriptase reactions from stationary-phase cultures of cells containing plasmids pGEM3 (control) and pPKL133, respectively. In the middle is shown the corresponding DNA sequence reactions employing the same oligonucleotide as the primer. (B). Overview of the sequence of the region upstream of the *gntP* gene. Primer extension signals are indicated by asterisks. A potential ribosomal binding site, −10 and −35 polymerase recognition sites, and a CAP binding site are indicated by R.B., −10, −35, and CAP, respectively. (C). Stem-loop model of the potential transcriptional terminator for the *gntP* gene.

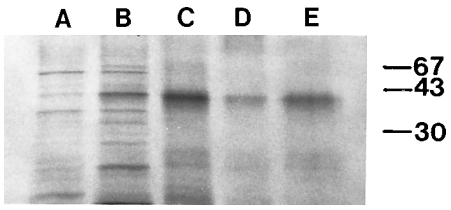

FIG. 4. SDS-polyacrylamide gel electrophoresis of the GntP protein expressed by the T7 polymerase-promoter system. Shown is [^{35}S]methionine labelling of protein from HB101 host cells, which, in addition to the T7 polymerase-encoding plasmid pGP1-2, harbored plasmid pGEM3 (vector control) or pPKL133. Lanes: A, total cell lysate, pGEM3, induced; B, total cell lysate, pPKL133, uninduced; C, total cell lysate, pPKL133, induced; D, outer membrane fraction, pPKL133, induced; E, inner membrane fraction, pPKL133, induced. Molecular mass standards are indicated in kilodaltons.

similarity to the *gntP* gene from *Bacillus subtilis* (12). Subcloning of the *E. coli gntP* gene allowed us to confirm the identity of this gene as coding for a gluconate permease, an identity based upon the ability of the gene to produce an approximately sized peptide, genetic complementation, and biochemical assay of gluconate transport activity in the recombinant *E. coli*. The *E. coli gntP* gene was found to be monocistronic and under catabolite repression control but, unexpectedly, was not induced by growth on gluconate.

A genetic comparison of the monocistronic *E. coli gntP* gene with the *B. subtilis gntP* gene shows the two to be very different, as the *B. subtilis gntP* gene is part of an operon and is cotranscribed with several other *gnt* genes, including the gene encoding 6-phosphogluconate dehydrogenase. The *gntP* gene (98.0 min) is oriented counterclockwise on the *E. coli* genome in the 1.9-kb region between the *fimH* gene, encoding the D-man-

```
                1                   2
ecognt MHVLNILWVVFGIGLMLVLNLKFKINSMVALLVAALSVGMLAGMDLMSLLHTMKAGFGNT  60
bacgnt MPLIIV---ALGILALLFLIMGLKLNTFISLLVVSFGVALALGMPFDKVVSSIEAGIGGT  57

          3                                   4
ecognt LGALAIIVVFGAVIGKLMVDSGAAHQIAHTLLARLGLRYVQLSVIIIGLIFGLAMFYEVA 120
bacgnt LGHIALIFGLGAMLGKLIADSGGAQRIAMTLVNKFGEKNIQWAVVIASFIIGIALFFEVG 117

           5                    6
ecognt FIMLAPLVIVIAAEAKIPFLKLAIPAVAAATTAHSLFPPQPGPVALVNAYGADMGMVYIY 180
bacgnt LVLLIPIVFAISRELKISILFLGIPMVAALSVTHGFLPPHPGPTAIAGEYGANIGEVLLY 177

           7                                   8
ecognt GVLVTIPSVICAGLILPKFLGNLERPT------PSFLKADQPVDMNNLPSFGVSILVPLI 234
bacgnt GFIVAVPTVLIAGPLFTKFAKKIVPASFAKNGNIASLGTQKTFNLEETPGFGISVFTAML 237

                                    9
ecognt PAIIMISTTIANIWLVK----DTPAWEVVNFIGSSPIAMFIAMVVAFVLFGTARGHDMQW 290
bacgnt PIIIMSVATIIDLLQETIGFADNGVLAFIRLIGNASTAMIISLLVAVYTMGIKRNIPVKT 297

                   10                                  11
ecognt VMNAFESAVKSIAMVILIIGAGGVLKQTIIDTGIGDTIGMLMSHGNISPYIMAWLITVLI 350
bacgnt VMDSCSTAISQIGMMLLIIGGGGAFKQVLINGGVGDYVADLFKGTALSPIILAWLIAAIL 357

                12                  13
ecognt RLATGQGVVSAMTAAGIISAAILDPATGQLVGVNPALLVLATAAGSNTLTHINDASFWLF 410
bacgnt RISLGSATVAALSTTGLVIP-LLGHSDVNL-----ALVVLATGAGSVIASHVNDAGFWMF 411

                          14
ecognt KGYFDLSVKDTLKTWGLLELVNSVVGLIIVLIISMVA                       447
bacgnt KEYFGLSMKETFATWTLLETIISVAGLGFILLLSLVV                       448
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FIG. 5. Comparison of sequences of GntP proteins from *E. coli* and *B. subtilis*. Identical amino acids are indicated by vertical lines. Predicted membrane-spanning segments are indicated by horizontal lines. Charged residues are indicated by + and −.

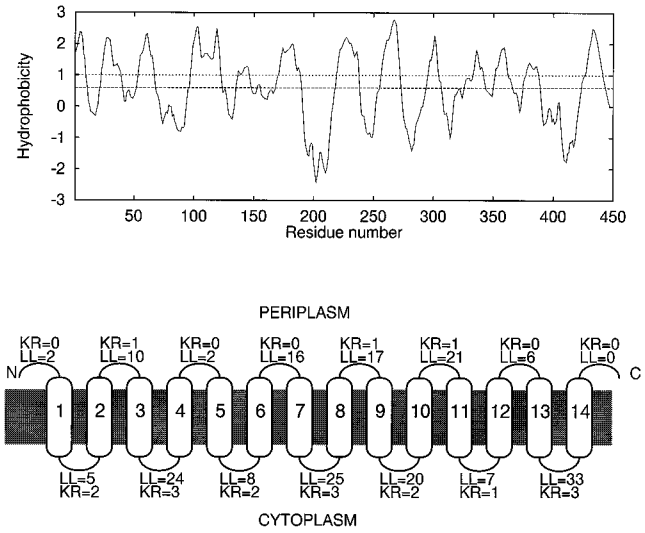

FIG. 6. Hydrophobicity plot (top) and predicted membrane topology (bottom) of the GntP protein. The hydrophobicity (11) was calculated over a trapezoid window with a full length of 15 and a core length of 11. The method employs two hydrophobicity cutoff values (indicated by horizontal lines), with peaks above the upper cutoff level considered certain and peaks between the upper and lower cutoff levels considered putative. The topology was predicted according to the method of von Heijne (29). The predicted model with 14 transmembrane segments is shown. The combined lysine and arginine content (KR) and length (LL) of each polar segment is indicated.

nose-specific adhesin of type 1 fimbriae (16), and the *uxuA* gene, involved in hexuronate catabolism, which are both oriented clockwise on the genome. It is unlikely that the *E. coli gntP* gene described in this work is the *gntS* gene of the GntII subsidiary system for gluconate metabolism since the *gntS* gene maps in the 95.9-min region and is closely linked to *fdp* (1). Thus, the *E. coli gntP* gene appears to be a fourth gluconate permease gene, in addition to *gntS* of the GntII system and *gntU* and *gntT* of the GntI system. While it is tempting to speculate, on the basis of proximity to the *uxu* genes involved in hexuronate metabolism, that the *E. coli gntP* gene codes for

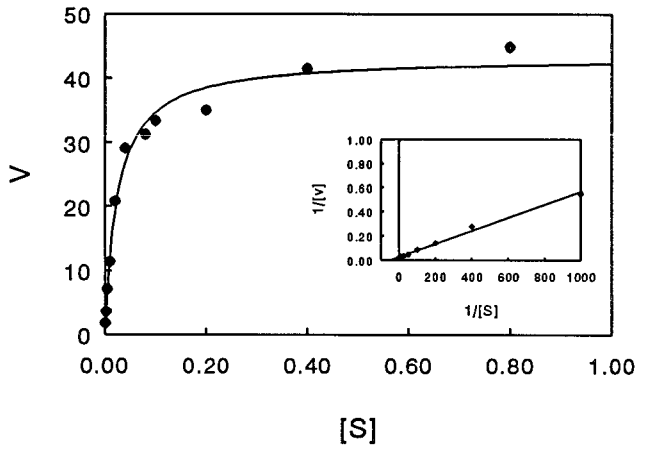

FIG. 7. Kinetics of gluconate transport by *E. coli* M6(pPKL133). Data are plotted as the least-squares fit to the hyperbola of velocity (V) versus substrate concentration ([S]). Each datum point represents the average of three separate determinations. The insert shows the corresponding Lineweaver-Burk plot. Substrate concentrations are micromolar, and units for velocity are given as nanomoles per minute per milligram of total cell protein.

TABLE 1. Inhibition of gluconate transport by alternative substrates

Competing sugar[a]	Rate (SD)[b]	% Wild-type value
None (control)	1.90 (± 0.17)	100
Gluconate	0.20 (± 0.09)	11
Glucuronate	1.58 (± 0.13)	83
Maltose	1.61 (± 0.08)	85
Lactose	1.37 (± 0.13)	72
Sucrose	1.57 (± 0.32)	83
Mannitol	2.74 (± 0.26)	144
Fructose	1.46 (± 0.03)	77
Galactose	1.55 (± 0.18)	82
Glucose	2.82 (± 0.37)	148

[a] Cells were preincubated in 200 μM competing sugar prior to measurement of uptake of 50 μM [^{14}C]gluconate.
[b] Rate is measured in nanomoles per minute per milligram of protein.

a glucuronate permease, the high affinity of GntP for gluconate (25 μM) and the relatively poor competition of unlabeled glucuronate for transport of radioactive gluconate seem to rule this out. In further support of the lack of involvement of *gntP* in glucuronate metabolism is the fact that regulation of *gntP* expression appears to be independent of neighboring genes.

A σ^{70} promoter of the 18-bp spacing class drives transcription of *gntP*. The promoter possesses a canonical CAP binding site that overlaps the rather poor −35 region with perfect spacing from the transcriptional start site. The *gntP* promoter is located 38 bp upstream of the start codon and the gene probably terminates at a rho-dependent terminator located about 30 bp downstream of the stop codon, giving rise to the *gntP* transcript with an apparent molecular size of 1.54 kb revealed by Northern blot analysis. The *gntP* transcript is expressed at high levels in the absence of added carbohydrate but is expressed only at very low levels in logarithmic-phase cells grown on glucose or gluconate, indicating a high degree of catabolite repression and lack of induction by gluconate.

Three lines of evidence confirmed that *gntP* does indeed encode a gluconate permease. First, expression of the *gntP* gene from a T7 promoter allowed labelling of a membrane-localized peptide of the appropriate size, when the aberrant mobility of integral membrane-localized proteins on polyacrylamide gels was taken into consideration. Second, the cloned *gntP* gene was able to genetically complement a lesion in gluconate transport and rescued growth of *E. coli* M6 on minimal gluconate medium. Third, *E. coli* M6(pPKL133) was capable of high-affinity transport of gluconate (apparent K_m of approximately 25 μM).

The deduced amino acid sequence of the *E. coli gntP* gene product was easily aligned with the *B. subtilis* protein; they are 37% identical and contain 447 and 448 amino acids, respectively. The *E. coli* GntP contains 48% hydrophobic amino acids and lacks a signal sequence, consistent with GntP being an integral cytoplasmic membrane protein. A hydrophobic moment analysis indicates that GntP probably contains 14 membrane-spanning domains and follows the positive-inside rule of von Heijne (29). Interestingly, a similar analysis of the *B. subtilis* GntP also points to a 14-membrane-spanning-domain model (data not shown). The possibility that GntP has 14-membrane-spanning domains is very interesting in light of the fact that the proposed consensus structure for a large family of transport proteins indicates that they possess 12 membrane-spanning domains (14). While the *E. coli* and *B. subtilis* GntP transporters show significant identity to one another, these gluconate permeases share little similarity (other than po-

tentially having 14 membrane-spanning domains) with the conserved amino acid sequence elements of the large family of transporters. The *E. coli gntP* does contain a conserved RXGRR motif at amino acid 94 but is otherwise lacking in conserved transporter sequence elements.

Finally, we turn our attention to the physiological role of the *gntP* gene product. GntP is clearly a gluconate transporter, with a high affinity for gluconate and a fairly broad specificity, including low affinities for glucuronate, several disaccharides, and some hexoses, but not glucose. Despite the apparent involvement of *gntP* in gluconate metabolism, this gene is not induced by growth on gluconate as are the genes of the GntI system (32) and the Entner-Doudoroff pathway (9), which are under control of a repressor encoded by the *gntR* gene. Expression of *gntP* in log-phase cells can be considered to be constitutive in the absence of gluconate or another catabolite-repressing carbon source. Perhaps the role of GntP is simply to reside in the membrane, ready to allow entry of gluconate into the cell in order to induce the genes required for gluconate metabolism that are under *gntR* control. This would be necessary if *gntR*-mediated control of the other gluconate permease genes of the GntI system is very tight. The *gntP* gene product would be dispensable after induction of the genes of gluconate metabolism, and catabolite repression of *gntP* would then be of no consequence. It is important to note that gluconate metabolism is not subject to tight catabolite repression and that gluconate and glucose can be cometabolized, but only in cells which have been previously exposed to gluconate (10). The requirement of preexposure to gluconate for cometabolism of glucose and gluconate is in keeping with the hypothesized role of GntP, to allow entry of gluconate into the cell for induction of other *gnt* genes. The observed catabolite repression of *gntP* by glucose would otherwise repress *gntP* expression and hence preclude entry of gluconate into the cells until the glucose was consumed.

GntP is a high-affinity gluconate transporter. Therefore, it might be that the biological role of this protein is to permit the host to take up gluconate when only tiny amounts are present. This would perhaps present the host with an advantage in a highly competitive environment like the gut. When the cell encounters higher concentrations of gluconate, *gntP* expression is shut off and the protein's role is taken over by the permeases of the GntI system, which are induced by gluconate.

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