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Cloning and Nucleotide Sequence of the Anaerobically Regulated *pepT* Gene of *Salmonella typhimurium*

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The anaerobically regulated *pepT* gene of *Salmonella typhimurium* has been cloned in pBR328. Strains carrying the *pepT* plasmid, pJG17, overproduce peptidase T by approximately 70-fold. The nucleotide sequence of a 2.5-kb region including *pepT* has been determined. The sequence codes for a protein of 44,855 Da, consistent with a molecular weight of ~46,000 for peptidase T (as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and gel filtration). The N-terminal amino acid sequence of peptidase T purified from a pJG17-containing strain matches that predicted by the nucleotide sequence. A plasmid carrying an anaerobically regulated *pepT*::*lacZ* transcriptional fusion contains only 165 bp 5′ to the start of translation. This region contains a sequence highly homologous to that identified in *Escherichia coli* as the site of action of the FNR protein, a positive regulator of anaerobic gene expression. A region of the deduced amino acid sequence of peptidase T is similar to segments of *Pseudomonas* carboxypeptidase G2, the *E. coli* peptidase encoded by the *iap* gene, and *E. coli* peptidase D.

The *Salmonella typhimurium pepT* gene encodes an aminotripeptidase that is produced at higher levels in cells grown anaerobically than in cells grown aerobically (32). The anaerobic expression of *pepT* and other *Salmonella* genes (13, 32) requires the product of *oxrA*, the *Salmonella* equivalent of the *Escherichia coli fnr* gene (11, 32). The product of *fnr* is a positive regulator of a family of genes that includes nitrate reductase, fumarate reductase, and nitrite reductase (14, 22, 27). Most genes regulated by *fnr* encode components of anaerobic respiratory pathways (29). Peptidase T has no obvious function in growth under anaerobic respiratory conditions, and the significance of *fnr*-dependent regulation of *pepT* is obscure. As part of an effort to understand the mechanism and physiological significance of the anaerobic regulation of *pepT*, we have cloned the *pepT* gene and determined its nucleotide sequence.

MATERIALS AND METHODS

Bacterial strains and plasmids, media, and growth conditions. The bacterial strains and plasmids used in this work are listed in Table 1. Media and growth conditions have been described elsewhere (32).

DNA manipulations. Standard methods (16) were used for preparation of chromosomal and plasmid DNA, restriction enzyme digestion, ligation, and agarose gel electrophoresis. P22 transduction (4) was used to move plasmids between *Salmonella* strains (25). DNA sequencing was carried out by the dideoxy method (26) with phage T7 DNA polymerase (Sequenase; U.S. Biochemicals). Most of the sequence was obtained by using single-stranded templates obtained from subclones into M13mp18 or M13mp19 (18, 35). Both strands were completely sequenced. Sequence analysis was carried out with the Bionet resource and the University of Wisconsin Genetics Computer Group programs (5).

Enzyme assays. β-Galactosidase activity was determined as described by Miller (19). Peptidase T was assayed, using Met-Gly-Gly as the substrate and high-performance liquid chromatography of reaction mixtures derivatized with trinitrobenzenesulfonyl chloride (33).

Purification of peptidase T. Peptidase T was purified from *S. typhimurium* TN2322 grown without shaking to stationary phase in minimal glucose medium supplemented with Casamino Acids (0.1%) and ampicillin (100 μg/ml). The harvested cells (26.4 g) were suspended in buffer (0.01 M Tris-HCl, pH 7.5) and disrupted by sonication. After centrifugation for 1 h at 27,000 × *g*, the supernatant was diluted to 68 ml with buffer and then 10 ml of protamine sulfate solution (2%) was added with stirring at 4°C over 1 h. Centrifugation at 12,000 × *g* for 10 min produced a pellet containing the peptidase T activity. This pellet was suspended in 140 ml of buffer, and the resulting suspension was centrifuged for 1 h at 27,000 × *g*. The supernatant was retained and, after dialysis overnight in buffer, applied to a Whatman DE-52 column (140-ml volume) equilibrated with buffer. This column was eluted with a NaCl gradient (0 to 0.4 M in buffer), and peptidase T was eluted at ~0.3 M salt. The pooled fractions containing peptidase T (209 ml) were concentrated to 6 ml (Amicon YM10 membrane) and applied to an Ultrogel AcA54 (LKB) column (475-ml total volume, equilibrated with 0.05 M Tris-HCl [pH 7.5]). Peptidase T eluted from this column at a position suggesting a molecular mass of ~47 kDa. The pooled active fractions (25 ml) were concentrated (Amicon Centriprep10) and exchanged into 0.02 M Tris-HCl (pH 7.5) with a Pharmacia PD10 column. This material was chromatographed on a Pharmacia MonoQ HR5/5 column equilibrated with 0.02 M Tris-HCl (pH 7.5) buffer and eluted with a 23-ml NaCl gradient (0 to 0.4 M). Peptidase T eluted at ~0.25 M salt. The active fractions were pooled, concentrated to 150 μl (Amicon Centriprep10), and chromatographed on a Superose-12 column (Pharmacia) equilibrated with 0.05 M Tris-HCl (pH 7.5). Peptidase T

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description
Strains	
TN1246	*leuBCD485 pepB11 pepN90 pepA16 ΔsupQ302(proAB pepD)*
TN1379	*leuBCD485*
TN2183	*leuBCD485 metA15 pepN90 pepA16 pepB11 pepP1 pepQ1 pepT7*::Mud1(X)
TN2262	*leuBCD485 pepT7*::MudJ
TN2322	*leuBCD485 metA15 pepN90 pepA16 pepB11 pepP1 pepQ1 pepT7*::Mud1(X) pJG17
TN2540 (DB4926)	*metE551 metA22 ilv-452 trpB2 hisC527*(Am) *galE496 xyl-404 rpsL120 flaA66 hsdL6 hsdSA29*
Plasmids	
pJG17	7.1-kb insert containing *pepT* in *Bam*HI site of pBR328
pJG38	15-kb insert containing *pepT7*::MudJ in *Bam*HI site of pBR322

eluted as a symmetrical peak and was concentrated to 0.92 mg/ml and frozen at −70°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the purified material showed only one band (molecular mass, 46 kDa) when the gel was stained with Coomassie blue. The purification yielded 12% recovery and a 145-fold increase in specific activity. This represents a purification factor of 860 relative to the level of activity found in extracts of cells grown under inducing conditions from strains carrying a single copy of *pepT*.

The amino acid sequence of the N-terminal region of the protein was determined by using an Applied Biosystems model 477A protein sequencer.

Nucleotide sequence accession number. GenBank has assigned the accession number M62725 to the *S. typhimurium pepT* sequence.

RESULTS AND DISCUSSION

Cloning of *pepT*. For cloning of the wild-type *pepT* gene, fragments (6.5 to 10 kb) from a *Sau*3A partial digest of chromosomal DNA from strain TN1246 (a $pepT^+$ strain carrying mutations in several other peptidase genes [31]) were ligated into the *Bam*HI site of pBR328, and the resulting plasmids were transformed into *E. coli* LE392 (16) with selection for ampicillin resistance. Plasmid DNA was prepared from a pool (~4,000 colonies) of these transformants and used to transform *S. typhimurium* TN2540, again with selection for ampicillin resistance. Approximately 20,000 transformants were pooled, and a P22 transducing lysate was prepared on this population. To identify plasmids carrying *pep* genes, this lysate was used to transduce the multiply peptidase-deficient strain TN2183, with selection for both chloramphenicol resistance and growth on Met-Gly-Gly as the Met source. To verify that Met-Gly-Gly utilization was plasmid encoded, colonies from these transduction plates were purified and transducing lysates were prepared and used to transduce TN2183 to chloramphenicol resistance. These transductants were checked for inheritance of the unselected Met-Gly-Gly utilization phenotype. Transductants from these crosses were tested for utilization of

TABLE 2. β-Galactosidase levels[a]

Strain (plasmid)	β-Galactosidase level (U)		Ratio (anaerobic/aerobic)
	Aerobic	Anaerobic	
TN2262 (no plasmid)	50	440	8.8
TN2262(pJG38)	860	6,100	7.1

[a] Cultures were grown in LB on a rotating wheel (aerobic) or standing in a filled screw-cap tube (anaerobic).

peptides other than Met-Gly-Gly. Several strains with a utilization pattern that suggested they contained plasmids producing peptidase T were identified, and one of these plasmids (pJG17, originally carried by strain TN2322) was characterized in detail.

A crude extract of TN2322 was electrophoresed in a nondenaturing polyacrylamide gel, and the gel was stained for peptidase activity (15), using Leu-Gly-Gly and Met-Gly-Gly as substrates. The gel showed two bands of activity, as observed previously with strains that overproduce peptidase T as a result of chromosomal mutations (31, 32). These bands had no activity toward the dipeptide Leu-Gly. These results strongly suggested that pJG17 codes for peptidase T. The pJG17 plasmid was transduced into a *polA* strain (34) with selection for chloramphenicol resistance. Since pBR328 cannot be maintained as a plasmid in a *polA* strain (12), these transductants contain plasmids inserted into the chromosome by homologous recombination between the *Salmonella* DNA in the plasmid and the corresponding chromosomal region (7). One of these transductants was purified and used as a recipient in a transduction cross with a strain carrying a mini-Tn*10* insertion (*zcf-3233*::Tn*10*Δ*16*Δ*17*) that is linked to *pepT* (13). Tetracycline-resistant transductants from this cross were tested for loss of chloramphenicol resistance conferred by the plasmid. Thirty percent (28 of 88) of the tetracycline-resistant transductants were chloramphenicol sensitive. As a control for spontaneous loss of the inserted plasmid, the same transduction was carried out by using as the donor a strain carrying *zad-3131*::Tn*10*Δ*16*Δ*17*, an insertion unlinked to *pepT*. Only 2 of 88 tetracycline-resistant transductants were chloramphenicol sensitive. These results indicate that the plasmid is integrated into the chromosome near *pepT* and must therefore carry an insert with homology to the *pepT* region. The levels of peptidase T in extracts of TN2322(pJG17) made from both aerobically and anaerobically grown cells were determined by using Met-Gly-Gly as

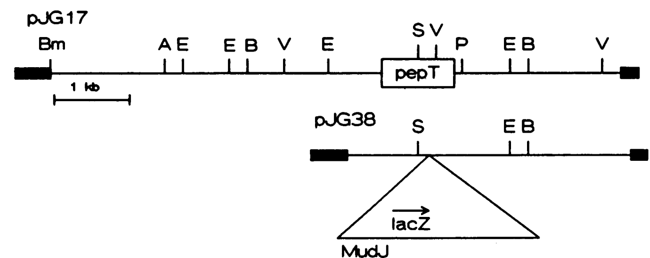

FIG. 1. Restriction maps of pJG17 and pJG38. Thick lines indicate vector DNA (pBR328 for pJG17 and pBR322 for pJG38); thin lines indicate inserted DNA. Abbreviations: A, *Ava*I; B, *Bgl*II; Bm, *Bam*HI; E, *Eco*RI; P, *Pst*I; S, *Sal*I; V, *Eco*RV. The MudJ element is not to scale.

```
        10                        30                        50
CAATCAGGCGAAGAACGGTTGTTTTACGCAGCCGGAAGGGCCAAGCAGCGTGAGGAATTC
        70                        90                       110
GCCATTGTTAATGGTCAAATCCAGTTGCGAAATGACCTCTTTGCCATCGAAACTTTTGCT
       130                       150                       170
AATTCCCGACAAAAGCACCAGCGGTGAAAGCGAACGCGGTTGATTATTCAATTTCTTACT
       190                       210                       230
CTGTCCCATGTAAACGCAACGGATGGCTGACCGCTGCGGGGTTTGTGGTTAACCACCTTA
       250                       270                       290
ATCACTCTTAATGAGGGCGGTCATTCTACGGCAAACCACTGTGATCGCCAATCCTTGTTG
       310                       330                       350
CGAATTACTGACTTAGCTTTATAGTCAGAAAGCGTGTCAAAGTGAAATATTCTTGTTTGC
       370                       390                       410
AGGGATAAAAGTGACCTGACGCAATATTTGTCTTTTCTTGCTTATTAATAATGTTGTCAC
       430                       450                       470
GAAAAGTGAGGGTGACTGCATGGATAAACTACTTGAGCGTTTTTTACACTACGTATCGCT
       S.D.             M  D  K  L  L  E  R  F  L  H  Y  V  S  L
                                                   10
       490                       510                       530
GGATACCCAATCAAAGTCGGGTGTTCGGCAGGTTCCCAGCACTGAGGGGCAGTGGAAGTT
 D  T  Q  S  K  S  G  V  R  Q  V  P  S  T  E  G  Q  W  K  L
             20                            30
       550                       570                       590
ACTACGTTTGCTCAAACAGCAGCTCGAAGAGATGGGGCTGGTTAACATTACATTAAGTGA
 L  R  L  L  K  Q  Q  L  E  E  M  G  L  V  N  I  T  L  S  E
             40                            50
       610                       630                       650
AAAAGGGACGTTGATGGCGACGCTCCCGGCCAATGTTGAGGGGGATATTCCCGCCATTGG
 K  G  T  L  M  A  T  L  P  A  N  V  E  G  D  I  P  A  I  G
             60                            70
       670                       690                       710
TTTTATCTCCCATGTGGATACCTCTCCGGATTTCAGCGGTAAAAACGTTAACCCGCAGAT
 F  I  S  H  V  D  T  S  P  D  F  S  G  K  N  V  N  P  Q  I
             80                            90
       730                       750                       770
TGTCGAGAATTATCGCGGCGGCGATATAGCATTAGGGATTGGCGATGAGGTGTTGTCACC
 V  E  N  Y  R  G  G  D  I  A  L  G  I  G  D  E  V  L  S  P
            100                           110
       790                       810                       830
CGTGATGTTCCCGGTACTGCATCAATTACTGGGACAGACGCTGATTACTACCGATGGTAA
 V  M  F  P  V  L  H  Q  L  L  G  Q  T  L  I  T  T  D  G  K
            120                           130
       850                       870                       890
GACATTGCTGGGCGCGGACGATAAAGCCGGCGTTGCGGAGATCATGACCGCGCTGGCGGT
 T  L  L  G  A  D  D  K  A  G  V  A  E  I  M  T  A  L  A  V
            140                           150
       910                       930                       950
GCTGAAAGGTAATCCTATTCCCCACGGCGACATTAAAGTGGCGTTTACGCCTGACGAAGA
 L  K  G  N  P  I  P  H  G  D  I  K  V  A  F  T  P  D  E  E
            160                           170
       970                       990                      1010
GGTAGGGAAAGGCGCGAAGCACTTCGATGTTGAGGCCTTTGGCGCGCAGTGGGCATATAC
 V  G  K  G  A  K  H  F  D  V  E  A  F  G  A  Q  W  A  Y  T
            180                           190
```

FIG. 2. Nucleotide sequence of region of pJG17 containing *pepT*. The underlined G at nucleotide 283 indicates the start of the insert in pJG38. A potential promoter site for the aerobic expression of *pepT* at nucleotides 298 to 303 (−35) and 320 to 325 (−10) is underlined. The sequence proposed to be the site of FNR action (Fig. 3) at nucleotides 364 to 391 is underlined. The proposed −10 region for this promoter at nucleotides 407 to 412 is indicated. A potential ribosome binding site is labeled S.D. The MudJ element of pJG38 is inserted between nucleotides 1308 and 1309. In addition to the inverted repeat present at the proposed FNR interaction site, the *pepT* promoter region contains an inverted repeat at nucleotides 285 to 291 and 298 to 303 and three appearances of the sequence AAAGTGA (nucleotides 339 to 345, 368 to 374, and 423 to 429). The N-terminal amino acid sequence determined from purified peptidase T is underlined.

```
      1030                      1050                      1070
GGTCGACGGCGGCGGCGTGGGAGAACTGGAGTTTGAAAACTTCAATGCCGCCTCGGTGAA
 V  D  G  G  G  V  G  E  L  E  F  E  N  F  N  A  A  S  V  N
            200                           210
      1090                      1110                      1130
TATCAAAATCGTCGGCAATAACGTGCATCCCGGTACGGCAAAAGGCGTGATGGTCAATGC
 I  K  I  V  G  N  N  V  H  P  G  T  A  K  G  V  M  V  N  A
            220                           230
      1150                      1170                      1190
GCTGTCGTTGGCGGCGAGGATTCACGCGGAAGTGCCGGCGGATGAAGCGCCTGAAACCAC
 L  S  L  A  A  R  I  H  A  E  V  P  A  D  E  A  P  E  T  T
            240                           250
      1210                      1230                      1250
CGAAGGTTACGAAGGGTTTTATCATCTGGCCAGCATGAAAGGCACCGTTGACCGGGCCGA
 E  G  Y  E  G  F  Y  H  L  A  S  M  K  G  T  V  D  R  A  E
            260                           270
      1270                      1290                      1310
AATGCACTACATCATTCGCGATTTCGACCGTAAGCAGTTTGAAGCGCGTAAACGCAAAAT
 M  H  Y  I  I  R  D  F  D  R  K  Q  F  E  A  R  K  R  K  M
            280                           290
      1330                      1350                      1370
GATGGAGATTGCCAAAAAAGTCGGTAAGGGGCTGCATCCGGACTGCTATATCGAACTGGT
 M  E  I  A  K  K  V  G  K  G  L  H  P  D  C  Y  I  E  L  V
            300                           310
      1390                      1410                      1430
GATTGAAGACAGTTATTACAATATGCGCGAAAAAGTAGTTGAACATCCGCATATTCTCGA
 I  E  D  S  Y  Y  N  M  R  E  K  V  V  E  H  P  H  I  L  D
            320                           330
      1450                      1470                      1490
TATCGCCCAGCAGGCCATGCGCGACTGTCATATTACGCCGGAGATGAAACCGATTCGCGG
 I  A  Q  Q  A  M  R  D  C  H  I  T  P  E  M  K  P  I  R  G
            340                           350
      1510                      1530                      1550
CGGTACAGACGGGGCGCAACTGTCGTTTATGGGTCTGCCATGTCCTAATCTCTTTACCGG
 G  T  D  G  A  Q  L  S  F  M  G  L  P  C  P  N  L  F  T  G
            360                           370
      1570                      1590                      1610
CGGATATAACTATCATGGTAAACATGAGTTTGTGACGCTGGAGGGGATGGAAAAAGCGGT
 G  Y  N  Y  H  G  K  H  E  F  V  T  L  E  G  M  E  K  A  V
            380                           390
      1630                      1650                      1670
ACAGGTGATTGTGCGTATCGCGGAGCTGACGGCTAAGCGCGGGCAGTAGCGAATGTTACG
 Q  V  I  V  R  I  A  E  L  T  A  K  R  G  Q
            400
      1690                      1710                      1730
CATTGTGGTGAAGCGTTTTATTATTTATGGCGGGATATTTTCCGCCATAAATTTTTCTGC
      1750                      1770                      1790
CTGGAGCGCCGAATATACGCCTTCCTGGTCCCAGCGCCAGCAACAATCTGCAGCATGTTT
      1810                      1830                      1850
TATGACTGGCGATGAAACCTGCATGACGTTTATTGATGATGCGGTGAGGCTGGCTCGCAG
      1870                      1890                      1910
CAATATGGCAAACGCTCAATACAGCTTGTTCGTAGCCTGCTATTACAAAGCGATATTTAT
      1930                      1950                      1970
CAATGGCTGGGTAAACCGGAGTTAACGCCGCAAATGCTGCTGCGAGCGCGGGCAATAATG
      1990                      2010                      2030
AAAACATTTCCGGCGGACACTTATCCGGGCGATCGGGCCGATATGTTTGAACACCTGGCG
      2050                      2070                      2090
GCCTTTAATGTTTACAGTGACGATCGTTATATAGAGTATTCACCGACAGAGCAGTGGCGA
      2110                      2130                      2150
TACGAGATCAAGGTTGATTACCGTCAGCAGATAGCCTGGCAAGAGCAGGCATTAACGTGG
      2170                      2190                      2210
CGTCTTAAAGACAAAAGGCATCGACGGAAGCATTGGTTTATACCTTAAACAGGATGCGCG
      2230                      2250                      2270
ACGCTTATTCTGATGCGCTGGAAGAGCGGGATGTCGAATGTGATAGTGCGCGTAAAGCCT
      2290                      2310                      2330
ATTATTTGGCGAAGATAGACGCAACGGAGCGGCAGTGGCTGAGCGTTATTTTACGAGATA
      2350                      2370                      2390
AACCTGGGATAATCGTGAGCGCGTCGCCTCTTTTTTGCAGCAGAAAGCGGATATTGCTTA
      2410                      2430                      2450
TAATGCAGGGCATATTAGCGAGGCGATTAATGCGCTAAGTCAGGCGCTAAAAATTCAGCA
      2470                      2490                      2510
AACGCTTTATGCGCGAATTCGGCGAAATGACGGTTGACAGCAATAATCTTGCTGGCTTTT
      2530                      2550
ATGCACAGGGCCATCTTATAAGGAAGCGAAA
```

the substrate (33) and compared with the levels in the parent strain not containing a plasmid. The specific activities (in units per milligram [33]) for TN1246 were 0.04 (aerobic) and 0.54 (anaerobic). TN2322(pJG17) showed specific activities of 9.3 (aerobic) and 39 (anaerobic) U/ml. These data show that the strain carrying pJG17 overproduces peptidase T and that peptidase T activity is anaerobically inducible in this strain. Taken together, these results show that pJG17 carries *pepT* and the sites necessary for its regulated expression.

Cloning of a *pepT*::*lacZ* fusion. In addition to the wild-type *pepT* gene, a *pepT*::*lacZ* transcriptional fusion, constructed by replacement (9) of the Mud1 insertion described previously (32) with MudJ (MudI1734 [3]), was cloned. Fragments

```
Consensus   A-A-TTGAT--A-ATCAAT--- -14-21 bp- "-10"
pepT        AAAAGTGACCTGACGCAATATT -15 bp- AATAAT
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FIG. 3. Comparison of the proposed *pepT* FNR site with the most recent *E. coli* consensus sequence (28). Bases underlined in the *pepT* sequence match the consensus. Other slightly different consensus sequences have been proposed (e.g., in reference 6). The proposed FNR site is located 15 to 21 bp upstream from the −10 region of most of the FNR-dependent promoters (6). A reasonable −10 site 15 bp downstream from the FNR site of *pepT* is indicated.

(14 to 19 kb) from a *Sau*3A partial digest of TN2262 were ligated into the *Bam*HI site of pBR322, and the resulting plasmids were transformed into *E. coli* LE392 (16) with selection for kanamycin resistance (encoded by the MudJ element). Plasmid DNA from 22 of these transformants was used to transform *Salmonella* strain DB4926 on nutrient agar–kanamycin–5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) plates. The 11 blue colonies were saved, and P22 transducing lysates were prepared on each. P22 lysates from 11 of these strains were used to transduce TN1379 to kanamycin resistance on MacConkey lactose plates. Ten of these strains yielded red transductants. These transductants were tested for anaerobic induction of β-galactosidase by determining the levels of activity in stationary-phase and exponential-phase cells (32). Four strains clearly contained plasmids that conferred elevated stationary-phase levels of β-galactosidase, and one of these (containing plasmid pJG38) was characterized further. The data in Table 2 suggest that this strain carries all of the *pepT*-linked sites necessary for anaerobic regulation.

Restriction analysis of pJG38 (Fig. 1) showed that this plasmid carries an insert of approximately 16 kb and locates the position and orientation of the MudJ insertion. The restriction map of pJG17 (Fig. 1) shows that the insert in this plasmid is approximately 7.1 kb long, and comparison with pJG38 indicates the location of *pepT* in pJG17.

Nucleotide sequence of *pepT*. The nucleotide sequence of a segment of pJG17 that includes *pepT* is shown in Fig. 2. An open reading frame encodes a protein with a molecular mass of 44,855 Da. This is consistent with the molecular masses determined by SDS-PAGE (46 kDa) and gel filtration (47 kDa). The N-terminal sequence of this open reading frame agrees with that determined for purified peptidase T. A potential ribosome binding site (GAGG) is located 8 bases 5′ to the translation start codon.

The chromosomal DNA in pJG38 begins at position 283 of the sequence shown in Fig. 2 and includes only 165 bp 5′ to the start of translation. Since production of β-galactosidase from pJG38 is anaerobically regulated, this 165-bp region must contain the sites necessary for this regulation. A site of imperfect dyad symmetry with significant similarity (Fig. 3) to the proposed FNR consensus recognition sequence (28) is located 15 bp upstream from a potential −10 sequence for sigma-70 promoters. This spacing is consistent with those observed for the FNR-dependent promoters discussed by Eiglmeier et al. (6). In addition to its presence in the proposed FNR site (nucleotides 368 to 374), the sequence AAAGTGA is present at two additional sites (nucleotides 339 to 345 and 423 to 429) in this region. The significance of this, if any, is unknown. As indicated in Fig. 2, another potential promoter site which might be responsible for the basal (aerobic) expression of *pepT* is located upstream from the proposed FNR site. Two regions of imperfect inverted repeat, also of unknown significance, are located 5′ to the proposed FNR site (nucleotides 285 to 303 and 305 to 331). A sequence potentially forming a mRNA stem-loop structure (nucleotides 1703 to 1732) followed by a run of T's that might function as a transcription terminator (24) is located downstream from the translation stop codon.

A search of the GenBank and EMBL sequence data bases using the FASTA program (23) turned up a potentially significant amino acid sequence similarity (27.5% sequence identity in a 211-amino-acid overlap) between a region of PepT and a region of carboxypeptidase G2 from a *Pseudomonas* strain (20) (Fig. 4). The gene encoding this enzyme was also determined to be the most closely related to *pepT* in a FASTA search of the nucleotide sequence data banks. Although the amino acid sequence identity is low, the fact that both proteins are bacterial metallopeptidases prompted further analysis. A subsequence of highest similarity (17 identities in 41 amino acids as aligned by the Bestfit program) was identified and used in a second search of the data banks. This search revealed a similar region in a third bacterial peptidase, the product of the *E. coli iap* gene. This locus encodes an enzyme (presumably an aminopeptidase) that is responsible for generating electrophoretically detectable variants of alkaline phosphatase (10). A comparison of this sequence with the sequence of *E. coli pepD* (8) also turned up a region of similarity. These proteins have similar functions (peptide bond hydrolysis), they are of roughly similar size (38 to 53 kDa), and the subsequence of high similarity is located in approximately the same position in each sequence (it begins between 110 and 137 amino acids from the N terminus). Peptidase T, carboxypeptidase G2, and peptidase D are known to be metallopeptidases, and it is possible that a common sequence motif represents a metal-binding region of the protein. The specificities of these enzymes are quite different, so it is likely that the sequence has nothing to do with substrate recognition.

The amino acid sequence was also compared to the sequences of several other peptidases from *E. coli* or *S. typhimurium* by using Genalign. No significant similarities were found between the sequence of *pepT* and those of *E. coli pepN* (1, 17), *pepA* (30), and *pepP* (36), *E. coli* (2) and *S. typhimurium* (21) *pepM*, *S. typhimurium dcp* (7a), and *S. typhimurium opdA* (formerly *optA*) (3a).

```
Iap    TLQGMDDNAAGLGVMLELAERLLKNTPTE.YGIRFVATSGEEE.GKLGA
PepT   TLLGADDKAGVAEIMTALAV.LKGNPIP.HGDIKVAFTPDEEVGK.GA
Cpg2   GPGIADDKGGNAVILHTLKL.LKEYGVRDYGTITVLFNTDEEKGSFGS
PepD   TTLGADNGIGMA...SALAV.LADENVV.HGPLEVLLTMTEEAGMDGA
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FIG. 4. Amino acid sequence similarities. The sequences shown are from Iap (amino acids 137 to 182), an *E. coli* peptidase involved in the generation of N-terminal sequence variants of alkaline phosphatase (10); peptidase T (PepT; amino acids 134 to 179); carboxypeptidase G2 (Cpg2; amino acids 136 to 179), a *Pseudomonas* enzyme that removes glutamate residues from folic acid and its derivatives and analogs (20); and peptidase D (PepD; amino acids 110 to 152), an *E. coli* dipeptidase (8).

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