The structure and function of the polyamine transport protein PotE was studied. Uptake of putrescine by PotE was dependent on the membrane potential. In contrast, the putrescine-ornithine antiporter activity of PotE studied with inside-out membrane vesicles was not dependent on the membrane potential (Kashiwagi, K., Miyamoto, S., Suzuki, F., Kobayashi, H., and Igarashi, K. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4529–4533). The $K_m$ values for putrescine uptake and for putrescine-ornithine antiporter activity were 1.8 and 73 $\mu$M, respectively. Uptake of putrescine was inhibited by high concentrations of ornithine. This effect of ornithine appears to be due to putrescine-ornithine antiporter activity because it occurs only after accumulation of putrescine within cells and because ornithine causes excretion of putrescine. Thus, PotE can function not only as a putrescine-ornithine antiporter to excrete putrescine, but also as a putrescine uptake protein.

Both the NH$_2$ and COOH termini of PotE were located in the cytoplasm, as determined by the activation of alkaline phosphatase and $\beta$-galactosidase by various PotE-fusion proteins. The activities of putrescine uptake and excretion were studied using mutated PotE proteins. It was found that glutamic acid 207 was essential for both the uptake and excretion of putrescine by the PotE protein and that glutamic acids 77 and 433 were also involved in both activities. These three glutamic acids are located on the cytoplasmic side of PotE, and the function of these three residues could not be replaced by other amino acids. Putrescine transport activities did not change significantly with mutations at the other 13 glutamic acid or aspartic acid residues in PotE.

Polyamines, aliphatic cations present in all living organisms, are known to be necessary for normal cell growth (1, 2). However, accumulation of excess polyamines causes inhibition of cell growth or a decrease in cell viability through inhibition of macromolecules, especially through inhibition of protein synthesis (3, 4). Furthermore, it has been reported that bis(ethyl)polyamine analogues cause inhibition of cell growth (5–8). The analogues could substitute for the functions of polyamines in various aspects, including the stimulation of protein synthesis at low concentrations and the inhibition of protein synthesis at high concentrations of analogues (9). These analogues accumulate in cells because they cannot be metabolized by spermidine/spermine $N^2$-acyltransferase and polyamine oxidase. Thus, metabolism and excretion of excess polyamines is necessary for cell growth. Indeed, excess polyamines induce spermidine/spermine $N^1$-acyltransferase (3, 10), and cells excrete any excess amount of polyamines (11, 12).

We obtained and characterized three clones of polyamine transport genes (pPT104, pPT77s, and pPT71) in *Escherichia coli* (13). The putrescine transport system encoded by pPT71 consists of one membrane protein (PotE) with 12 putative transmembrane segments (14) and is active in the excretion of putrescine through putrescine-ornithine antiporter activity (15). Because the PotE protein was first identified as the putrescine uptake-deficient protein (13), we started with the detailed mechanism of putrescine uptake and excretion by the PotE protein. We have also carried out site-directed mutagenesis studies to identify amino acid residues that contribute to the transport functions of the PotE protein. The results show that glutamic acids 207, 77, and 433, which are located at the cytoplasmic side of PotE, are involved in both the uptake and excretion of putrescine.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, and Culture Conditions**—A polyamine-requiring mutant, *E. coli* MA261 (16), provided by Dr. W. K. Maas, New York University School of Medicine, and its polyamine uptake-deficient mutant KK313 potF::Km (17) were grown in medium A in the absence of polyamines as described previously (18). A proton-translocating ATPase mutant, KK313 potF::Km attP, was derived from *E. coli* KK313 potF::Km by transduction of a P1 phage-infected lysate of *E. coli* DE8 (ΔatpB-atpC(C) int::Tn10, Ref. 19) and was grown in N$^{-}$C$^{-}$ medium (20) to deplete ATP. *E. coli* JM105 (21), TG1 (21), DH5α (21), and CC118 (22) were cultured in a 19-amino acid supplemented medium (11) containing 1% glycerol, 2YT, LB, and LB medium (21), respectively. Plasmids pPT71 containing SpeI and potF genes and pPT71.3 containing potE gene were prepared as described previously (14). pUCpotE was prepared by inserting the 1.6-kb $\beta$-lactamase fragment of pPT71 into the same restriction site of pUC119 (TakaRa Biomedicals, Japan). Then, pMWpotE was prepared by inserting the 1.6-kb SphI-BamHI fragment of pUCpotE into the same restriction site of pMW119 (Nippon Gene, Japan). The strains and plasmids used are listed in Table I.

**Mutagenesis of potE Gene**—To prepare potE mutants, the 1.6-kb PstI-BamHI fragment of pPT71 was inserted into the same site of pUC19 (23). Site-directed mutagenesis was carried out by the method of Sayers et al. (24) with a Sculptor$^{TM}$ in vitro mutagenesis system (Amersham Corp.), using the oligonucleotides shown in Table II. The mutated 1.6-kb SphI-BamHI fragments were isolated from the replicative form of M13 and religated into the same site of pMW119. Mutations were confirmed by DNA sequencing (25) using the M13 phage system (23) with synthesized primers.

**Construction of potE-phoA Fusion Plasmids and Assay for Alkaline Phosphatase (PhoA)—**Plasmid pAP2022 carrying phoA with a single amino acid substitution at the cleavage site of the signal sequence (arginine (CGG) at +1 to glycine (GGC)) was supplied by Dr. H. Tokuda.

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The three products (a, b, and c) were digested with NheI and BamHI, E. coli strains and plasmids used

| Strain or plasmid | Relevant characteristics | Comments, source, or Refs. |
|-------------------|--------------------------|---------------------------|
| MA261             | speB speC thr ieu ser thi | Polyamine requiring mutant, Cunningham-Rundles and Maas (16) |
| MA261 potA        | speB speC thr ieu ser thi | Polyamine requiring mutant, Cunningham-Rundles and Maas (16) |
| potF::Km          | speB speC thr ieu ser thi | Polyamine requiring mutant, Cunningham-Rundles and Maas (16) |
| DeltaB-aptC        | speB speC thr ieu ser thi | Polyamine requiring mutant, Cunningham-Rundles and Maas (16) |

**TABLE I**

E. coli strains and plasmids used

| Oligonucleotides used in this study |
|-----------------------------------|
| Mutant | Primer sequence |
|--------|-----------------|
| E36Q   | TGC CAC ACT TGG GCT AGC TTT GTC G |
| E36A   | TGG TCC CGA CGG CGG CGA TTA CCG C |
| E77Q   | AAA GCA TAC TGG GCA TAA CCG C |
| E77A   | CGA AAG CAT ATG CGG CAT ACC C |
| E77D   | CGG AAA TAG TGG GCA TAA CCC |
| E77N   | ACC GAA AGC ATA GTG GGT GAT ACC GCC |
| E11A3  | GGC GAG CAG TGG GGT GTA |
| D179N  | TTC CAG GAA TTC AGC TAC G |
| D179A  | ATT GCA GAA AAG GTA CAG |
| E207Q  | CAC GCA GAC TGG ACA ACA G |
| E207A  | ACA CGG AGC CGG CAC AAG |
| E207D  | GCA CAC GAG TGG ACA AGA CAG |
| E207N  | GCA CAC GAA TCC AGA CCA AGA A |
| D210N  | TTC ACC ATA GTA TTA GCA |
| D210C  | TTC CAC TAC AGC TAT GTC ACC AC G |
| E217Q  | GTC CGG GTG TCT CCA GTA CAT CA |
| E217A  | TTC CGG GTG TCT CAC ATC CAC |
| E220Q  | GCA CAT TAC GCT GGG GTT CCC |
| E220A  | ACA ATT AGC GGG GTT TCT |
| E253Q  | GTC ACC TGC ACT TGG |
| E253A  | GTC TGG CAC ATT GTC |
| E253J  | CTT CCA CTC GAG TGG AGA C |
| E272Q  | CTT TAC CCA CTT GAG TGG GTA |
| E272A  | CTT AAC CTC GGG CGG GAA |
| D305N  | TAG CCT TCA TGG GAG GAA CAT TAA AA |
| D305A  | AGT AGC CTT CAG AGT AAG A |
| E306Q  | AAG TAG CCT TCA TCA GAT GAA |
| E306A  | GAA GTA GCC TGC ACT TGA AGA |
| D320N  | ACC GTG GCA TCT ATT GTG |
| D320A  | CCG TGC ACC GGC GGG GTT ACT TGG GT |
| F408Q  | ATG GTC TCT GGG CAT GAT G |
| F408A  | AGC ATG GGC TGG CGG CGG GAT GAG TA |
| F409Q  | CAG CAT GGC TGC TCC GGA GTA TGA |
| F433Q  | TAT TAC TGA GCT GAG GTC GT |
| F433A  | ATT TTT CAG TGC AAA CCG C |
| F433D  | TTA ATT TTA TAG TGC AAG CAG GGT |
| F433N  | TTT ATT TTT CAG TGT AAA CCG TGG TGA |

**TABLE II**

The mutated nucleotides are underlined.

| Mutant | Primer sequence |
|--------|-----------------|
| F433N  | TTT ATT TTT CAG TGT AAA CCG TGG TGA |

University of Tokyo. Replacement of Arg-1 by Gly-1 of PhoA protein did not change the activity (26). The phoA gene was amplified from pPA2022 by PCR (polymerase chain reaction) using the following primers: (a) potE522-phoA, 5'-TGGCGCTAGCGCCACAGAAGA-3' and 3'-TTGGTCTGTAATTCGCCAGTTCCG-5' (PC1), (b) potE529-phoA, 5'-TCAACCGCGCACAGGCAAGAAT-3' and 5'-CCAAGCATGCCGCACCAGAAATACAGA-3' and PC1, and (c) potE534-phoA, 5'-CTCGCTGTTTATCAAATTGCTGAAA-3' and PC1. These three products (a, b, and c) were digested with NheI and BamHI, EagI and BamHI, and Csp451 and BamHI, respectively. Then, the potE-phoA fusion plasmids that encode the fusion proteins differing in the number of amino acids in PotE protein (PotE525-PhoA, PotE529-PhoA, and PotE304-PhoA) were prepared by inserting the above fragments into the same restriction sites of pMWpote E253A, pMWpote E290A, and pMWpote D305N (Table II). Other potE-phoA fusion plasmids (pMWpote99-phoA, pMWpote34-phoA, pMWpote77-phoA, pMWpote115-phoA, pMWpote147-phoA, pMWpote181-phoA, pMWpote216-phoA, pMWpote348-phoA, pMWpoteE434-phoA) were made by the following methods. The truncated potE genes in the different sizes with NheI cutting site in the 3'-end were prepared by PCR, using pMWpote as template, and PotI-NheI fragments were isolated. Then the fragment was ligated together with the 3.1- and 3.1-kb fragments obtained by PotI-NheI digestion of pMWpote252-phoA.

Assay for PhoA activity of E. coli CC118 carrying the fusion plasmids was performed using 0.1 ml of cell suspension and 0.04% 2-nitrophenyl phosphate by the method of Michaelis et al. (27). The PhoA activities were calculated as follows: unit activity = 1000 × (A_{405} - 1.75 × A_{350})/(time (min) × A_{500} × cell suspension volume (ml)).

Construction of potE-lacZ Fusion Plasmids and Assay for β-Galactosidase—Plasmid pMC1871 carrying lacZ gene was obtained from Pharmacia Biotech Inc. The lacZ gene was amplified from pMC1871 by PCR using the following primers: (a) potE522-lacZ, 5'-TGGCGCTAGCGCCACAGAAGA-3' and 3'-ATTATTTGGCGCCGCTCCTCCATCAAGC-5' (GC1), (b) potE529-lacZ, 5'-TCAACCGCGCACAGGCAAGAAT-3' and GC1, and (c) potE304-lacZ, 5'-CTTCGCTTGTTTACAAATTGCTGAAA-3' and GC1. These three products (a, b, and c) were digested with NheI and BamHI, EagI and BamHI, and Csp451 and BamHI, respectively. Then the potE-lacZ fusion plasmids that encode the fusion protein differing in the number of amino acids in PotE protein (PotE525-β-Gal, PotE529-β-Gal, and PotE304-β-Gal) were prepared by inserting the above fragments into the same restriction sites of pMWpote E253A, pMWpote E290A, and pMWpote D305N (Table II). Other potE-phoA fusion plasmids (pMWpote68-lacZ, pMWpote77-lacZ, pMWpoteE147-lacZ, pMWpoteE181-lacZ, and pMWpoteE216-lacZ) were made by the following methods. The truncated potE genes in the different sizes with Csp451 cutting site in the 3'-end were prepared by PCR, using pMWpote as template, and SplI-Csp451 fragments were isolated. Then the fragment was ligated with the 7.4-kb fragment obtained by SplI-Csp451 digestion of pMWpote 340-lacZ. Assay of β-galactosidase of E. coli DH5a carrying the fusion plasmids was performed by the method of Miller (28).

Putrescine Uptake by Intact Cells—E. coli KK313 potF::Kan pMWpote cells grown in medium A were suspended in buffer 1 containing 0.4% glucose, 62 mM potassium phosphate, pH 7.0, 1.7 mM sodium citrate, 7.6 mM (NH₄)₂SO₄, and 0.41 mM MgSO₄ to yield a protein concentration of 0.1 mg/ml. The cell suspension (0.48 ml) was preincubated at 30 °C for 5 min, and the reaction was started with the addition of 20 μl of 0.25 mM [¹⁴C]putrescine (370 MBq/mmol). After
incubation at 30°C for 30 s to 6 min, the cells were collected on membrane filters (cellulose acetate, 0.45 μm; Advantec Toyo), and the filters were washed three times with a total of 12 ml of buffer 1. The radioactivity on the filters was assayed with a liquid scintillation spectrometer.

**Putrescine Uptake by Inside-out Membrane Vesicles—**E. coli JM105/pMWpotE cells were cultured in the presence of 0.5 mM isopropyl-β-D-thiogalactopyranoside. Inside-out membrane vesicles were prepared by French press treatment of the cells suspended in 100 mM potassium phosphate buffer, pH 6.6, 10 mM EDTA containing 2.5 mM ornithine (15). The reaction mixture (0.1 ml) for the uptake by inside-out membrane vesicles contained 10 mM Tris-HCl, pH 8.0, 10 mM potassium phosphate buffer, pH 8.0, 0.14 M KCl, 50 μM [3H]putrescine (1.48 GBq/mmol), and 100 μg of inside-out membrane vesicle protein. The reaction mixture was incubated at 22°C for 5 min without the substrate, and the reaction was started by the addition of the substrate. After incubation at 22°C for 20 s to 1 min, membrane vesicles were collected on membrane filters (cellulose nitrate, 0.45 μm; Advantec Toyo) and washed, and their radioactivities were measured with a liquid scintillation spectrometer (15).

**Measurement of Polyamine and Protein Contents—**The polyamine content in E. coli was determined by high performance liquid chromatography as described previously (29). Protein content was determined by the method of Lowry et al. (30).

**Western Blot Analysis of PotE Protein—**Rabbit antibody for the PotE protein was prepared according to the method of Ponett et al. (31) using the multiple antigenic peptide, SDEGFYPKIFSRTVK, which corresponds to amino acids 304–318 of the PotE protein (14). For Western blot analysis of the PotE protein, inside-out membrane vesicles (20 μg protein) were separated by SDS-polyacrylamide gel electrophoresis on a 12% acrylamide gel and transferred to Immobilon transfer membranes (Milli-pore). The PotE protein was detected with ProteinBlot Western blot AP systems (Promega), except that 0.2% Triton X-100 was used instead of 0.05% Tween 20 (32).

**Measurement of ATP Content, Δφ, and ΔpH—**Assay of ATP was performed by the luciferase enzyme system (33). ATP was extracted with 0.2 M HClO4 and measured after neutralization with 1 M KOH containing 50 mM K2HPO4. Δφ and ΔpH were measured in parallel experiments by determining the relative distribution of [3H]tetraphenylphosphonium bromide and [7-14C]benzoic acid, respectively, across the membrane according to the method of Joshi et al. (34). Correction for the nonspecific binding of tetraphenylphosphonium bromide or benzoic acid was made by treating a sample with 10 μM carbonyl cyanide m-chlorophenylhydrazone and subtracting this value from that of the experimental samples. The Nernst equation and the Henderson-Hasselbalch equations were used to calculate Δφ (mV, negative inside) and ΔpH (mV, alkaline inside) (35).

**RESULTS**

**Putrescine Transport by PotE Protein—**The properties of putrescine uptake by the PotE protein were compared with those of putrescine excretion by the putrescine-ornithine antiporter activity of the PotE protein. We previously reported that energy is not required for the excretion of putrescine (15). The energy requirement of putrescine uptake by the PotE protein was examined using the mutant E. coli KK313 potF::Km atp/pMWpotE, transformed with pMWpotE. In this mutant, the proton-translocating ATPase (19) and spermidine and putrescine uptake systems encoded by potFGH and atp (17) are lacking. Thus, putrescine uptake of the cells was catalyzed by the PotE protein, and the cells were energized by the addition of glucose or succinate. As shown in Fig. 1, putrescine uptake was dependent on the membrane potential, because the uptake activity and the membrane potential were similarly affected by the addition of glucose and succinate. This contrasts with the effect of glucose and succinate on the ATP content of cells because glucose increases ATP levels by 10-fold, whereas succinate slightly increases ATP content (37). The membrane potential dependence was also confirmed by the inhibition of putrescine uptake by carbonyl cyanide m-chlorophenylhydrazone, an inhibitor of proton circulation (Fig. 1).

Ornithine uptake by PotE protein was not observed when a low concentration of ornithine (10 μM) was used as substrate (data not shown). However, uptake of putrescine was inhibited by high concentrations of ornithine (100–250 μM) after accumulation of putrescine within cells. The Km value for the putrescine uptake was 1.8 μM, whereas the Km values for antiporter activities of putrescine and ornithine were 73 and 108 μM, respectively, when measured in inside-out membrane vesicles that contained 2.5 mM ornithine or putrescine. Thus,
inhibition by ornithine was probably based on the putrescine-ornithine antiporter activity.

Although the uptake of putrescine was inhibited by NEM (N-ethylmaleimide) (Fig. 1), the putrescine-ornithine antiporter activity was not inhibited by NEM. The results suggest that NEM affects the membrane potential but does not directly alter the function of the PotE protein. The optimal pH of putrescine uptake by intact cells was 6.5 and that of the antiporter activity by inside-out membrane vesicles was 9.2. The results suggest that the excretion of putrescine is not so effective when the difference between intracellular and extracellular pH is small. It may be that protons enter the cell together with ornithine when the PotE protein functions as an antiporter.

Next, we examined whether putrescine uptake by the PotE protein can stimulate cell growth of the polyamine-requiring mutant KK313 potF::Km by culturing the mutant, and the mutant expressing PotE after transformation with pPT71.3, in the absence and presence of 5 μM putrescine. As shown in Table III, the PotE protein caused the accumulation of polyamines (putrescine and spermidine) in cells and the stimulation of cell growth (1.94-fold), determined from the decrease in the generation time.

Topology of PotE Protein—It has been reported that fusions of the secreted protein alkaline phosphatase (PhoA) to an integral cytoplasmic membrane protein of E. coli show different activities depending on where the PhoA is fused to the membrane protein. Fusions to positions in or near the periplasmic domain led to high PhoA activity, whereas those to positions in the cytoplasmic domain gave low activity (22). As shown in Table IV, PotE34-, PotE115-, PotE181-, PotE252-, PotE259-, PotE348-, and PotE410-PhoA fusion proteins showed high PhoA activity, whereas PotE9-, PotE77-, PotE147-, PotE216-, PotE304-, PotE380-, and PotE434-PhoA proteins gave low activity. In contrast, β-galactosidase fusion proteins lose activity if the cells attempt to export them (38). Although PotE8-, PotE78-, PotE147-, PotE216-, and PotE304-β-GAL fusion proteins showed high activity, PotE182-, PotE252-, and PotE259-β-GAL proteins gave low activity. The results indicate that both the NH₂ and COOH termini are located in the cytoplasm (see Fig. 5) and that PotE has the same topology as other membrane proteins having 12 transmembrane segments such as lactose permease (39), melibiose permease (40), and the metal-tetracycline/H⁺ antiporter (41).

Identification of Amino Acids Involved in the Transport Activity—There are 12 glutamic acids and 4 aspartic acids in the PotE protein. In the PotD protein, which is a substrate binding protein of the spermidine-preferential uptake system encoded by the potABCD operon, four acidic amino acids are involved in binding of spermidine (42, 43). To identify the amino acids involved in the transport activity of PotE, we prepared mutated PotE proteins in which acidic amino acids were replaced by neutral amino acids using site-directed mutagenesis. Putrescine uptake activity was decreased greatly with mutated PotE proteins E207Q and E207A. Residue Glu-207 is

3 The mutated PotE protein E207Q contains glutamine instead of glutamic acid at position 207.
located in the hydrophilic region between transmembrane segments VI and VII. The uptake activity also decreased significantly with mutated PotE proteins E77Q, E77A, E433Q, and E433A. Residues Glu-77 and Glu-433 are located in the hydrophilic region between transmembrane segments II and III and in the COOH-terminal, respectively (see Fig. 5). We constructed a double mutated PotE protein (E77Q and E433Q) and examined its putrescine uptake activity. The decreased activity was additive in the double mutant (data not shown). Putrescine uptake activity did not change significantly with other mutated PotE proteins (Fig. 2). The amount of mutated PotE on membranes measured by Western blot analysis was almost the same as that of normal PotE protein (data not shown). Thus, mutations at Glu-77, Glu-207, and Glu-433 affect the activity of PotE and not the expression of the protein in membranes. The amount of PotE protein in E. coli cells transformed with the vector pMW119 was negligible, suggesting that the genomic speF-potE operon located at 16 min on E. coli chromosome (14) is not expressed efficiently at neutral extracellular pH.

Putrescine excretion (putrescine-ornithine antiporter activity) was measured using inside-out membrane vesicles (Fig. 3). Excretion decreased greatly with mutated PotE E207Q, and significantly with mutated PotE E77Q and E433Q, similar to effects of these mutants on putrescine uptake (Fig. 2). Excretion did not change significantly with other mutated PotE proteins (Fig. 3).

To confirm the importance of glutamic acids Glu-207, Glu-77, and Glu-433, each was replaced by several amino acids (A, D, N, and Q). As shown in Fig. 4, even aspartic acid could not substitute for glutamic acid at these positions. A secondary structure model of PotE protein is shown in Fig. 5. The three critical glutamic acid residues on the PotE protein are located on the cytoplasmic side.

**DISCUSSION**

It has been reported that there are three basic amino acid/decarboxylated derivative antiporters on prokaryotic membranes, they are the ornithine-puerticine (15), lysine-cadaverine (44), and histidine-histamine (45) antiporters. These proteins play important roles in the generation of a proton motive force (45), neutralization of low extracellular pH (44), and supply of carbon dioxide (46). In this study, we clarified that the ornithine-putrescine antiporter also functions as a putrescine uptake system that is dependent on the membrane potential.

It is of interest to know how the uptake and excretion of putrescine are regulated by the PotE protein. It is already known that there are two ATP-dependent putrescine uptake systems: the spermidine-preferential and putrescine-specific uptake systems (13, 17, 47). Furthermore, the expression of speF-potE operon is weak at neutral extracellular pH (Fig. 2 and Ref. 48). Thus, the contribution of the PotE protein to putrescine uptake is small under standard culture conditions. On the other hand, the PotE protein is the only protein known to be involved in the excretion of putrescine. The protein functions as an antiporter for ornithine (or lysine)-putrescine and putrescine-putrescine (15). Thus, PotE has broad substrate specificity from the outside. When putrescine had accumulated in cells, putrescine was actually excreted from the cells by the PotE protein to maintain the optimal amount of polyamines (15). It remains to be clarified, however, why the $K_{m}$ values for uptake of putrescine ($1.8 \mu M$) and excretion of putrescine ($73 \mu M$) are so different.

Our results clearly show that Glu-207, -77, and -433, which are located at the cytoplasmic side of PotE, are involved in both the uptake and excretion of putrescine. The functions of Glu-207, -77, and -433 in the PotE protein could not be replaced by any of four other amino acids, including aspartic acid. These residues may contribute directly to a binding site for putrescine and/or ornithine on PotE. Because the amino acid sequence of a putative PotE protein in Hemophilus influenzae has been reported recently (49), the sequence homology between the two PotE proteins was compared. As shown in Fig. 5, glutamic acids 207 and 77 and their surrounding amino acid sequences were conserved between two proteins (bold letters, Fig. 5). However, glutamic acid 433 was replaced by aspartic acid, and the surrounding amino acid sequence was different, in the PotE protein of H. influenzae (outline letters, Fig. 5). Thus, putrescine may recognize these acidic amino acids and their surrounding amino acids in each PotE protein. It has also been shown that main functional amino acids of the lactose/H$^{+}$ symporter (50, 51) and metal tetracycline/H$^{+}$ antiporter (52) are located on the cytoplasmic side of the proteins.

The amino acid sequence homology between the PotE protein from E. coli and that from H. influenzae (49) was 77%. The homology was more strongly observed in the NH$_{2}$-terminal region (especially the hydrophilic regions) than in the COOH-terminal region (Fig. 5). As for the transmembrane segments, the segments I and VII were identical, and segments II, III,
VIII, and XII were well conserved between *E. coli* and *H. influenzae*. According to the model of the lactose/H\(^{+}\) symporter (53), six transmembrane segments among the 12 segments may form the transport passage. If this is applicable to the PotE protein, segments I, II, III, VII, VIII, and XII may form the transport passage for putrescine and ornithine.

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