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Nucleotide Sequence of the *btuCED* Genes Involved in Vitamin B_{12} Transport in *Escherichia coli* and Homology with Components of Periplasmic-Binding-Protein-Dependent Transport Systems

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The products of the *btuCED* region of the *Escherichia coli* chromosome participate in the transport of vitamin B_{12} across the cytoplasmic membrane. The nucleotide sequence of the 3,410-base-pair *Hin*dIII-*Hin*cII DNA fragment carrying a portion of the *himA* gene and the entire *btuCED* region was determined. Comparison of the location of the open reading frames with the gene boundaries defined by transposon insertions allowed the assignment of polypeptide products to gene sequences. The *btuC* product is a highly nonpolar integral membrane protein of molecular weight 31,683. The distribution of hydrophobic regions suggests the presence of numerous membrane-spanning domains. The *btuD* product is a relatively polar but membrane-associated polypeptide of M_r 27,088 and contains segments bearing extensive homology to the ATP-binding peripheral membrane constituents of periplasmic binding protein-dependent transport systems. Other regions of this protein are similar to portions of the outer membrane vitamin B_{12} receptor. The *btuE* product (M_r 20,474) appears to have a periplasmic location. It has the mean hydropathy of a soluble protein but lacks an obvious signal sequence. The cellular locations and structural and sequence homologies of the Btu polypeptides point to the similarity of these three proteins to components of binding protein-dependent transport systems. However, the dependence on a periplasmic vitamin B_{12}-binding protein has not yet been demonstrated.

Vitamin B_{12} is transported into cells of *Escherichia coli* by means of a high-affinity, energy-dependent uptake process (13). Transport is initiated by the binding of the vitamin to an outer membrane receptor protein, encoded by the *btuB* gene (18, 38). The *tonB* product is necessary for the energy-dependent release of receptor-bound vitamin B_{12} (3, 31). Iron chelate uptake systems also employ specific outer membrane receptor proteins and *tonB* function (29). The use of receptors allows these relatively large and scarce growth factors to be translocated across the outer membrane more efficiently than by diffusion through porin channels.

Little information is available concerning the passage of vitamin B_{12} or the iron chelates across the cytoplasmic membrane. Genetic studies of vitamin B_{12} and siderophore uptake revealed the requirement for additional components acting after the receptors and TonB. Three genes (*fhuBCD*) are necessary for ferric hydroxamate entry (14); ferrienterochelin uptake requires *fepBC* function in addition to the *fepA*-coded receptor (30). In the case of vitamin B_{12}, three linked genes, *btuCED*, have been identified (11, 12). In their absence, vitamin B_{12} is accumulated in an energy-dependent manner, but, in contrast to the wild-type situation, all of the transported compound is lost from the cell upon chase with excess nonradioactive substrate or disruption of the outer membrane by osmotic shock or treatment with chelators (31). Furthermore, there is little conversion of transported cyanocobalamin into the usual metabolic species encountered in wild-type strains.

The *btuCED* region has been cloned, and the locations of *btuC* and *btuD* were defined by further subcloning and transposon mutagenesis. The two genes were separated by approximately 800 base pairs (bp) (11). Transposon insertions in this central region (*btuE*) produced a deficiency in vitamin B_{12} transport and utilization, as did those in *btuC* or *btuD* (12). A maxicell system allowed identification of the products of *btuC*, *btuD*, and *btuE* as having apparent molecular weights of 26,000, 29,000, and 22,000, respectively (12). Cellular localization studies indicated that the BtuC and BtuD polypeptides were membrane associated, whereas some of the BtuE polypeptide was released from maxicells by osmotic shock or spheroplast formation, suggesting a periplasmic location. Previous studies demonstrated that an M_r 22,000 vitamin B_{12}-binding protein was released by osmotic shock (6, 37).

Some active transport systems in *E. coli* depend on periplasmic substrate-binding proteins and require phosphate bond energy (4). Nucleotide sequencing of the genes encoding several of these transport system (those for histidine [20], maltose [9, 15, 16], phosphate [36], and oligopeptides [21]) revealed some common properties of the constituent proteins. In addition to the periplasmic binding proteins, there are two very nonpolar integral membrane proteins and an ATP-binding peripheral membrane protein. There is genetic evidence from the histidine transport system that all of these components are necessary for transport function and that the periplasmic protein interacts with the peripheral membrane protein (1).

In this communication we present the nucleotide sequence of the *btuCED* region. Portions of the predicted BtuD polypeptide exhibited sequence homology to the conserved parts of the peripheral membrane proteins that might be involved in nucleotide binding. The BtuC polypeptide displayed weak structural homology to some of the integral membrane transport proteins. These similarities in location and structure prompted the conclusion that the BtuCED proteins comprise an active transport system for the move-

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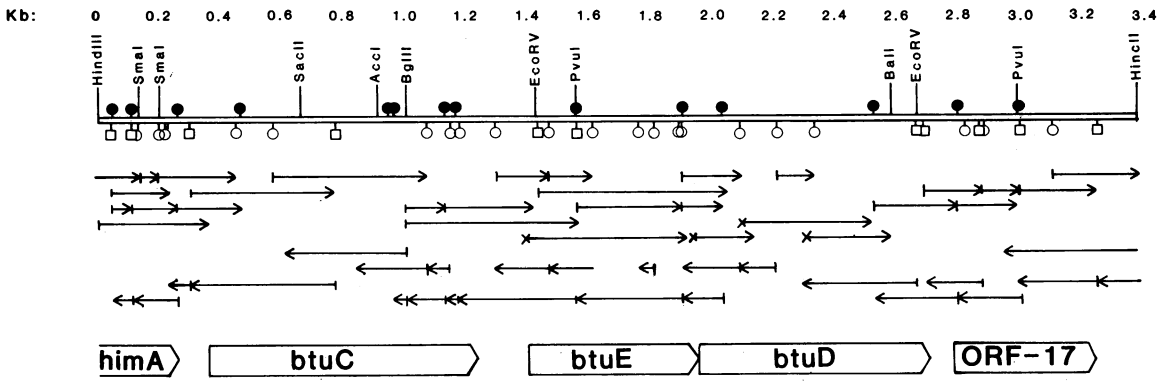

FIG. 1. Restriction map and sequencing strategy of the 3.4-kilobase (kb) *Hin*dIII-*Hin*cII fragment containing *btuCED*. The restriction map was constructed by analysis of restriction fragments of plasmid pLCD25 and by incorporation of the sequencing data. Some restriction endonuclease cleavage sites are indicated by their mnemonics; symbols are used to designate sites for *Sau*3A (●), *Hpa*II (○), and *Taq*I (□). The extent and direction of the sequences obtained from each fragment are represented by the length of each arrow. Each reading started at the restriction site indicated by the vertical line, except for those with an X at the left-hand end, which were derived by *Exo*III-generated deletions. The location and direction of the open reading frames are identified at the bottom of the figure.

ment of vitamin B_{12} across the cytoplasmic membrane. This transport system bears a resemblance to binding-protein-dependent systems, but the role and existence of a periplasmic vitamin B_{12}-binding protein remains to be demonstrated.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. Plasmid pLCD25 carries the 3.4-kilobase *Hin*dIII-*Hin*cII fragment in pBR322 (11). Plasmids carrying various portions of the insert were previously described (11, 12). Phages M13mp8 and M13mp9 and their derivatives were propagated in *E. coli* JM101 (27).

Determination of nucleotide sequence. DNA fragments of the 3.4-kilobase *Hin*dIII-*Hin*cII fragment carrying the *btuCED* region were prepared by digestion of the insert in pLCD25 with *Sau*3A, *Taq*I, or *Hpa*II and were randomly cloned into appropriate sites of phage M13mp8 or M13mp9 (25). Subclones obtained in one vector were inverted by ligation of the *Hin*dIII-*Eco*RI insert-containing fragment from the replicative form of each recombinant phage into these sites in the other vector. The *Hin*dIII-*Bgl*II (from pLCD16), *Bgl*II-*Hin*cII (from pLCD27), *Bgl*II-*Bal*I (from pLCD26), and *Bal*I-*Hin*cII fragments were also cloned into these phages (11).

The 2.4-kilobase *Bgl*II-*Hin*cII region was cloned between the *Bam*HI and *Eco*RI sites of phage M13mp8, making use of the *Eco*RI site present on plasmid pLCD27 adjacent to the *Hin*cII site (11). To generate deletions extending from the *Bgl*II site, the replicative form of this recombinant phage was opened by treatment with *Sal*I and *Pst*I. As described by Henikoff (19), nuclease ExoIII cannot initiate cleavage at the 3′ overhang at the *Pst*I site. After 1, 2, and 3 min of incubation, exonuclease action was stopped, and the DNA was treated successively with nuclease S1 and T4 DNA ligase as described previously (19). The deletions thereby generated extended into the insert while retaining the primer-binding sequences and M13 replication functions.

DNA sequencing employed the enzymatic dideoxy chain termination method (33) with deoxyadenosine 5′-(α-[^{35}S]thio)triphosphate as described previously (5, 18). Sequence readings were obtained across all of the restriction sites used for subcloning, and every character was read from at least two restriction fragments.

Sequence data were handled with the DBUTIL program of Staden (35); homology searches employed the FASTP program of Lipman and Pearson (24). Estimation of the relatedness of possible conserved regions made use of the PAM250 amino acid replacement odds matrix of Dayhoff et al. (10). This yields the probability that a given amino acid would be replaced by any of the other amino acids over a fixed period of time. A score of +2 means that the pair would be expected to occur 1.6 times as frequently in related proteins as random chance would predict.

RESULTS AND DISCUSSION

Nucleotide sequence of the *btuCED* region. Figure 1 shows the restriction map of the 3,410-bp *Hin*dIII-*Hin*cII insert carried in plasmid pLCD25 and the strategy employed for determination of its nucleotide sequence. All of the sequence except 83 nucleotides was determined from both strands of the DNA. The nucleotide sequence of the noncoding strand of the fragment is given in Fig. 2, starting from the second *Sma*I site and extending for 2,600 bp through *btuCED* to the start of ORF-17, the coding region for an M_r 17,287 polypeptide of unknown function.

Identification of coding regions. The open reading frames were correlated with the *btu* genes by a comparison of their locations to the gene boundaries defined by Tn*1000* insertions (11, 12). These insertions showed that *btuC* started near the *Sma*I site at position 1 and ended near position 1,000 (halfway between the *Bgl*II and first *Eco*RV sites). The *btuE* gene began near the first *Eco*RV site at position 1,231, and the end of *btuD* was near the other *Eco*RV site at position 2,460. The other ends of these two genes could not be precisely defined owing to the lack of sufficient transposon insertions and restriction sites in that area. These gene boundaries defined by Tn*1000* insertions agreed very well with the locations of the open reading frames.

The reading frame entering the fragment from the *Hin*dIII end and extending past the *Sma*I site to nucleotide 54 matched the coding sequence for the *himA* gene described by Miller (28) and by Mechulam et al. (26). Its termination codon is followed by a region of dyad symmetry similar to rho-independent transcription termination sites (32).

The *btuC* coding region probably starts with the ATG at position 156, which is preceded by a sequence (AGCAGAA) having partial complementarity to the 3′ end of 16S rRNA

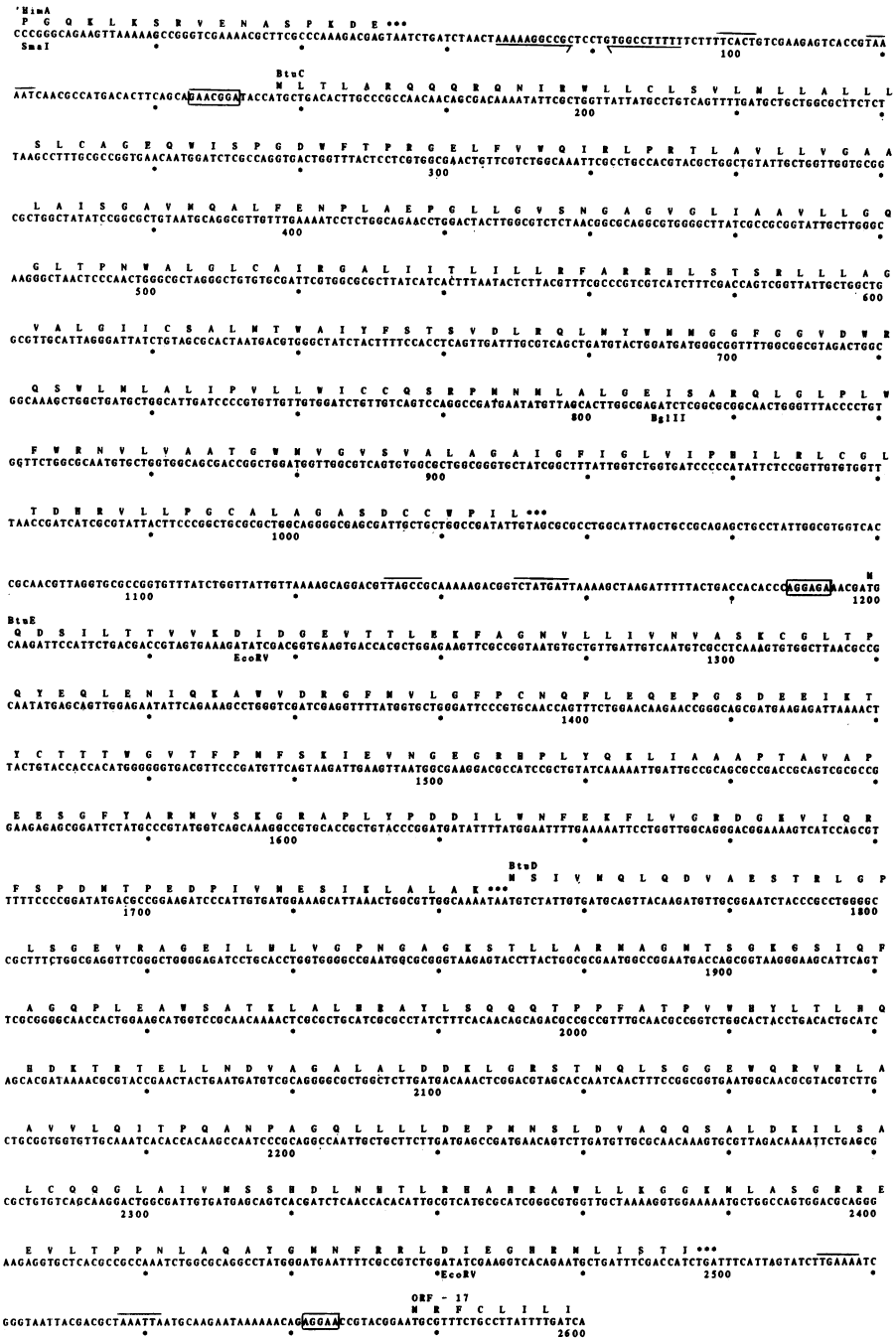

FIG. 2. DNA sequence of *btuCED*. The noncoding strand is presented, and the predicted polypeptide sequence of the open reading frames is shown in one-letter code above the DNA sequence. The numbering begins at the second *Sma*I site located at the 3′ end of *himA*. Some regions of local dyad symmetry are underlined with arrows. Lines are drawn over potential promoter sequences, and putative Shine-Dalgarno sequences are enclosed in boxes.

(Shine-Dalgarno sequence [34]). Other possible initiation codons at positions 222 and 384 lack this hallmark of a translation initiation site. Upstream from this coding region is a potential promoter sequence with a −35 region (TTCACT) separated by 15 bp from a −10 region (TAAAAT). The *btuC* coding region extends for 879 bp, encoding a polypeptide of 292 amino acids. The *btuC* termination codon (UAG) at position 1,032 was followed by a 68-bp region containing extensive lengths of dyad symmetry and alternative base pairings. This region might be involved in termination, but evidence for this is not yet available.

The *btuE* coding sequence probably starts with the ATG at position 1,198, lying 163 bp from the end of *btuC*. A Shine-Dalgarno sequence (AGGAG) precedes this initiation codon, albeit having only a spacing of five nucleotides. The next possible start (GTG at position 1,245) has a poorer ribosome-binding sequence (GACGG). The *btuE* reading frame continues for 552 bp to the TAA at position 1,747,

encoding a polypeptide of 183 amino acids. The *btuE* gene is preceded by at least two potential transcription initiation regions. The presence of these potential promoters could explain the partial polarity engendered by insertions in the *btu* genes (12).

The sequence TAATG at position 1,747 can serve both as a termination codon for *btuE* and as an initiation codon for *btuD*. The 750-bp *btuD* coding region extends past the *Eco*RV site to position 2,498, encoding a polypeptide of 249 amino acids. The coding region for a 154-amino-acid polypeptide of unknown function extends rightward from position 2,576. Its product, termed ORF-17, is not involved in either the regulation or function of the vitamin B_{12} transport system (12).

The direction of translation proceeds from *himA* toward *btuD*, which represents the counterclockwise direction relative to the genetic map, in agreement with the direction of transcription determined from the orientation of *btuD*::*lac* operon fusions (11). The usage of most of the available codons for translation of the three *btu* genes (data not shown) is characteristic of weakly expressed genes in *E. coli* (17).

Table 1 presents the amino acid compositions of the predicted BtuCED polypeptides.

BtuC polypeptide. The BtuC polypeptide is predicted to contain 292 amino acids and have a molecular weight of 31,683. It has a net positive charge (+11) and is extremely nonpolar, containing about 10.6% charged amino acids and a mean hydropathy (0.92, using the values of Kyte and Doolittle [23]) similar to that of bacterial integral cytoplasmic membrane proteins. Notable features are the high leucine content (20% by number) and the absence of lysine.

The *btuC* product detected in maxicells is membrane associated (12). Its lower apparent molecular weight from sodium dodecyl sulfate-polyacrylamide gel electrophoresis, 26,000, can be attributed to the increased binding of sodium dodecyl sulfate to very hydrophobic portions, as previously observed for the *lacY* and *malG* products (8, 9). The distribution of charged amino acids is not even, with many of the positively charged residues in clusters.

There is very little conservation of amino acid sequence among BtuC and the integral membrane components of

TABLE 1. Amino acid composition of the predicted BtuCDE polypeptides

Amino acid	No. of residues (mol %)		
	BtuC	BtuD	BtuE
Ala	32 (11.0)	27 (10.8)	12 (6.6)
Arg	18 (6.2)	15 (6.0)	6 (3.3)
Asn	6 (2.1)	8 (3.2)	6 (3.3)
Asp	5 (1.7)	10 (4.0)	10 (5.5)
Cys	10 (3.4)	1 (0.4)	3 (1.6)
Gln	12 (4.1)	18 (7.2)	8 (4.4)
Glu	5 (1.7)	10 (4.0)	15 (8.2)
Gly	30 (10.3)	21 (8.4)	13 (7.1)
His	3 (1.0)	10 (4.0)	1 (0.6)
Ile	20 (6.9)	9 (3.6)	11 (6.0)
Leu	60 (20.6)	39 (15.7)	15 (8.2)
Lys	0 (0.0)	8 (3.2)	12 (6.6)
Met	11 (3.8)	10 (3.6)	6 (3.3)
Pro	12 (4.1)	11 (4.4)	12 (6.6)
Ser	15 (5.1)	17 (6.8)	8 (4.4)
Thr	10 (3.4)	14 (5.6)	12 (6.6)
Trp	14 (4.8)	4 (1.6)	3 (1.6)
Tyr	2 (0.7)	3 (1.2)	5 (2.7)
Val	19 (6.5)	12 (4.8)	15 (8.2)

TABLE 2. Conserved sequences in the peripheral membrane components of binding protein-dependent transport systems

Protein	Residue	Conserved sequence
BtuD	19	LSGEVRAGEILHLVGPNGAGKSTLLARMAGM
HisP	25	VSLQARAGDVISIIGSSGSGKSTFLRCINFL
Mal K	22	INLDIHEGEFVVFVGPSGCGKSTLLRMIAGL
PstB	29	INLDIAKNQVTAFIGPSGCGKSTLLRTFMKM
OppD1 (Ec)	43	VTLRLYEGETLGVVGESGCGKSTFARAIIGL
OppD2 (Ec)	42	LNFSLRAGETLGIVGESGSGKSQTAFALMGL
OppD (St)	40	LNFTLRAGETLGIVGESGSGKSQSRLRLMGL
BtuD	127	LSGGEWQRVRLAAVVLQITPQANPAGQLLLLDEPMNSLDVAQQSALDKILSAL
HisP	154	LSGGQQQRVSIARALAMEPDILLFDEPTSALDPELVGEVLRIMQQL
MalK	134	LSGGQRQRVAIGRTLVAEPSVFLLDEPLSNLDAALRVQMRIEISRL
PstB	152	LSGGQQQRLCIARGIAIRPEVLLLDEPCSALDPISTGRIEELITEL
OppD1 (Ec)	165	FSGGQCQRIGIARALILEPKLIICDEPVSALDVSIQAQVVNLLQQL
OppD2 (Ec)	169	FSGGMRQRVMIAMALLCRPKLLIADEPTTALDVTVQAQIMTLLNEL
OppD (St)	167	FSGGMRQRVMIAMALLCRPKLLIADEPTTALDVTVQAQIMTLLNEL

TABLE 3. Similar sequences present at two sites in BtuD and BtuB, the outer membrane receptor[a]

Protein	Position	Similar sequence	Relatedness index[b]	% Identity
BtuB	162	DKTRVTLLGDYAHTHGYDVVAYGNTGTQ	1.39	39
BtuD	100	DKTRTELLNDVAGALALDDKL-GRSTNQ		
BtuB	396	PNLGQLYGFYGNPNLDPE	2.11	50
BtuD	224	PNLAQAYGMN-FRRLDIE		

[a] Identical residues are indicated by colons.
[b] The relatedness index is the average of the log odds for 250 PAMs (probabilities of accepted mutations), as described by Dayhoff et al. (10).

periplasmic-binding-protein-dependent transport systems, PstA, PstC (36), HisM, HisQ (20), MalF (15), MalG, OppC (*Salmonella* sp.) (9), and OppB (*E. coli*; S. Short, personal communication). Aligning these proteins from their amino termini, with no gaps inserted, revealed less than 10% identity between any pair; this is what would be expected from random alignment of the sequences. The pairs of proteins in the same transport system were no more closely related to each other than to any of the other proteins, although short regions of similarity could be found. One of these regions is near the amino terminus. The other was previously described by Dassa and Hofnung (9) as being 80 to 90 residues from the carboxyl terminus and including the consensus sequence EAA---G---------I-LP. This sequence is not present in BtuC (or in its other two possible reading frames), although weakly related sequences are present in this area.

The distribution of potential membrane-spanning regions in BtuC were predicted by using hydropathy (23) or membrane propensity (22) indices (data not shown). The bulk (at least 50%) of BtuC and the other integral membrane proteins could be embedded in the membrane. In the case of BtuC, six or seven potential transmembrane segments are apparent. The other proteins contain four to seven such regions. There was no obvious conservation of the length of the transmembrane segments, their position in the primary sequence, or the distance between them.

BtuD polypeptide. The deduced BtuD polypeptide contains 249 amino acids and has a molecular weight of 27,088, close to that of 29,000 determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It is relatively polar (21.3% charged residues, +13 net charge) and lacks extensive stretches of uncharged amino acids, consistent with a peripheral association with the membrane (12). The mean hydropathy value was −0.13.

Two regions in BtuD exhibit considerable homology to portions of the peripheral membrane components of binding-protein-dependent transport systems (Table 2). Within the conserved regions, BtuD exhibits 38 to 46% identity with HisP, MalK, PstB, and OppD. The maximum relatedness within these regions among those proteins was only 52%, with each of the proteins showing about the same degree of divergence from any other. Most replacements were conservative. Homology of the total polypeptide sequences out to 246 residues, with gaps inserted only to bring the main homology regions into register, revealed 15 to 21% identity of BtuD with the other analogous proteins. A maximum of 26% identity was seen for the comparison of HisP and PstB, and a maximum of 42% identity was seen for OppD from *E. coli* and *Salmonella typhimurium*. The nucleotide sequences encoding the conserved regions were also strongly conserved, exhibiting 72 to 90% identity. These two conserved regions have been suggested to comprise the ATP-binding domain of these proteins, since similar sequences are present in several ATPases (2, 21). The sequences between these two sites are not conserved, although the distances between them are similar.

Two other regions of BtuD might be related to portions of BtuB, the vitamin B_{12} receptor in the outer membrane (Table 3). Comparing BtuB and BtuD, the two regions of 30 and 18 residues displayed 40 and 50% identity, with Dayhoff scores of 1.43 and 2.06, respectively.

BtuE polypeptide. The BtuE polypeptide synthesized in a maxicell system migrates as a major band of M_r 22,000 and a minor band of M_r 20,000. The deduced BtuE protein contains 183 amino acids and has a molecular weight of 20,474, which is slightly lower than that of the product made in maxicells. The net charge was −6, with 24.0% charged residues. The mean hydropathy value was −0.13. Although the protein appears to be located in the periplasmic space, a signal sequence expected for a periplasmic protein was not apparent. It will be necessary to determine the amino-terminal sequence of the BtuE protein to show whether processing of the polypeptide occurs. The low level of expression of these genes requires use of an expression system to obtain sufficient protein for this determination.

Comparison with a library of protein sequences revealed that BtuE exhibited significant homology only with bovine glutathione peroxidase, with 41% identity over a region of 156 amino acids.

Conclusions. The *btuCED* region has been characterized with respect to nucleotide sequence, polypeptide-coding capacity, complementation activity, and effect of its gene amplification on vitamin B_{12} transport. The location of the genes defined by transposon insertions correlated well with the location of the open reading frames. The cellular locations of the *btu* polypeptides, determined in maxicells, showed that these proteins were periplasmic or membrane associated. Sequence or structural relatedness to components of periplasmic-binding-protein-dependent transport systems was apparent. We therefore conclude that the three polypeptides comprise cytoplasmic membrane transport system. This conclusion is consistent with the previous observation of an M_r 22,000 periplasmic vitamin B_{12}-binding protein (6, 37). The capacity of the BtuE polypeptide to bind vitamin B_{12} is under investigation. The genetic studies indicated that BtuE is not essential for transport, although it does seem to be involved in the transport process in an auxiliary manner.

Studies of the energetics of vitamin B_{12} uptake revealed a dependence on both the proton motive force and on phosphate bond energy (7, 31). Binding-protein-dependent transport systems are characterized by their utilization of phosphate bond energy, possibly in the form of ATP (4, 21), and

the transport across the cytoplasmic membrane could account for the requirement for phosphate bond energy. Vitamin B_{12} accumulation in BtuC$^-$ mutants is energy dependent, but the accumulated substrate is readily released from the cell by treatments that do not result in release of the vitamin from wild-type cells. If these mutants are deficient in transport across the cytoplasmic membrane, as indicated by the results presented here, then the substrate must be accumulating in the periplasmic space, suggesting that the receptor is capable of carrying out active transport across the outer membrane. Presumably, this step is dependent on the proton motive force. It will be of interest to see whether the iron chelate uptake systems are of a similar nature.

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LITERATURE CITED

1. **Ames, G. F.-L., and E. N. Spudich.** 1976. Protein-protein interaction in transport: periplasmic histidine-binding protein J interacts with P protein. Proc. Natl. Acad. Sci. USA **73:** 1877–1881.
2. **Amzel, L. M., and P. L. Pedersen.** 1983. Proton ATPases: structure and mechanism. Annu. Rev. Biochem. **52:**801–824.
3. **Bassford, P. J., C. Bradbeer, R. J. Kadner, and C. A. Schnaitman.** 1976. Transport of vitamin B_{12} in *tonB* mutants of *Escherichia coli*. J. Bacteriol. **128:**242–247.
4. **Berger, E. A., and L. A. Heppel.** 1973. Different mechanisms of energy coupling for the shock-sensitive and shock-resistant amino acid permeases of *Escherichia coli*. J. Biol. Chem. **249:**7747–7755.
5. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer-gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80:**3963–3965.
6. **Bradbeer, C., J. S. Kenley, D. R. DiMasi, and M. Leighton.** 1978. Transport of vitamin B_{12} in *Escherichia coli*. Corrinoid specificities of the periplasmic B_{12}-binding protein and of energy-dependent B_{12} transport. J. Biol. Chem. **253:**1347–1352.
7. **Bradbeer, C., and M. L. Woodrow.** 1976. Transport of vitamin B_{12} in *Escherichia coli*: energy dependence. J. Bacteriol. **128:**99–104.
8. **Buchel, D. E., B. Gronenborn, and B. Muller-Hill.** 1980. Sequence of the lactose permease gene. Nature (London) **283:** 541–545.
9. **Dassa, E., and M. Hofnung.** 1985. Sequence of gene *malG* in *E. coli* K12: homologies between integral membrane components from binding protein-dependent transport systems. EMBO J. **4:**2287–2293.
10. **Dayhoff, M. O., R. M. Schwartz, and B. C. Orcutt.** 1978. A model for evolutionary change in proteins, p. 352. *In* Atlas of protein sequence and structure, vol. 5, suppl. 3. National Biomedical Research Foundation, Silver Spring, Md.
11. **DeVeaux, L. C., and R. J. Kadner.** 1985. Transport of vitamin B_{12} in *Escherichia coli*: cloning of the *btuCD* region. J. Bacteriol. **162:**888–896.
12. **DeVeaux, L. C., D. S. Clevenson, C. Bradbeer, and R. J. Kadner.** 1986. Identification of the BtuCED polypeptides and evidence for their role in vitamin B_{12} transport in *Escherichia coli*. J. Bacteriol. **167:**920–927.
13. **DiGirolamo, P. M., and C. Bradbeer.** 1971. Transport of vitamin B_{12} in *Escherichia coli*. J. Bacteriol. **106:**745–750.
14. **Fecker, L., and V. Braun.** 1983. Cloning and expression of the *fhu* genes involved in iron(III)-hydroxamate uptake by *Escherichia coli*. J. Bacteriol. **156:**1301–1314.
15. **Froshauer, S., and J. Beckwith.** 1984. The nucleotide sequence of the gene for *malF* protein, an inner membrane component of the maltose transport system of *Escherichia coli*. J. Biol. Chem. **259:**10896–10903.
16. **Gilson, E., H. Nikaido, and M. Hofnung.** 1982. Sequence of the *malK* gene in *E. coli* K12. Nucleic Acids Res. **10:**7449–7458.
17. **Grosjean, H., and W. Fiers.** 1982. Preferential codon usage in prokaryotic genes: the optimal codon-anticodon interaction energy and the selective codon usage in efficiently expressed genes. Gene **18:**199–209.
18. **Heller, K., and R. J. Kadner.** 1985. Nucleotide sequence of the gene for the vitamin B_{12} receptor protein in the outer membrane of *Escherichia coli*. J. Bacteriol. **161:**904–908.
19. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28:** 351–359.
20. **Higgins, C. F., P. D. Haag, K. Nikaido, F. Ardeshir, G. Garcia, and G. Ferro-Luzzi Ames.** 1982. Complete nucleotide sequence and identification of membrane components of the histidine transport operon of *S. typhimurium*. Nature (London) **298:** 723–727.
21. **Higgins, C. F., I. D. Hiles, K. Whalley, and D. J. Jamieson.** 1985. Nucleotide binding by membrane components of bacterial periplasmic binding protein-dependent transport systems. EMBO J. **4:**1033–1040.
22. **Kuhn, L. A., and J. S. Leigh, Jr.** 1985. A statistical technique for predicting membrane protein structure. Biochim. Biophys. Acta **828:**351–361.
23. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157:**105–132.
24. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227:**1435–1441.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Mechulam, Y., G. Fayat, and S. Blanquet.** 1985. Sequence of the *Escherichia coli pheST* operon and identification of the *himA* gene. J. Bacteriol. **163:**787–791.
27. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene **19:**269–276.
28. **Miller, H. I.** 1984. Primary structure of the *himA* gene of *Escherichia coli*: homology with DNA-binding protein HU and association with the phenylalanyl-tRNA synthetase operon. Cold Spring Harbor Symp. Quant. Biol. **49:**691–698.
29. **Neilands, J. B.** 1982. Microbial envelope proteins related to iron. Annu. Rev. Microbiol. **36:**285–309.
30. **Pierce, J. R., C. L. Pickett, and C. F. Earhart.** 1983. Two *fep* genes are required for ferrienterochelin uptake in *Escherichia coli*. J. Bacteriol. **155:**330–336.
31. **Reynolds, P. R., G. P. Mottur, and C. Bradbeer.** 1980. Transport of vitamin B_{12} in *Escherichia coli*. Some observations on the role of the gene products of *BtuC* and *TonB*. J. Biol. Chem. **255:**4313–4319.
32. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13:**319–353.
33. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
34. **Shine, J., and L. Dalgarno.** 1974. The 3′ terminal sequences of *Escherichia coli* 16S rRNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71:**1342–1346.
35. **Staden, R.** 1980. A new computer method for the storage and manipulation of DNA gel reading data. Nucleic Acids. Res. **8:**3673–3694.

36. **Surin, B. P., H. Rosenberg, and G. B. Cox.** 1985. Phosphate-specific transport system of *Escherichia coli*: nucleotide sequence and gene-polypeptide relationships. J. Bacteriol. **161:** 189–198.
37. **Taylor, R. T., S. A. Norrell, and M. L. Hanna.** 1972. Uptake of cyanocobalamin by *Escherichia coli* B: some characteristics and evidence for a binding protein. Arch. Biochem. Biophys. **148:**366–381.
38. **White, J. C., P. M. DiGirolamo, M. L. Fu, Y. A. Preston, and C. Bradbeer.** 1973. Transport of vitamin B_{12} in *Escherichia coli*: isolation and properties of the initial B_{12}-binding site. J. Biol. Chem. **248:**3978–3986.