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PEB1, the Major Cell-binding Factor of *Campylobacter jejuni*, Is a Homolog of the Binding Component in Gram-negative Nutrient Transport Systems*

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The protein PEB1 (28 kDa) is a common antigen and a major cell adherence molecule of *Campylobacter jejuni* and *Campylobacter coli*. We created a bank of chromosomal DNA fragments of *C. jejuni* strain 81-176 using λgt11. Screening this bank in *Escherichia coli* Y1090 cells with antibody raised against purified PEB1 enabled us to isolate and to purify a clone with a 2.6-kilobase insert expressing an immunoreactive protein of 28 kDa. DNA sequencing revealed that the insert contains three complete and two partial open reading frames (ORFs), designated 5′ to 3′ as ORFs A–E. The *peb*1A gene (ORF D) contains 780 bases encoding a 259-residue polypeptide having a calculated molecular mass of 28,181 Da. The peptide sequence starting at residue 27 matches that determined from amino-terminal sequencing of mature PEB1 from *C. jejuni*. The first 26 residues contain typical signal peptidase I and II cleavage sites. The deduced amino acid composition and pI of the recombinant mature protein are similar to those determined for purified PEB1. Gene bank searches indicated significant overall homology of *peb*1A and ORF C with operons for amino acid transport systems in other Gram-negative organisms. *peb*1A is homologous to the binding components of systems such as *gln*H (27.8%) and *his*J (28.9%), whereas ORF C has nearly 50% identity to *gln*Q and *his*P. Thus, PEB1 could be involved both in binding to intestinal cells and in amino acid transport.

Campylobacter jejuni and the closely related species *Campylobacter coli* are important causes of diarrheal disease in humans worldwide (1–3). These organisms exhibit considerable serotypic diversity with >50 O antigens (4) and a similar number of heat-labile antigens (5). However, evidence from natural infections in developing countries (6, 7), from raw milk drinkers in the United States (8), and from experimental infections in volunteers (9) and monkeys (10) suggests that immunity to *C. jejuni* is induced by recurrent exposure; thus, it may be possible to develop a vaccine against *C. jejuni* enteritis. We have previously identified two antigenic proteins, PEB1 (28 kDa) and PEB3 (30 kDa), from *C. jejuni* that are commonly recognized by convalescent sera from patients with sporadic *C. jejuni* diarrhea (11) and that may be vaccine candidates.

PEB1 is conserved in all *C. jejuni* and *C. coli* isolates and is located on the surface of *C. jejuni* cells as identified by immunogold electron microscopy (12), indicating that it is a good target for the immune system (11). PEB1 (CBF1) plays a major role in adherence to HeLa cells, suggesting that it may be involved in *Campylobacter* colonization of the intestine (13). PEB1 is a lysine-rich basic (pI 8.5) protein without methionine at its amino terminus, suggesting that a leader peptide is cleaved during PEB1 maturation (11); the amino terminus of PEB1 has no significant homology to other known proteins (11). We thus undertook the molecular cloning and sequencing of the gene encoding PEB1 to determine its primary sequence, to understand its post-translational modification and intracellular transport, and because large-scale production of recombinant PEB1 in *Escherichia coli* will facilitate functional and immunological studies.

We report the cloning and sequencing of the PEB1 structural gene (which we name *peb*1A) from *C. jejuni* strain 81-176. The deduced amino acid sequence indicated that PEB1 has a cleaved 26-amino acid leader peptide; the complete molecule exhibits significant homology to *Enterobacteriaceae* glutamine-binding protein (*gln*H) (14), lysine/arginine/ornithine-binding protein (LAO)[1] (15), and histidine-binding protein (*his*J) (16) and may be part of a homologous operon.

EXPERIMENTAL PROCEDURES

Enzymes and Chemicals—Restriction endonucleases, calf intestinal alkaline phosphatase, IPTG, and affinity-purified goat antibody to rabbit IgG conjugated with alkaline phosphatase were obtained from Boehringer Mannheim. Other important reagents were bacteriophage T4 DNA ligase (New England BioLabs, Inc.), nitrocellulose BA-85 (Schleicher & Schuell), 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Sigma), and Sepharose CL-2B (Pharmacia LKB Biotechnology Inc.).

Bacterial Strains—*C. jejuni* strain 81-176 (ATCC 55026), used for PEB1 production and genomic DNA preparation, was isolated from an outbreak of *Campylobacter* diarrhea and has been demonstrated to be a virulent strain in monkeys (10) and in volunteers (9). *E. coli* strains Y1088, Y1089, and Y1090 have been described (17), and XL1-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L13662.

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[1] The abbreviations used are: LAO, lysine/arginine/ornithine-binding protein; IPTG, isopropyl-1-thio-β-D-galactopyranoside; kb, kilobase(s); bp, base pair(s); PCR, polymerase chain reaction; ORF, open reading frame.

Blue (Stratagene, La Jolla, CA) was used for transformations.

Preparation of λ Bank with Insert DNA from C. jejuni—A genomic library was prepared from *C. jejuni* strain 81-176 as previously described (18). In brief, purified DNA (800 μg) was sheared by sonication. DNA fragments of 1.0–10 kb were isolated and ligated to *Eco*RI linkers and then to dephosphorylated λgt11 arms. The ligation mixture was added to a λ-packaging mixture (Gigapack, Stratagene) and titered on Y1088 cells.

Immunological Methods—Polyclonal antiserum to PEB1 purified from strain 81-176 was raised in a hyperimmunized rabbit as previously described (11). This serum recognizes heterologous PEB1 antigens in all *C. jejuni* and *C. coli* strains, but has essentially no reactivity against other *C. jejuni* or *C. coli* antigens. Immunological screening of the *C. jejuni* library was performed as previously described by Gotschlich *et al.* (19). SDS-polyacrylamide gel electrophoresis was performed on lysates, proteins were transferred to nitrocellulose membranes, and the blots were developed by an immunoenzymatic method (11).

Subcloning and Physical Mapping of Insert—For expression and mapping of the insert, the original clones in λgt11 were digested with *Eco*RI, and the inserts were separated in low-melting-point agarose and ligated into the *Eco*RI site of phosphatase-treated pUC19. The ligation mixture was used to transform competent XL1-Blue *E. coli* cells, and carbenicillin-resistant transformants were isolated (20). Recombinant plasmids were purified (21) and digested with restriction endonucleases (22). Western blotting was performed with antiserum to PEB1 to identify expressed proteins.

DNA Analysis—The *C. jejuni* DNA insert in pUC19 (pPB119) was digested with exonuclease III to generate a series of nested deletion mutants (23) and also by bidirectional endonuclease deletion based on the restriction map. The nucleotide sequence of plasmid DNA was determined on both strands by the dideoxynucleotide chain termination reaction (24) and was analyzed with DNASTAR software to define open reading frames and restriction sites. The amino acid sequence of the deduced gene product was analyzed for hydrophobicity with the algorithm of Kyte and Doolittle (25) and for secondary structure with the algorithm of Garnier *et al.* (26). Nucleic acid and amino acid homologies to GenBank and EMBL data bases were examined with Nuscan and Proscan programs using the method of Pearson and Lipman (27).

Amplification of pebIA Gene from C. jejuni Isolates Using Polymerase Chain Reaction (PCR)—Oligonucleotides were synthesized on a Milligen 7500 automated DNA synthesizer. PCR was performed with bacterial chromosomal DNA concentrations of 1 ng/μl and with primer annealing at 54 °C for 1 min, extension at 72 °C for 2 min, and denaturation at 94 °C for 1 min for 30 cycles. PCR products were electrophoresed on 1% agarose gel and purified (GeneClean, Bio101, Inc., La Jolla, CA) for restriction digestions.

Southern Blot Analysis—Restriction fragments generated by *Hin*dIII digestion of whole chromosomal DNA on 0.7% agarose gel were transferred to nylon membranes after denaturation and neutralization according to the method of Southern as described (22). Probe was labeled with [^{32}P]dATP by random priming, and hybridization was performed in 50% formamide buffer overnight at 42 °C (22).

RESULTS

Detection of Recombinant Bacteriophage Expressing C. jejuni PEB1 Protein—The λgt11 bank of genomic DNA from *C. jejuni* strain 81-176 in Y1088 cells yielded 8.2×10^5 plaque-forming units with a 76.2% insertion rate. After amplification in Y1090 cells, the bank was screened with an *E. coli*-absorbed rabbit antiserum to purified PEB1 from strain 81-176 (11) to detect expression of recombinant clones bearing PEB1 antigens. Two positive plaques were detected from a 10-cm diameter Petri dish containing ~10^5 plaques. These two clones were plaque-purified and amplified on Y1090 cells to provide high titer stocks for further study.

Characterization of Recombinant Protein in λgt11—To further characterize these two clones, we constructed lysogens of each in *E. coli* Y1089 cells. The lysogens grown with IPTG and analyzed by immunoblot with antiserum to PEB1 both produced an immunoreactive product migrating at ~28 kDa (Fig. 1), essentially identical in apparent molecular mass to

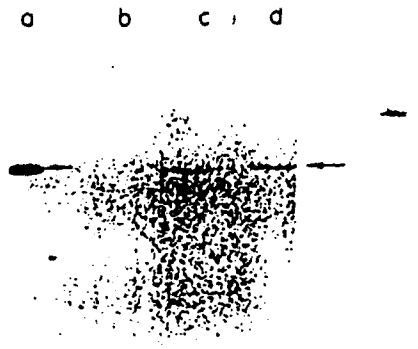

FIG. 1. Immunoblot of lysates of lysogenized *E. coli* Y1089 cells producing recombinant *C. jejuni* antigens. *Lane a*, cells of *C. jejuni* strain 81-176; *lane b*, cells of *E. coli* strain Y1089 containing λgt11 without an insert; *lane c*, cells of lysogenic clone 1; *lane d*, cells of lysogenic clone 2. *E. coli* cells in *lanes b–d* were cultured overnight in the presence of 2 mM IPTG to induce expression of genes downstream of the *lacZ* promoter in λgt11. A band migrating at ~28 kDa (indicated by *arrow*) was recognized by rabbit antiserum to purified PEB1 (1:10,000) (11) in clones 1 and 2, but not in the strain harboring λgt11 alone.

PEB1 produced by *C. jejuni* strain 81-176. The DNA from each clone was purified, and the inserts were excised following *Eco*RI digestion and resolved by agarose gel electrophoresis. Each clone contained a single insert of 2.6 kb.

Characterization of C. jejuni Insert DNA—The 2.6-kb insert in clone 1 was subcloned into pUC19, and transformants were screened by immunoblot using antiserum to PEB1. The plasmids from two transformants producing immunoreactive molecules migrating at ~28 kDa had the insert in opposite orientations and were designated pPB119 (Fig. 2) and pPB219. Both strains expressed the molecule in the absence of IPTG, but IPTG enhanced expression in pPB119, indicating an effect mediated by the β-galactosidase promoter of the vector. *Hin*dIII and *Nco*I deletion mutants of pPB119 (pPB203 and pPB11) both expressed the full-length 28-kDa protein (Fig. 2).

Nucleotide Sequence of pebIA—The nucleotide sequence of the 2687-kb insert determined according to the strategy shown in Fig. 2 yielded three complete and two partial open reading frames (ORFs), which were designed 5′ to 3′ as ORFs A–E (Fig. 3). ORF A is a partial ORF encoding 21 amino acids, ending with TAA at positions 65–67. Between ORFs A and B, there are 15 nucleotides containing a putative (28) ribosomal binding site (ACGA, positions 72–75) 7 nucleotides upstream from the ATG codon initiating ORF B. No putative transcriptional terminator was found in this region, suggesting that ORFs A and B may be cotranscribed. ORF B is 795 nucleotides, encoding a 264-residue polypeptide, ending with TAA at positions 875–877. Following ORF B is a 128-nucleotide noncoding sequence containing an inverted repeat that could form a stem-loop structure ($\Delta G = -9.0$) (Fig. 3) (29). ORF C begins with an unusual start codon (TTG) at positions 1006–1008 (30, 31). A putative ribosomal binding site (AGGA) is located 6 nucleotides upstream from the TTG codon. There is a sequence (TAAAAT) resembling the −10 consensus sequence in *E. coli* (TAtAaT) that is 35 bases upstream from the ribosomal binding site, and 20 nucleotides farther upstream, there is a sequence (TTGAAG) resembling the −35 consensus sequence in *E. coli* (TTGACa) (32). ORF C is 729 nucleotides, encoding a polypeptide of 242 amino acids, ending at positions 1732–1734 with TAA. ORF D follows ORF C after a 21-nucleotide noncoding region. A putative ribosomal binding site (AGGA) is located 6 nucleotides upstream from

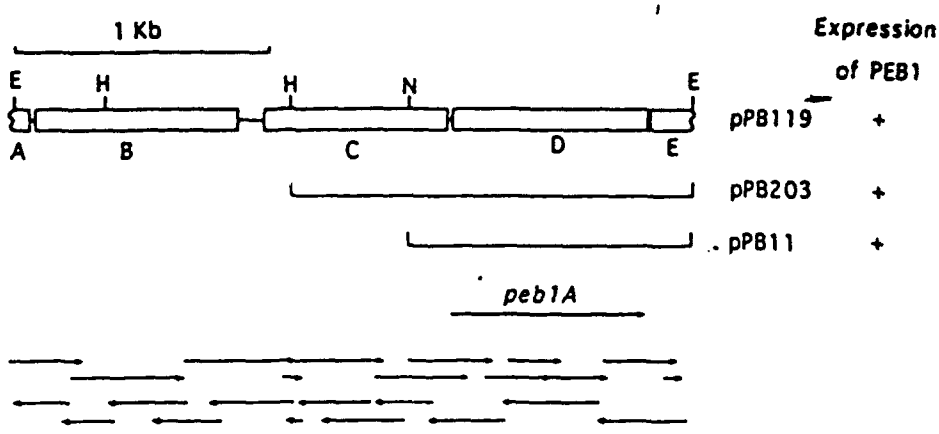

FIG. 2. **Restriction map of pPB119 and sequencing strategy for *peb*1A gene.** Restriction sites are shown above the 2.6-kb insert (*E*, *EcoRI*; *H*, *HindIII*; *N*, *NcoI*). Three complete ORFs, B-D, and two partial ORFs, A and E, are indicated below the insert. The *large arrow* represents the direction of transcription of *peb*1A. pPB203 and pPB11 are deletion mutants of pPB119. *Solid arrows* represent sequences obtained from deletion mutants, and *dotted arrows* from primer sequencing.

the start codon (ATG) for ORF D at position 1756. ORF D (*peb*1A) is 780 nucleotides, terminated by TAA at positions 2533-2535, and encodes a polypeptide of 259 amino acids with a molecular mass of 28.18 kDa. One base downstream of ORF D, truncated ORF E begins; only the first 50 amino acids of this ORF can be deduced from the insert. Since no potential transcriptional terminators were found among ORFs C-E, it is possible that these ORFs are cotranscribed using a common promoter located upstream from ORF C. No ORF >300 nucleotides was found in the complementary strand.

Signal Sequence of PEB1—The amino-terminal amino acid sequence of mature native PEB1 (11) is identical to the deduced sequence from ORF D beginning at residue 27, indicating that mature PEB1 has a 26-residue cleaved signal sequence. Overall, the 26-residue signal peptide has a calculated molecular weight of 2742 and is similar in structure to a typical signal peptide (33, 34). Arg and Lys at positions 4 and 5, respectively, form its positively charged head; the next 9 residues form a hydrophobic core, followed by Gly, an α-helix breaker, 10 residues upstream from the cleavage site. A typical structure for signal peptidase I cleavage (33, 34) occurs between Ala^{26} and Ala^{27}, followed by negatively charged Glu^{28}. Immediately following the cleavage site, 8 of 13 residues are polar. A second conserved signal peptidase-processing structure (Leu^{15}-Gly^{16}-Ala^{17}-Cys^{18}) homologous to signal peptidase II cleavage sites was located in which Cys is essential, Leu is highly conserved, and small amino acids between Leu and Cys such as Gly, Ala, Ser, and Val are preferred (35).

Amino Acid Composition and Codon Usage—Although ORF D (*peb*1A) encodes a deduced protein of 28.2 kDa, the deduced molecular mass of mature PEB1 (27th to 259th amino acid residue) is 25.5 kDa. The pI (8.51) of the deduced mature protein is nearly identical to the experimentally derived pI (8.5) of the mature protein (11). The amino acid composition determined for the mature protein from *C. jejuni* strain 81-176 (11) and that deduced for *peb*1A (27th to 259th residue) are in general agreement, with a few exceptions. The deduced mature protein contains 39 basic amino acid residues (33 Lys, 4 Arg, and 2 His) and 35 acidic residues (22 Asp and 13 Glu), indicating a net positive charge consistent with both the determined and predicted pI values. Cysteine is not found either in PEB1 or in the deduced mature *peb*1A gene product. Examination of the deduced amino acid sequence indicates relatively uniform distribution of positive and negative charges over the length of the molecule. Hydrophobic amino acids with no polar side chains represent 50% of the residues. The GC content of *peb*1A is 31.66%; A or T represents 88% (229/260) of the third-position nucleotides. This GC content and codon usage are consistent for *Campylobacter* DNA (18, 36-38), but as expected, are significantly different from *E. coli* (39).

Secondary Structure of peb1A Gene Product—Secondary structure calculations (26) for the deduced mature protein indicate that 78% of the 233 residues are in the α-helical conformation. The deduced signal peptide is entirely in the α-helical conformation. Using the method of Kyte and Doolittle (25), the only major hydrophobic region (Leu^{7}-Ala^{24}) is located in the leader peptide (data not shown); the other minor hydrophobic regions are randomly distributed over the entire molecule, but none is sufficiently long for membrane spanning.

Homologies of PEB1 to Other Proteins—A search of the National Biomedical Research Foundation (PIR 21.0) showed 27.8% identity of the deduced *peb*1A product to *E. coli* glutamine-binding protein precursor (*gln*H) (14), 22.9% identity to *Salmonella typhimurium* LAO (15), and 28.9% identity to *S. typhimurium* histidine-binding protein (*his*J) (16, 40). Searches of a variety of regions of PEB1 show no significant homologies to other known proteins. The amino acid composition, molecular mass, and secondary structure are similar between PEB1 and *gln*H, *his*J, and LAO; however, PEB1 is significantly more basic than these other proteins. A pairwise alignment of the primary sequence did not show consecutive identical regions of >4 amino acid residues between PEB1 and *gln*H or LAO (Fig. 4). The relationship of PEB1 with amino acid-binding proteins was further confirmed by the homology of ORF C to other members of operons for glutamine and histidine transport systems. ORF C shares nearly 50% identity with the proteins *gln*Q and *his*P (Fig. 5), which serve as membrane receptors for the binding proteins *gln*H and *his*J, respectively. Both *gln*Q and *his*P, like ORF C, begin with uncommon start codons such as TTG and GTG (14, 15, 41). ORF E, the third member of the putative PEB1 operon, did not share significant homology with other known proteins in the limited sequence that was identified.

ORF B shares an overall 22-24% identity with a number of heat shock proteins belonging to the hsp70 (42-45) and hsp90 (46) families, such as the 78-kDa glucose-regulated protein of yeast, which facilitates the assembly of multimeric protein complexes inside the endoplasmic reticulum and binds immunoglobulin heavy chain (42, 45). Homology was found also between mouse brain microtubule-associated protein (47) and ORF A (45% identity) and ORF B (24% identity) (Fig. 6).

Conservation of peb1A Gene among C. jejuni Strains—We next sought to determine the conservation of *peb*1A among *Campylobacter* strains by Southern hybridization since PEB1 is apparently present in all *C. jejuni* strains examined, and a closely related molecule is found in *C. coli* (11). Initial analyses used as the probe a 702-bp PCR product from pPB119

```
ORF A
  His Leu Lys Pro Met Ser Leu Lys Glu Ile Lys Lys Glu Ile          14
G CAT TTA AAA CCT ATG AGC TTA AAA GAA ATT AAA AAA GAA ATT          43

                                                    ORF B
Val Asn Phe Ile Asp Gln Asp Och                     Met Glu Lys     3
GTA AAT TTT ATT GAT CAG GAT TAA TAAAAGGAAAAATTGC ATG GAA AAA       91
                                 S.D.

Lys Ile Thr Pro Ser Glu Leu Glu Leu Asn Glu Phe Ile Lys Ile       18
AAA ATA ACT CCT AGC GAA TTG GAA CTT AAT GAA TTT ATA AAA ATT       136

Ile Asn Glu Met Ser Gly Ile Asp Leu Thr Asp Lys Lys Asn Ile       33
ATC AAC GAA ATG AGT GGT ATT GAT TTA ACC GAT AAA AAA AAT ATA       181

Leu Ala Leu Lys Leu Asn Lys Phe Leu Glu Gly Thr Asn Thr Lys       48
CTA GCT TTA AAG TTG AAT AAA TTT CTT GAA GGA ACT AAT ACT AAA       226

Asn Phe Ser Glu Phe Leu Gly Lys Leu Lys Ser Asn Arg Gln Leu       63
AAT TTT TCC GAA TTT TTG GGA AAA TTA AAA AGC AAT AGA CAA CTT       271

Lys Gln Glu Thr Leu Asp Phe Val Thr Ile Gly Glu Thr Tyr Phe       78
AAA CAA GAA ACT TTA GAT TTT GTA ACC ATA GGT GAA ACT TAT TTT       316

Leu Arg Glu Leu Ala Gln Leu Lys Glu Ile Ile Tyr Tyr Ala Lys       93
TTA AGA GAA TTG GCT CAA TTG AAA GAA ATA ATT TAT TAT GCC AAA       361

Ser Leu Glu Lys Arg Val Asn Ile Leu Ser Ala Pro Cys Ser Ser      108
AGC TTA GAA AAG AGA GTA AAT ATC CTA AGC GCC CCT TGT TCA AGT       406

Gly Glu Glu Val Tyr Ser Leu Ala Leu Leu Ala Ala Gln Asn Phe      123
GGA GAA GAA GTA TAT TCT TTG GCA TTA TTG GCT GCA CAG AAT TTT       451

Ile Lys Asp Met Tyr Ile Leu Gly Val Asp Ile Asn Ser Ser Val      138
ATT AAA GAT ATG TAT ATT TTA GGC GTT GAT ATT AAT TCA AGT GTG       496

Ile Glu Lys Ala Lys Leu Gly Lys Tyr Gln Gly Arg Thr Leu Gln      153
ATT GAA AAA GCA AAA CTT GGA AAA TAT CAA GGA AGA ACT TTA CAG       541

Arg Leu Ser Glu Ser Glu Lys Arg Arg Phe Phe Leu Glu Ser Glu      168
CGA TTG AGC GAG AGT GAA AAA AGA AGG TTT TTT TTA GAA AGC GAA       586

Asp Lys Phe Tyr Thr Ile Asn Lys Asn Glu Leu Cys Thr Cys Lys      183
GAT AAA TTT TAT ACT ATT AAT AAA AAT GAG CTT TGT ACT TGT AAA       631

Phe Glu Leu Cys Asn Val Phe Glu Glu Lys Phe Ser Arg Leu Gly      198
TTT GAA CTT TGC AAT GTT TTT GAA GAA AAA TTT TCA AGA TTG GGA       676

Lys Phe Asp Ile Ile Ala Ser Arg Asn Met Ile Ile Tyr Phe Asp      213
AAA TTT GAT ATT ATA GCT TCT AGA AAT ATG ATT ATT TAT TTT GAT       721

His Glu Ser Lys Leu Lys Leu Met Glu Arg Phe His Arg Ile Leu      228
CAT GAA TCA AAA CTA AAA CTT ATG GAG AGG TTT CAT AGA ATT TTA       766

Asn Asp Lys Gly Arg Leu Tyr Val Gly Asn Ala Asp Leu Ile Pro      243
AAT GAT AAA GGA AGG CTT TAT GTT GGC AAT GCT GAT TTA ATT CCA       811

Glu Thr Ile Tyr Phe Lys Lys Ile Ser Leu Gln Glu Val Phe Thr      258
GAG ACT ATT TAT TTT AAA AAG ATT TCT CTC CAA GAG GTG TTT ACT       856

Met Lys Lys Tyr Lys Phe Och                                     264
ATG AAA AAG TAT AAA TTC TAA AAATTACTAAAAGTTACACTTTGGAAATTTA      908

                    -35                         -10
TTAGTAAAAATAAGTTACATTTTGAAGTAGTTTTCTTTATTTAATGATAAAATAATTTC      967

                                          ORF C
                                          Met Ile Glu Leu Lys     5
AATTAATTTTATATTTAGCTAAAAATAAAGGAAAAAAC TTG ATT GAA TTA AAA      1020
                                  S.D.

Asn Val Asn Lys Tyr Tyr Gly Thr His His Val Leu Lys Ile Phe      20
AAT GTA AAC AAA TAC TAC GGA ACT CAT CAT GTT CTA AAG ATA TTT     1065

Asn Leu Ser Val Lys Glu Gly Glu Lys Leu Val Ile Ile Gly Pro      35
AAT CTT TCT GTT AAA GAA GGT GAG AAG CTT GTT ATT ATA GGT CCA     1110

Ser Gly Ser Gly Lys Ser Thr Thr Ile Arg Cys Met Asn Gly Leu      50
AGT GGA AGT GGA AAA AGT ACA ACT ATC CGT TGC ATG AAT GGG CTT     1155

Glu Glu Val Ser Ser Gly Glu Val Val Val Asn Asn Leu Val Leu      65
GAA GAA GTT AGT TCA GGA GAG GTC GTA GTT AAC AAT CTT GTT TTA     1200

Asn His Lys Asn Lys Ile Glu Ile Cys Arg Lys Tyr Cys Ala Met      80
AAT CAT AAA AAT AAA ATT GAA ATT TGC CGA AAA TAT TGT GCA ATG     1245

Val Phe Gln His Phe Asn Leu Tyr Pro His Met Thr Val Leu Gln      95
GTT TTT CAG CAT TTT AAT TTA TAT CCA CAT ATG ACG GTT TTG CAA     1290

Asn Leu Thr Leu Ala Pro Met Lys Leu Gln Lys Lys Ser Lys Lys     110
AAT TTG ACC TTA GCT CCA ATG AAA CTT CAA AAA AAA TCT AAA AAA     1335

Glu Ala Glu Glu Thr Ala Phe Lys Tyr Leu Lys Val Val Gly Leu     125
GAA GCT GAA GAA ACA GCT TTT AAG TAT TTA AAA GTT GTA GGT TTG     1380

Leu Asp Lys Ala Asn Val Tyr Pro Ala Thr Leu Ser Gly Gly Gln     140
CTG GAT AAA GCA AAT GTT TAT CCA GCA ACC CTT TCA GGT GGA CAA     1425
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FIG. 3. Nucleotide and deduced amino acid sequences of 2687-bp pPB119 fragment containing *peb1A*. The DNA sequence was determined for both strands as described under "Experimental Procedures." The three-letter amino acid code and the termination codon TAA (*Och*) are indicated above each triplet nucleotide codon. Nucleotides for pPB119 and amino acids for each open reading frame are numbered on the right of each line. The ribosomal binding sites (Shine-Dalgarno (*S.D.*)) and the putative promoter are indicated, and the *boldface* portions of the DNA sequence represent inverted repeat sequences that may serve as a transcriptional terminator. The *boldface* amino acid sequence in ORF D was determined by amino-terminal sequencing of mature PEB1 *from C. jejuni* (11).

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Gln Gln Arg Val Ala Ile Ala Arg Ser Leu Cys Thr Lys Lys Pro        155
CAA CAA CGC GTT GCT ATA GCA AGA TCA CTT TGT ACT AAA AAA CCC       1470

Tyr Ile Leu Phe Asp Glu Pro Thr Ser Ala Leu Asp Pro Glu Thr        170
TAT ATT TTA TTT GAT GAA CCT ACT TCA GCC CTT GAT CCA GAA ACC       1515

Ile Gln Glu Val Leu Asp Val Met Lys Glu Ile Ser His Gln Ser        185
ATA CAA GAG GTT TTA GAT GTA ATG AAA GAA ATT TCA CAT CAA AGC       1560

Asn Thr Thr Met Val Val Val Thr His Glu Met Gly Phe Ala Lys        200
AAT ACT ACC ATG GTG GTT GTT ACA CAC GAA ATG GGT TTT GCA AAA       1605

Glu Val Ala Asp Arg Ile Ile Phe Met Glu Asp Gly Ala Ile Val        215
GAA GTA GCA GAT AGG ATT ATT TTT ATG GAA GAT GGT GCT ATT GTG       1650

Glu Glu Asn Ile Pro Ser Glu Phe Phe Ser Asn Pro Lys Thr Glu        230
GAA GAA AAT ATT CCT AGT GAA TTT TTC TCA AAT CCA AAA ACT GAA       1695

Arg Ala Arg Leu Phe Leu Gly Lys Ile Leu Lys Asn Och               242
AGA GCG CGA CTC TTT TTA GGG AAA ATT CTT AAA AAT TAA CCAAAAT       1741

                    ORF D
                    Met Val Phe Arg Lys Ser Leu Leu Lys Leu Ala    11
TGAAAGGAGAAAAA ATG GTT TTT AGA AAA TCT TTG TTA AAG TTG GCA        1788
    S.D.

Val Phe Ala Leu Gly Ala Cys Val Ala Phe Ser Asn Ala Asn Ala        26
GTT TTT GCT CTA GGT GCT TGT GTT GCA TTT AGC AAT GCT AAT GCA       1833

Ala Glu Gly Lys Leu Glu Ser Ile Lys Ser Lys Gly Gln Leu Ile        41
GCA GAA GGT AAA CTT GAG TCT ATT AAA TCT AAA GGA CAA TTA ATA       1878

Val Gly Val Lys Asn Asp Val Pro His Tyr Ala Leu Leu Asp Gln        56
GTT GGT GTT AAA AAT GAT GTT CCG CAT TAT GCT TTA CTT GAT CAA       1923

Ala Thr Gly Glu Ile Lys Gly Phe Glu Val Asp Val Ala Lys Leu        71
GCA ACA GGT GAA ATT AAA GGT TTC GAA GTA GAT GTT GCC AAA TTG       1968

Leu Ala Lys Ser Ile Leu Gly Asp Asp Lys Lys Ile Lys Leu Val        86
CTA GCT AAA AGT ATA TTG GGT GAT GAT AAA AAA ATA AAA CTA GTT       2013

Ala Val Asn Ala Lys Thr Arg Gly Pro Leu Leu Asp Asn Gly Ser       101
GCA GTT AAT GCT AAA ACA AGA GGC CCT TTG CTT GAT AAT GGT AGT       2058

Val Asp Ala Val Ile Ala Thr Phe Thr Ile Thr Pro Glu Arg Lys       116
GTA GAT GCG GTG ATA GCA ACT TTT ACT ATT ACT CCA GAG AGA AAA       2103

Arg Ile Tyr Asn Phe Ser Glu Pro Tyr Tyr Gln Asp Ala Ile Gly       131
AGA ATT TAT AAT TTC TCA GAG CCT TAT TAT CAA GAT GCT ATA GGG       2148

Leu Leu Val Leu Lys Glu Lys Lys Tyr Lys Ser Leu Ala Asp Met       146
CTT TTG GTT TTA AAA GAA AAA AAA TAT AAA TCT TTA GCT GAT ATG       2193

Lys Gly Ala Asn Ile Gly Val Ala Gln Ala Ala Thr Thr Lys Lys       161
AAA GGT GCA AAT ATT GGA GTG GCT CAA GCT GCA ACT ACA AAA AAA       2238

Ala Ile Gly Glu Ala Ala Lys Lys Ile Gly Ile Asp Val Lys Phe       176
GCT ATA GGT GAA GCT GCT AAA AAA ATT GGC ATT GAT GTT AAA TTT       2283

Ser Glu Phe Pro Asp Tyr Pro Ser Ile Lys Ala Ala Leu Asp Ala       191
AGT GAA TTT CCT GAT TAT CCA AGT ATA AAA GCT GCT TTA GAT GCT       2328

Lys Arg Val Asp Ala Phe Ser Val Asp Lys Ser Ile Leu Leu Gly       206
AAA AGA GTT GAT GCG TTT TCT GTA GAC AAA TCA ATA TTG TTA GGT       2373

Tyr Val Asp Asp Lys Ser Glu Ile Leu Pro Asp Ser Phe Glu Pro       221
TAT GTG GAT GAT AAA AGT GAA ATT TTG CCA GAT AGT TTT GAA CCA       2418

Gln Ser Tyr Gly Ile Val Thr Lys Lys Asp Asp Pro Ala Phe Ala       236
CAA AGT TAT GGT ATT GTA ACC AAA AAA GAT GAT CCA GCT TTT GCA       2563

Lys Tyr Val Asp Asp Phe Val Lys Glu His Lys Asn Glu Ile Asp       251
AAA TAT GTT GAT GAT TTT GTA AAA GAA CAT AAA AAT GAA ATT GAT       2508

                                    ORF E
Ala Leu Ala Lys Lys Trp Gly Leu Och   Met Asn Glu Ser Val          5
GCT TTA GCG AAA AAA TGG GGT TTA TAA T ATG AAT GAA AGT GTA       2551

Gly Phe Val Glu His Leu Arg Gln Ile Leu Thr Ser Trp Gly Leu        20
GGT TTT GTT GAA CAT TTA AGA CAA ATT CTT ACT TCT TGG GGT TTA       2596

Tyr Aps Glu Asn Ser Ile Ser Pro Phe Ala Val Trp Lys Phe Leu        35
TAT GAT GAA AAT AGT ATA AGC CCT TTT GCG GTA TGG AAA TTT TTA       2641

Asp Ala Leu Asp Asn Lys Asp Ala Phe Ile Asn Gly Phe Ile Tyr        50
GAT GCT TTG GAT AAT AAA GAT GCT TTT ATT AAT GGT TTT ATT TAT G     2687
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FIG. 3C—*continued*

(primers: 5′-GCAGAAGGTAAACTTGAGTCTATT-3′ (bp 1834–1857) and 5′-TTATAAACCCCATTTTTTCGCTAA-3′ (complementary to bp 2512–2535), corresponding to the start and end of the sequence encoding mature PEB1). Under high stringency conditions, this probe hybridized to a single 1.8-kb *Hin*dIII-digested chromosomal fragment from all three *C. jejuni* strains, but not to the other *Campylobacter* strains examined (Fig. 7). When the same pair of primers was used in PCR analysis, a 702-bp PCR product was amplified from all three *C. jejuni* strains tested, as predicted, but from none of the *C. coli*, *Campylobacter lari*, or *Campylobacter fetus* strains tested (Fig. 8*A*). Restriction digestion of the *peb*1A PCR products amplified from each of the three *C. jejuni* strains demonstrated identical patterns (Fig. 8*B*), exactly as

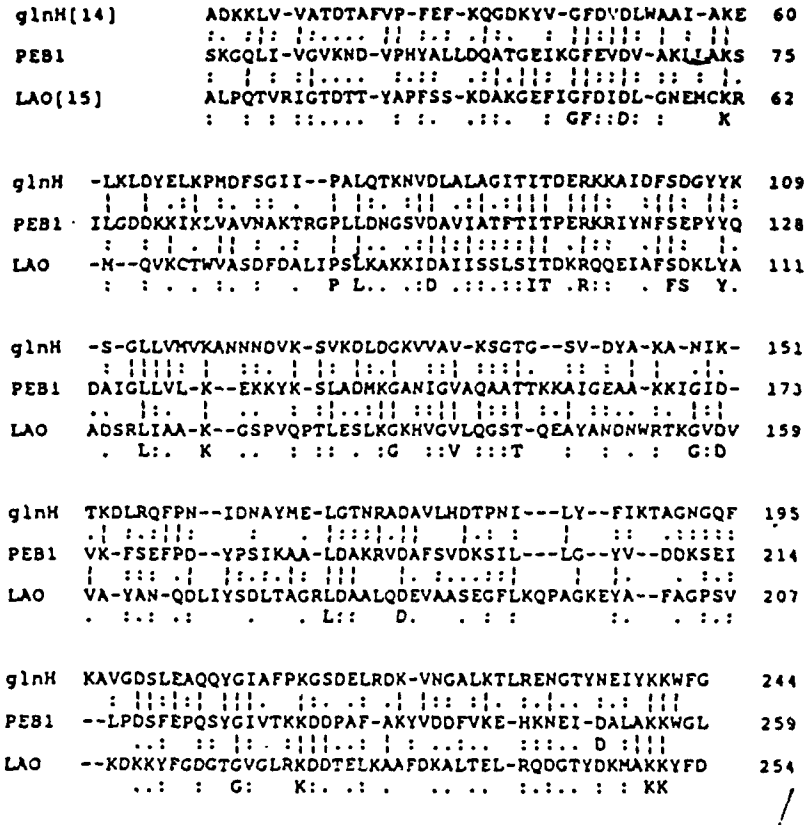

FIG. 4. Pairwise alignment of PEB1 with two amino acid-binding proteins. Gap penalty = 4, deletion penalty = 5, and similarity is defined by PAM 250 matrix, as described (57). Indicated are identity (|) and conservative substitution for hydrophobicity and charge (:) and size (.). For PEB1 *versus glnH*, there is 29.3% identity in a 233-amino acid overlap, and for PEB1 *versus* LAO, there is 23.7% identity in a 232-amino acid overlap.

expected from sequence analysis, indicating the high degree of conservation of the *peb*1A gene among *C. jejuni* strains.

DISCUSSION

PEB1, a surface-exposed conserved antigen in *C. jejuni* and *C. coli* that is commonly recognized by convalescent sera from infected patients and is involved in the binding of *C. jejuni* to eukaryotic cells, is possibly a vaccine candidate (11–13). In this study, we found that *peb*1A, the gene cloned using antibody to PEB1, is a homolog of the binding component in bacterial amino acid transport systems. Since a role for amino acid transport systems in bacterial pathogenesis has not been reported before, we sought to determine whether the properties of PEB1 and those of the recombinant *peb*1A product are similar. Although we have not yet performed studies to evaluate the role of the recombinant *peb*1A protein as a cell-binding factor, the following evidence establishes its identity to the native cell-binding factor PEB1. 1) *E. coli* transformed with pPB119 (containing *peb*1A) expressed a protein similar in electrophoretic migration, deduced isoelectric point, and amino acid composition to PEB1 from *C. jejuni*. The amino-terminal sequence determined by peptide analysis of mature PEB1 matches that deduced from the *peb*1A DNA sequence. A leader peptide was predictable (and observed) since PEB1 does not have an amino-terminal methionine and is an exported protein. The deduced molecular mass of the mature *peb*1A product is 25.5 kDa, slightly less than that determined by SDS-polyacrylamide gel electrophoresis (28 kDa), which could be due to the slower migration of a basic protein that has fewer net negative charges per residue. That the DNA sequence predicts Ala for the first position of the mature protein whereas amino-terminal sequencing showed Gly may be artifactual since the chromatographic behaviors of these 2 amino acids during peptide sequencing are similar. 2) We have purified the recombinant *peb*1A protein to homogeneity. Antibody to PEB1 recognized the purified *peb*1A protein.[2] 3) We have specifically mutated the *peb*1A gene from wild-type *C. jejuni* strain 81-176 by allelic replacement. If *peb*1A encodes a protein other than PEB1, mutating *peb*1A should not affect expression of PEB1. Using immunoblotting with antibody to PEB1, we found the PEB1 band in strain 81-176, but not in the isogenic *peb*1A⁻ mutant.[2]

Protein sequence comparisons of PEB1 indicated homology to members in the LAO superfamily, including products of the *gln*H, LAO, and *his*J genes. These function as amino acid-binding proteins as part of periplasmic amino acid transport systems in Gram-negative bacteria. Within the superfamily, the *his*J and LAO products share the highest homology, reflecting their binding of basic amino acids (histidine for *his*J and arginine for LAO), and share a common cellular receptor, *his*P (15). Similar to *gln*H, PEB1 exhibits an overall identity of ~25% to other superfamily members. However, other evidence strengthens the hypothesis that PEB1 belongs to this superfamily. Mature PEB1 and the other three proteins all are 25–26 kDa, and all contain a cleaved amino-terminal signal peptide, a high percentage of lysine, and conserved Lys-Lys sequences near the carboxyl terminus. The presence of a signal peptide and the absence of transmembrane domains in the mature protein are consistent with each being secreted beyond the cytoplasmic membrane. In the superfamily, PEB1 is most closely related to *gln*H in both sequence similarity and hydrophobicity distribution, especially between residues 58 and 163.

In several periplasmic binding protein-dependent transport systems (14, 41, 51–53), the binding protein and the membrane-associated components are each encoded in the same operon containing three or four structural genes. Genomic organization of ORFs A–E in pPB119 indicates that the 2.6-kb insert contains two partial operons separated by a noncoding region between ORFs B and C. The putative transcrip-

[2] Z. Pei and M. J. Blaser, unpublished data.

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ORF C      MIElk-NV---nKyYGthhVLklfnLsvkeGEklVIIGPSGSGKSTtiR  45
glnQ[14]   MIEfK-NV---sKhfGptqVLhnIdLniaqGEVvVIIGPSGSGKSTlLR  45
hisP[40]   MsEnKlNVidlhKryGeheVLkgvsLqanaGdVisIIGssGSGKSTfLR  49
consensus  mIe-Klnvid--k--g---vlk-i-L----Gev-vIIGpSGSGKST-lR

ORF C      CmNgLE---E---vsSG--evvV---nnL-VlnhKnkieicRkycaMVF  82
glnQ       CINkLE---E--I-tSGdlI--V---DGLkVndpKvdeRLiRqeagMVF  83
hisP       CINfLEkpsEgsIvvnGqtInlVrdkDGqlkvadKnqlRLlRtrltMVF  98
consensus  CiN-LEkpsEgsiv-sG--i--Vrdkdgl-v---Kn--rl-R----MVF

ORF C      QhFnLyPHmTvLqNltlaPmklqkksKkEAEetAfkyLkvVGLldkA-n  131
glnQ       QqFyLfPHltaLENVMfgPlrVrGanKeEAEklArelLAKVGLaERA-h  131
hisP       QhFnLwsHmTvLENVMeaPiqVlGlsKqEArerAvkyLAKVGldERAqg  147
consensus  QhFnL-pHmTvLeNvm-aP--v-g-sK-EAee-A-kyLakVGl-erAq-

ORF C      vYPatLSGGQQQRVAIARsLctKkpyiLFDEPTSALDPEtiqEVLdVMke  180
glnQ       hYPseLSGGQQQRVAIARALAvKPkmmLFDEPTSALDPELrhEVLkVM-Q  180
hisP       kYPvhLSGGQQQRVsIARALAmePevlLFDEPTSALDPELvgElLriM-Q  196
consensus  -YP--LSGGQQQRVaIARaLa-kp---LFDEPTSALDPEl--EvL-vMkq

ORF C      ishqsntTMVvVTHEmGFAkeVAdRiIFmedGaIvEeniPseffsNPkte 230
glnH       dLAEEGmTMViVTHEiGFAekVAsRliFidkGrIaEdGnPqvLikNPpSq 230
hisP       qLAEEGkTMVvVTHEmGFArhVsthvIFlhqGkIeEeGaPeqLfgNPqSp 246
consensus  -laeeg-TMVvVTHEmGFA--Va-r-IF---G-I-Eeg-P--lf-NP-s-

ORF C      RarlFLgkilkn 242
glnQ       RLQeFLqhvs   240
hisP       RLQrFLkgslk  257
consensus  Rlq-FL---lkn
```

FIG. 5. Homology between ORF C and *glnQ* and *hisP*. On the consensus line, *upper-case letters* represent residues conserved in all three molecules at that position, and *lower-case letters* represent residues conserved in two of the three molecules.

```
                                                    ORF B
ORF A      HLKPHSLKEIKKEIVNFIDQD 21                 MEKK-ITPS 8
MAP1B[47]  PRKEEVKKEIKKEIKKEERKELKKEVKKETPLKDAKKEVKKEEKKEVKKE 722

ORF B      ELELNEFIKII--NEMSGIDLTDKKNILALKLNKFLEGTNTKNFSEFLGK  56
MAP1B      EKEPKKEIKKISKDIKKSTPQSDTKKPSALKPKVAKKEESTKKEPLAAGK  772

ORF B      LKSNRQLK------QETLDFVTIGETYFLRELAQLKEIIYYAKSLEKRVN  100
MAP1B      LKDKGKVKVIKKEGKTTEAAATAVGT--AATTAAVVAAAGIAAS--GPVK  818

ORF B      ILSAPCS--SGEEVYSLALLAAQNFIKDMYI-LGVDI--NSSVIE-KAKL  144
MAP1B      ELEAERSLMSSPE----DLTKDFEELKAEEIDVAKDIKPQLELIEDEEKL  864

ORF B      GKYQ-GRTLQRLSESEKRRFFLESEDKFYTINKNE-LCTCKFE-LCNVFE  191
MAP1B      KETQPGEAYVIQKETEVSKGSAESPDEGITTTEGEGECEQTPEELEPV--  912

ORF B      EKFSRLGKFDIIASRNMIIYF-------DHESKLKLMERFHRILNDKGRL  234
MAP1B      EK---QGVDDIEKFEDEGAGFEESSETGDYEEK-AETEEAEEPEEDGEDN  958

ORF B      YVGNADLIPETIYFKKISLQEVFTHKKYKF  264
MAP1B      ASGSASKHSPT-EDDESAKAEADVHLKEKR  987
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FIG. 6. Homology between ORFs A and B and mouse brain microtubule-associated protein (*MAP1B*) (47). Parameters used were gap penalty = 1, gap size penalty = 0.05, and joining penalty = 20.

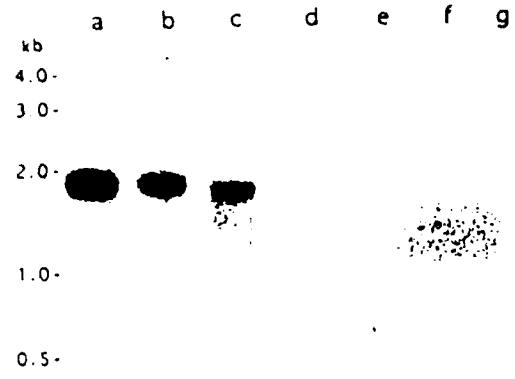

FIG. 7. Southern hybridization showing conservation of *peb*1A gene in *C. jejuni* chromosomal DNA digested with *HindIII*. A 702-bp PCR product corresponding to the DNA sequence of mature PEB1 was used as probe. *Lane a–c*, *C. jejuni* strains 81-176, 85-H, and 81-95, respectively; *lanes d* and *e*, *C. coli* strains D126 and D730, respectively; *lanes f* and *g*, *C. fetus* strains 23D and 84-91, respectively. Molecular size markers (in kilobases) are shown to the left.

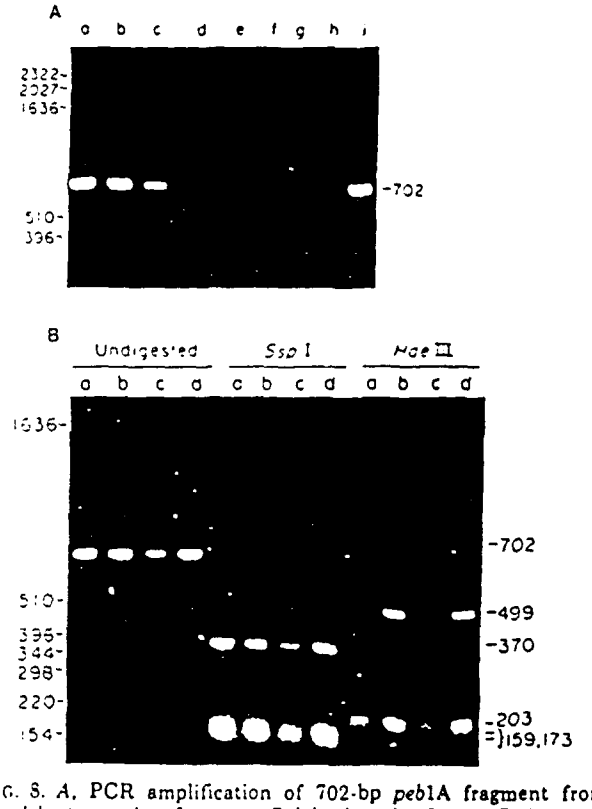

FIG. 8. *A*, PCR amplification of 702-bp *peb*1A fragment from *Campylobacter* strains. *Lane a–c*, *C. jejuni* strains 81-176, D1916, and 85AC, respectively; *lanes d* and *e*, *C. coli* strains D126 and D1035, respectively; *lanes f* and *g*, *C. lari* strains D110 and D67, respectively; *lane h*, *C. fetus* strain 23D; *lane i*, *E. coli* with pPB119. A 702-bp PCR-amplified product was found in all *C. jejuni* strains (*arrow*), but not in the other *Campylobacter* species. *B*, restriction pattern of 702-bp PCR products from *C. jejuni* strains. The 702-bp PCR products were undigested or were digested with *Ssp*I or *Hae*III. Strain 81-176 is shown in *lanes a* and *d* (undigested) and *c* (*Ssp*I-digested), strain D1916 in *lanes b* (undigested) and *a* and *d* (*Ssp*I-digested), and strain 85AC in *lanes c* (undigested), *b* (*Ssp*I-digested), and *a* (*Hae*III-digested). *Ssp*I cleaved the 702-bp PCR products from each strain into 370-, 173-, and 159-bp fragments, and *Hae*III cleaved the PCR products from each strain into 499- and 203-bp fragments, indicating that the *peb*1A gene is highly conserved in *C. jejuni*.

tional terminator in this region indicates a potential 3′-end of the operon containing ORFs A and B. The putative promoter just downstream indicates the 5′-end of the operon containing ORFs C, D (*peb*1A), and E. Support for this hypothesis includes the following. 1) The putative promoter is the only promoter found upstream from ORFs C–E, which could be responsible for *lacZ*-independent transcription of ORF D in pPB219; 2) there is no transcriptional terminator identified between ORFs C and D and between ORFs D and E; and 3) in *E. coli* and *S. typhimurium*, the genes homologous to ORFs C and D are randomly aligned and cotranscribed (14–16). A putative function of ORF E is unknown at present.

Although the homology between PEB1 and amino acid-binding proteins is significant, PEB1 is unique since all identified amino acid-binding proteins in bacterial transport systems are located in the periplasmic space, whereas PEB1 is exposed on the bacterial surface (12). Members of the LAO superfamily all contain a leader peptide processed by signal peptidase I, which enables these molecules to cross the cytoplasmic membrane. PEB1 also has such a processing site; as demonstrated by both the characteristics of the deduced PEB1 sequence and the evidence for cleavage at this site in *C. jejuni* (11). In addition, PEB1 contains a putative signal peptidase II cleavage site. Signal peptidase II processes lipoprotein precursors acylated on the free sulfhydryl group of cysteine by cleavage of the peptide bond at the amino-terminal side of cysteine. The acyl chains of the lipoprotein often anchor the polypeptide to membranes (54). Nearly all secreted proteins in bacteria have only one signal peptidase cleavage site processed by either signal peptidase I or II. However, the endoglucanase precursor of *Pseudomonas solanacearum* has a signal sequence of 45 residues with two processing sites (55). The endoglucanase is modified by fatty acylation at Cys^{20}, cleaved by signal peptidase II, and exported across the inner membrane. The lipoprotein intermediate is then cleaved at the signal peptidase I-like site (Ala^{45}-Ala^{46}) during export across the outer membrane. Since PEB1 also contains two signal peptidase-processing sites, the location of PEB1 on the bacterial surface suggests that *C. jejuni* could utilize similar mechanisms to export this protein across both the cytoplasmic and outer membranes. If this hypothesis is true, we would expect that mature PEB1 is not a lipoprotein since the final cleavage at Ala^{27} eliminates any amino acid residues located between residues 1 and 26, including the putatively acylated Cys^{20}. This hypothesis was supported by the evidence that mature PEB1 begins at Ala^{27} and that amino-terminal sequencing was not blocked by lipid (11).

We speculate that the major cell-binding factor PEB1 may have a common evolutionary origin with periplasmic amino acid-binding proteins, from which PEB1 gains the binding capacity. Since the genome of *C. jejuni* is only half the size of that of *E. coli* or *Salmonella* (56), *C. jejuni* may use particular proteins for multiple purposes. Two-step cleavage of the PEB1 leader peptide may distinguish it from these amino acid-binding proteins and make it accessible to the bacterial surface to perform cell binding functions as well.

The codon usage for PEB1 shows strong third-position AT preference; consequently, Arg, Asn, Cys, Gln, His, and Tyr are single-codon amino acids, which has been previously observed for *C. jejuni* serine hydroxymethyltransferase (*glyA*) (48). Knowledge of this phenomenon will be helpful in designing oligonucleotide probes to clone other *C. jejuni* proteins for which antibody probes are not available.

ORF C begins with TTG, which although not common, has been previously identified (30, 31, 41, 49, 50). The ORF C and *glnQ* and *hisP* gene products are highly homologous, and their

ORFs also begin with uncommon initiation codons (14, 15). Experimental replacement of TTG with ATG led to a 2.5–3.7-fold increase in protein translation (49, 50). The low translation efficiency when TTG is the initiating codon suggests a possible conserved mechanism for control of expression of this family of homologous proteins.

Although both *C. jejuni* and *C. coli* strains contain PEB1 homologs (11), using primers corresponding to the amino and carboxyl termini of mature PEB1, we PCR-amplified the expected fragment from *C. jejuni* strains, but not from *C. coli* strains. Amino-terminal differences in the *C. jejuni* and *C. coli* PEB1 homologs (11) correlate with the failure of both amplification and hybridization of the *peb*1A gene with *C. coli* strains. In contrast to *C. coli* strains, the primary sequence of *peb*1A must be highly conserved among *C. jejuni* strains, as shown by the conserved PCR product restriction profiles, again indicating its importance to the organism.

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