*Acta Medica et Biologica*
*Vol. 48, No.1, 11-18, 2000*

# The Enterohemorrhagic *Escherichia coli* (EHEC)-Hemolysin Genes of a Shiga Toxin 1 (Stx1)- and Stx2-Producing, Serotype O128 *Escherichia coli* Strain with a Greatest Hemolytic Activity

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*Received February 7 2000; accepted March 21 2000*

**Summary. Most strains of enterohemorrhagic *Escherichia coli* (EHEC) belonging to serotypes O157, O26, O111, and O128, isolated from humans in Japan also produced EHEC-hemolysin (EHEC-hly), the hemolytic activity of whom was detected on blood agar containing washed but not unwashed sheep erythrocytes. Of those, Shiga toxin 1 (Stx1)- and Stx2-producing strains belonging to serotypes O128:H2 and O128:H12 produced EHEC-hly in much greater quantities than did the other strains. The two strains carried the EHEC-hly genes on the 120-kbp plasmid. The entire EHEC-hly region (carrying genes C, A, B, and D) of the serotype O128:H12 strain was cloned into pACYC184 and sequenced. The EHEC O128-hly genes showed a close similarity to the the EHEC O157-hly genes of more than 98.3% at the nucleotide level (more than 97.7% at the amino acid level). However, the EHEC O128-hlyB (the ATP-binding transmembrane protein acting as a transporter for hemolysin [hlyA]) lacked one amino terminal methionine, in contrast to the repeated amino terminal methionines in the EHEC O157-hlyB. The O128- and O157-hlyAs shared similar RTX repeats and acylation sites.**

**Key words**—enterohemorrhagic *Escherichia coli* (EHEC), serotype O128, EHEC-hemolysin, ABC-transporter.

## INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) (alternatively called Shiga toxin (Stx)-producing *E. coli*, STEC) of serotype O157:H7 was identified as an important food-borne bacterial pathogen in the United States in 1982$^{1)}$. EHEC belonging to serotype O157:H7 is associated with hemolytic uremic syndrome (HUS) and is now recognized as one of the important causes of emerging infectious diseases$^{2,3)}$. EHEC includes not only serotype O157:H7 (the most prevalent serotype), but other serotypes including O26, O111, O145, and O128 have also been implicated in human infections$^{2)}$. In Japan, during 1996, large outbreaks of EHEC (O157:H7) infections occurred with more than 17,877 people infected (mostly primary school children), more than 1,795 patients hospitalized, and 12 fatalities$^{4)}$.

EHEC belonging to serotype O157:H7 carries a ca. 90-kbp plasmid (pO157) that encodes for hemolysin$^{5-8)}$. This hemolysin, called EHEC-hemolysin (EHEC-hly) or enterohemolysin, has a unique hemolytic manner in that hemolysis is observed on blood agar containing washed but not unwashed sheep erythrocytes$^{9)}$, in sharp contrast to $\alpha$-hemolysin, which is detected even on blood agar containing unwashed sheep erythrocytes. EHEC-hly and $\alpha$-hemolysin are genetically and immunologically distinct hemolysins. $\alpha$-hemolysin is produced from approximately 50% of *E. coli* isolates from extraintestinal infections$^{10)}$ or from porcine STEC strains$^{9)}$. EHEC-hly appears to have clinical importance, because it occurs in most EHEC isolates belonging to serotype O157:H7$^{9)}$ and is

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reactive to sera of patients with HUS[5].

The entire nucleotide sequence of the EHEC-hly operon of EHEC strains belonging to serotype O157:H7 has been determined; it consisted of the *hlyC*, *hlyA*, *hlyB*, and *hlyD* genes[6–8], similar to the $\alpha$-hemolysin operon. However, the hemolysin transport system does not work well in EHEC belonging to serotype O157:H7[5], and the effective secretion of EHEC-hly requires the addition of the hlyB/hlyD/tolC-based secretion system of $\alpha$-hemolysin[5]. In this study, we investigated the EHEC-hly genes of EHEC strains belonging to serotype O128:H2 and O128:H12, which produced EHEC-hly to much greater extents than did EHEC strains belonging to serotype O157:H7.

## MATERIALS AND METHODS

### Bacterial strains

Stx1- and Stx2-producing EHEC strain K17 (serotype O128:H2), Stx1- and Stx2-producing EHEC strain F60 (serotype O128:H12), and other EHEC strains examined in this study were isolated on occasions of outbreaks of EHEC infections in 1991 and 1996 in Japan, respectively. *E. coli* HB101 is a hybrid between *E. coli* K-12 and *E. coli* B and lacks restriction ability. *E. coli* 20S0 (K12 derivative strain) carrying the pTH10 plasmid was constructed previously[11]; pTH 10 is a self-transmissible plasmid with temperature-sensitive replication and codes for resistance to ampicillin (as a result of the ampicillin resistance transposon Tn*1*), tetracycline, and kanamycin.

### Media and bacterial growth

For bacterial growth, we used L broth[12] as a liquid medium. Incubations were performed for 18 to 20 h at 37°C with agitation (unless otherwise noted). MacConkey agar (Eiken Chemical, Tokyo) and tryptic soy agar (Difco Laboratories, Detroit, Mich. USA) were used as solid media.

### Hemolysis assay

Bacteria were streaked on tryptic soy agar supplemented with 5% fresh, washed, defibrinated sheep erythrocytes and 10 mM $CaCl_2$[9]. The inoculated plates were then incubated for 3 to 24 h at 37°C. Hemolysis occurred under and around the streaked bacterial colonies, and was macroscopically examined[9].

### Plasmid analysis and transformation with plasmid DNA

Plasmid analysis was carried out essentially by a published method[13] as described previously[11]. Plasmid DNA was analyzed by electrophoresis in 0.3, 0.5, or 0.7% agarose gel with reference plasmid DNAs of known molecular size (including the NR1 plasmid of 94.5 kbp[14]). DNA was then stained with ethidium bromide. Transformation of *E. coli* HB101 with plasmid DNA was done as described previously[11].

### Tn*1* labeling of plasmids

For labeling of plasmids with Tn*1*, EHEC strains were mixed with *E. coli* 20S0 carrying pTH10 (Tn*1* donor), EHEC strains carrying pTH10 were selected, and then EHEC strains carrying Tn*1*-labeled plasmids were chosen, essentially as described previously[11].

### PCR assay

For the detection of the EHEC-hlyA gene (EHEC-*hlyA*), PCR analysis was carried out as described previously[15] with a primer set of hlyA1 (5'-GGTGCAGCAGAAGAAAAAGTTGTAG) and hlyA4 (5'-TCTCGCCTGATAGTGTTTGGTA), generating a 1,551-bp product[5]. Amplified PCR products were analyzed by gel electrophoresis with 1 or 2% agarose and stained with ethidium bromide. *Hin*dIII digests of $\lambda$ DNA (Boehringer Mannheim Biochemicals, Indianapolis, Ind. USA) were used as molecular size standards.

### DNA cloning and sequencing

Cloning of the EHEC-hly genes into pACYC184 (specifying resistance to chloramphenicol and tetracycline) was done as described previously[16]. DNA size was determined using 8–48 kb DNA size standard (Bio-Rad Laboratories, Hercules, CA) and 1 kb DNA ladder (Life Technologies, Gaithersburg, MD) as molecular size standards. DNA sequences were determined using the *Taq* dyedeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and a 373A DNA sequencer (Applied Biosystems) according to the manual.

## RESULTS

### Hemolytic phenotypes of EHEC strains

When examinations using blood agar plates contain-

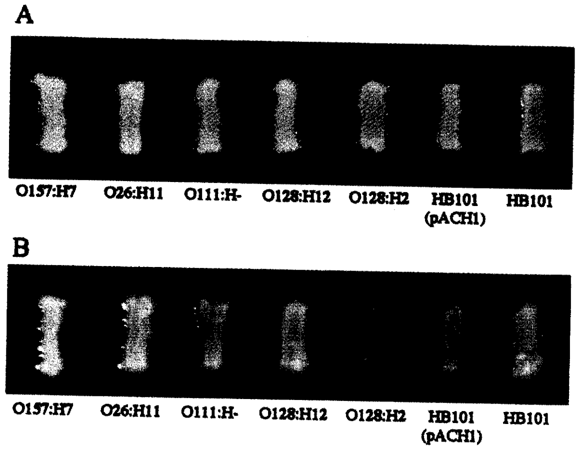


**Fig. 1.** Hemolytic phenotypes of EHEC strains belonging to various serotypes and *E. coli* HB101 carrying pACH1. Streaked blood agar plates containing washed sheep erythrocytes (**A**) or unwashed sheep erythrocytes (**B**) were incubated for 24 h at 37°C. Data shown are representative of the greatest hemolytic activity in each serotype. Bacterial strains belonging to serotype O128:H12 and O128:H2 were F60 and K17, respectively. pACH1 is a pACYC184 derivative carrying the EHEC-hly genes of strain F60 (text).

ing washed sheep erythrocytes were performed, 145 of the 148 strains (98.0%) belonging to serotype O157:H7, all of the 6 strains belonging to serotype O26, the 2 strains belonging to serotype O111, and the 2 strains belonging to serotype O128 were positive for hemolysis. The hemolytic phenotype of the two EHEC strains belonging to serotype O128 was much greater than that of any of the EHEC strains belonging to serotype O157:H7, O26, or O111, as shown in Fig. 1A. No hemolysis was observed on blood agar containing unwashed sheep erythrocytes (Fig. 1B).

### Plasmids encoding for the EHEC-hly production

Strains F60 (serotype O128:H12) and K17 (serotype O128:H2) had two species of plasmids with molecular sizes of 120 kbp and 7.5 kbp (Fig. 2A). The plasmids were labeled with Tn*1*, transferred into *E. coli* HB101 by transformation, and examined for EHEC-hly. The EHEC-hlyA gene was located on the 120-kbp plasmid (pO128-60 for strain F60 and pO128-17 for strain K17) (Fig. 2B).

### Cloning of the EHEC-hly genes of strain F60

DNA of the Tn*1*-labeled pO128-60 plasmid (designated pO128-60-314) was purified, digested with *Bam*HI and the fragments were inserted into the *Bam*HI site

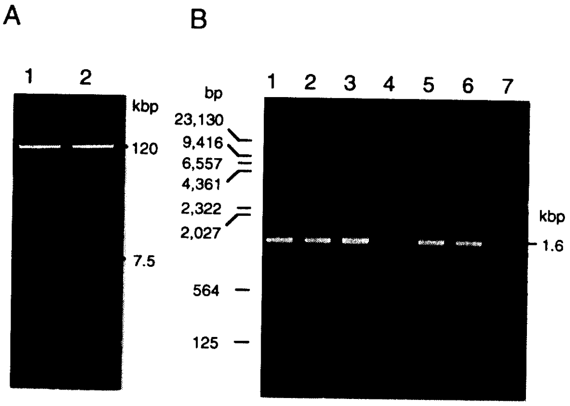


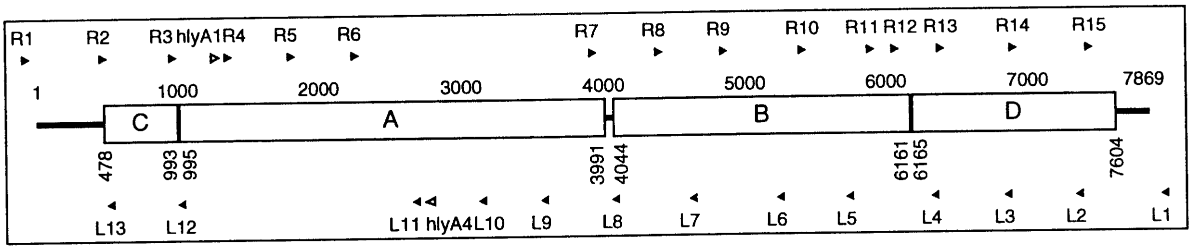
**Fig. 2.** Plasmid profiles and EHEC-hly gene location in strains K17 (serotype O128:H2) and F60 (serotype O128:H12). In A, plasmid DNA was analyzed by electrophoresis in 0.5% agarose gel and stained with ethidium bromide. Lane 1, strain K17; lane 2, strain F60. In B, to detect the EHEC-hly gene (EHEC-*hlyA*), the PCR was conducted with the primer set hlyA1 and hlyA4[5], and the PCR products were analyzed by electrophoresis in 1% agarose gel and stained with ethidium bromide. *Hin*dIII digests of λ DNA were used as molecular size standards. Bacterial strains examined: lane 1, serotype O157:H7 strain (U8); lane 2, strain K17; lane 3, *E. coli* HB101 carrying the Tn*1*-labeled 120-kbp plasmid (pO128-17-31) of strain K17; lane 4, *E. coli* HB101 carrying the Tn*1*-labeled 7.5-kbp plasmid (pO128S-17-11) of strain K17; lane 5, strain F60; lane 6, *E. coli* HB101 carrying the Tn*1*-labeled 120-kbp plasmid (pO128-60-314) of strain F60; lane 7, *E. coli* HB101 carrying the Tn*1*-labeled 7.5-kbp plasmid (pO128S-60-33) of strain F60.

of pACYC184. Subsequently, *E. coli* HB101 was transformed with the ligated DNA and chloramphenicol-resistant, EHEC-hly$^+$ clones were chosen. Such a clone, which was susceptible to tetracycline and ampicillin, contained a recombinant plasmid (24.5 kbp in size) consisting of a 20-kbp fragment of pO128-60 and a pACYC184 fragment; this plasmid was designated pACH1. Hemolysis by *E. coli* HB101 carrying pACH1 was visible after 3 h of incubation at 37°C; no hemolysis was observed on blood agar containing unwashed erythrocytes (Fig. 1B).

The 7,869-bp region of pACH1 was sequenced and the EHEC-hly genes were determined (Fig. 3). The EHEC-hly operon of EHEC strain F60 consisted of the four genes (C, A, B and D), just like that of EHEC belonging to serotype O157:H7.

### Sequence comparison of the EHEC-hly genes

Series of single base substitutions were found in the EHEC-hlyC, A, B, and D genes of serotype O128,



| | | | |
|---|---|---|---|
| R1 | 5'-(-27)CAGAGAAACGGGCGGAATCAGTC | L1 | 5'-(7903)CCATAAGCTTTGTATCATTTATAC |
| R2 | 5'-(468)TTTAAACATTATGAAATCTAATGC | L2 | 5'-(7356)GTGTATATGGATATGCATCTACTT |
| R3 | 5'-(924)CCATCATGAACTTATGTCTGA | L3 | 5'-(6859)GATACCTGATGCTCATACAGGCTA |
| R4 | 5'-(1312)GTATCAGAAAGTTGGGAGTAAAAT | L4 | 5'-(6334)GAACGTCTGGATACTGGTGTTTCA |
| R5 | 5'-(1763)GCGGGTATCGAACTGACAACTCAG | L5 | 5'-(5732)TGTAATATTATCGATAATACTACGA |
| R6 | 5'-(2206)TGAGCACGTTGCAGATAAATTCGC | L6 | 5'-(5234)ATGTGCTCCCATCCATAGACTAATT |
| R7 | 5'-(3883)TTCTTCAGTTTCAGGGTTCGATTC | L7 | 5'-(4628)TATTGTGATAATATTTAACGTTG |
| R8 | 5'-(4358)ACTAACTTTTAGTCGGGATGAGTT | L8 | 5'-(4093)GCAAGCAATATCAGTGCATAC |
| R9 | 5'-(4806)AGGGAACTGGAGCAAATTCGTAAT | L9 | 5'-(3600)CCAACTCTCTTAAATGCGATATCA |
| R10 | 5'-(5347)AAGTTGGTATTTCTGTTGAGCGCC | L10 | 5'-(3151)CTTACCGCCTTTGAATTCATCATC |
| R11 | 5'-(5848)GCGTTGGTCTTTCAGGAGGGCAGC | L11 | 5'-(2692)AGACCATGTATCCTTACCATTTAC |
| R12 | 5'-(6018)ATTGCACACAGGTTGTCAACTGTA | L12 | 5'-(1041)GTCGATGTCGCATTGTTGAAAATG |
| R13 | 5'-(6331)GTTCTCATTTTGTTATATGGAGTA | L13 | 5'-(543)ATGCAGAGGAGAACAGGCCCATAG |
| R14 | 5'-(6849)CATCAGGTATCACAGGAAGGAAGA | | |
| R15 | 5'-(7386)AATATAACTGCAGATTCTGTTTCT | | |

**Fig. 3.** Sequencing strategy of the EHEC-hly genes of strain F60 belonging to serotype O128:H12. The box labeled with letters C, A, B, and D represents the EHEC-*hlyC*, EHEC-*hlyA*, EHEC-*hlyB*, and EHEC-*hlyD* genes, respectively. R1 to R15 and L1 and L13 are primers constructed and used to determine the nucleotide sequence in this study. Primers hlyA1 and hlyA4[5)] were also employed. The nucleotide sequence of the region 1 to 7,869 was determined in this study (GenBank accession number AB032930).

when compared with that of serotype O157:H7 or O111 (Table 1).

The EHEC O128-hlyC gene (encoding for the hlyC protein responsible for activation of prohemolysin through acylation) differed from the EHEC O157-hly C gene by 9 nucleotides, resulting in 4 amino acid changes in the predicted gene product.

For the genes responsible for the transport of hemolysin across the bacterial membranes, the EHEC O128-hlyB gene (encoding for the ATP-binding transmembrane protein) differed from the EHEC O157-hlyB by 16 nucleotides, resulting in one amino acid-shorter sequence (at the amino terminus) with 2 amino acid substitutions in the predicted gene product (Fig. 4). The EHEC O128-hlyD gene (encoding for another transporter protein) differed from the EHEC O157-hlyB by 15 nucleotides, resulting in 4 amino acid changes in the predicted gene product.

The EHEC O128-hlyA gene, encoding for hemolysin (hlyA) differed from the EHEC O157-hlyA gene by 44 nucleotides, resulting in 15 amino acid changes. The EHEC O128-hlyA and EHEC O111-hlyA genes showed 98.3% similarity at the nucleotide level (51 nucleotide differences) and 97.9% similarity at the amino acid level (21 amino acid differences), indicating that the EHEC O128-hlyA gene is closer to the EHEC O157-hlyA gene than to the EHEC O111-hlyA gene. Among the available EHEC-hlyA sequences, the acylation site and RTX sequences were well conserved (Fig. 5).

## DISCUSSION

EHEC-hly was first described by Beutin et al.[9)]. It is closely associated with EHEC[9)]. Hemolysis due to EHEC-hly is visible after overnight incubation at 37°C, while hemolysis due to $\alpha$-hemolysin is visible only after 3 to 4 h of incubation at 37°C[9)]. In this study, hemolysis by *E. coli* HB101 carrying pACH1 (encoding for EHEC-hly) was visible after 3 h of incubation at 37°C.

The EHEC O128-hly and EHEC O157-hly regions shared the same gene organization with a high sequence similarity. Although the reason why two different sequences have been reported for the EHEC O157-hly genes with respect to strain EDL 933 is not known, one sequence reported by Burland et al.[7)] was identi-

**Table 1.** EHEC-hly genes of strains belonging to serotypes O157:H7, O111:H-, and O128: H12

| STEC serotype (strain) | Length of the gene and predicted gene product (homology) | | | | Origin of strain | Source or reference |
|---|---|---|---|---|---|---|
| | HlyC | HlyA | HlyB | HlyD | | |
| O157:H7 (EDL 933) | 516 bp<br>171 aa | 2997 bp<br>998 aa | 2121 bp<br>706 aa | 1440 bp<br>479 aa | Hamburger | AF 074613[a], reference[7] |
| O157: H7 (EDL 933) | 516 bp<br>(99.6%)<br>171 aa<br>(99.4%) | 2997 bp<br>(99.7%)<br>998 aa<br>(99.2%) | 2121 bp<br>(100%)<br>706 aa<br>(99.9%) | 1440 bp<br>(99.7%)<br>479 aa<br>(99.4%) | Hamburger | X 86087[a], Y 07545[a], reference[5,6] |
| O157:H7 (RIMD 0509952) | 516 bp<br>(100%)[b]<br>171 aa<br>(100%)[b] | 2997 bp<br>(100%)<br>998 aa<br>(100%) | 2121 bp<br>(100%)<br>706 aa<br>(100%) | 1440 bp<br>(100%)<br>479 aa<br>(100%) | Human | AB 011549[a], reference[8] |
| O111:H- (78/92) | ND[c] | 2997 bp<br>(99.4%)<br>998 aa<br>(98.8%) | ND[c] | ND[c] | Human | X 94129[a], reference[17] |
| O128:H12 (F 60) | 516 bp<br>(98.3%)<br>171 aa<br>(97.7%) | 2997 bp<br>(98.5%)<br>998 aa<br>(98.5%) | 2118 bp<br>(99.2%)<br>705 aa<br>(99.6%) | 1440 bp<br>(99.0%)<br>479 aa<br>(99.2%) | Human | This study, AB 032930[a] |

[a] GenBank accession number. [b] Alternative assign: 492 bp, 163 aa [AB 011549[a]]. [c] ND, not determined.

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                    hlyA
                                                                         hlyB
                                                                          1
            ...ThrValStop                                                MetMetSerLys...
0157:H7     ...ACTGTCTGAgactaaaagaggaggtgatggccattccaggaggaatggtcatcgttatATGATGAGTAAA...
                                *                                          *
0128:H12    ...ACTGTCTGAgactaaaagcggaggtgatggccattccaggaggaatggtcatcgttatataATGAGTAAA...
            ...ThrValStop                                                   MetSerLys...
                                                                             1

                                                                   α-hlyB   MetAspSer...
                                                                             1
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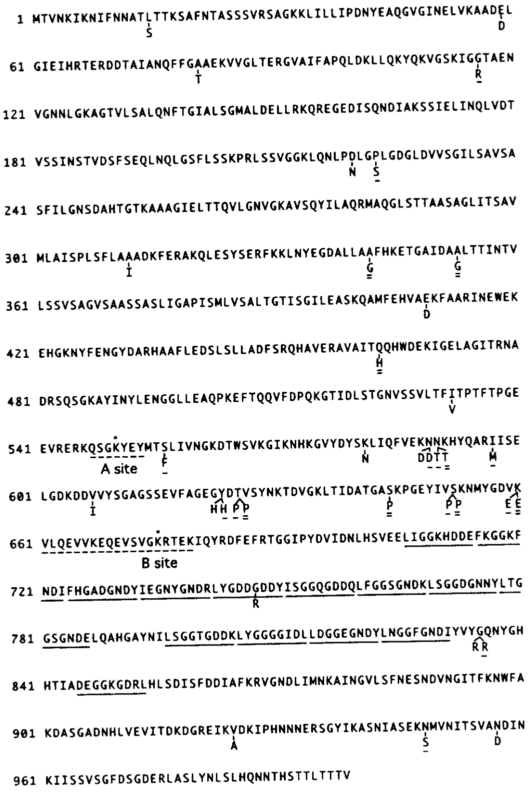
**Fig. 4.** The nucleotide sequence at the 5'-region of the EHEC-hlyB gene and comparison of the amino terminal amino acid sequences of hlyB among EHEC O157-hly, EHEC O128-hly, and α-hemolysin. The data of EHEC belonging to serotype O157:H7 and α-hemolysin are from reference[7] and reference[18], respectively. Asterisks (*) indicate nucleotide difference between the EHEC O157-hly and EHEC O128-hly regions.



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  1 MTVNKIKNIFNNATLTTKSAFNTASSSVRSAGKKLILLIPDNYEAQGVGINELVKAADEL
                  S                                           D

 61 GIEIHRTERDDTAIANQFFGAAEKVVGLTERGVAIFAPQLDKLLQKYQKVGSKIGGTAEN
                        T                                  R

121 VGNNLGKAGTVLSALQNFTGIALSGMALDELLRKQREGEDISQNDIAKSSIELINQLVDT

181 VSSINSTVDSFSEQLNQLGSFLSSKPRLSSVGGKLQNLPDLGPLGDGLDVVSGILSAVSA
                                           N  S

241 SFILGNSDAHTGTKAAAGIELTTQVLGNVGKAVSQYILAQRMAQGLSTTAASAGLITSAV

301 MLAISPLSFLAAADKFERAKQLESYSERFKKLNYEGDALLAAFHKETGAIDAALTTINTV
               I                             G          G

361 LSSVSAGVSAASSASLIGAPISMLVSALTGTISGILEASKQAMFEHVAEKFAARINEWEK
                                                    D

421 EHGKNYFENGYDARHAAFLEDSLSLLADFSRQHAVERAVAITQQHWDEKIGELAGITRNA
                                              H

481 DRSQSGKAYINYLENGGLLEAQPKEFTQQVFDPQKGTIDLSTGNVSSVLTFITPTFTPGE
                                                       V

             •
541 EVRERKQSGKYEYMTSLIVNGKDTWSVKGIKNHKGVYDYSKLIQFVEKNNKHYQARIISE
                   F                        N      DDTT     M
           A site

601 LGDKDDVVYSGAGSSEVFAGEGYDTVSYNKTDVGKLTIDATGASKPGEYIVSKNMYGDVK
          I              HH PP                 P       PP     EE

                  •
661 VLQEVVKEQEVSVGKRTEKIQYRDFEFRTGGIPYDVIDNLHSVEELIGGKHDDEFKGGKF
                 B site

721 NDIFHGADGNDYIEGNYGNDRLYGDDGDDYISGGQGDDQLFGGSGNDKLSGGDGNNYLTG
                              R

781 GSGNDELQAHGAYNILSGGTGDDKLYGGGGIDLLDGGEGNDYLNGGFGNDIYVYGQNYGH
                                                          RR

841 HTIADEGGKGDRLHLSDISFDDIAFKRVGNDLIMNKAINGVLSFNESNDVNGITFKNWFA

901 KDASGADNHLVEVITDKDGREIKVDKIPHNNNERSGYIKASNIASEKNMVNITSVANDIN
                           A                       S        D

961 KIISSVSGFDSGDERLASLYNLSLHQNNTHSTTLTTTV
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**Fig. 5.** Sequence comparison of EHEC O157-hlyA, EHEC O128-hlyA, and EHEC O111-hlyA. The entire amino acid sequence of EHEC O157-hlyA (GenBank accession number AF074613) is shown in the figure. In the case of EHEC O128-hlyA, only amino acids that differ from those of EHEC O157-hlyA are shown below the EHEC O157-hlyA sequence; they are indicated by amino acids without underlines. The amino acids underlined once are different residues in EHEC O111-hlyA (GenBank accession number X94129), and those underlined twice are different residues in another EHEC O157-hlyA sequence (GenBank accession numbers X86087 and Y07545). Dotted lines indicate sequences homologous to the acylation site sequence for α-hemolysin[19]; Lys-550 and Lys-675 (marked with a dot) are predicted sites for acylation in EHEC-hlyA. The RTX repeats (glycine- and aspartate-rich $Ca^{2+}$-binding repeats[20]) in EHEC O157-hlyA have been assigned in reference[5].

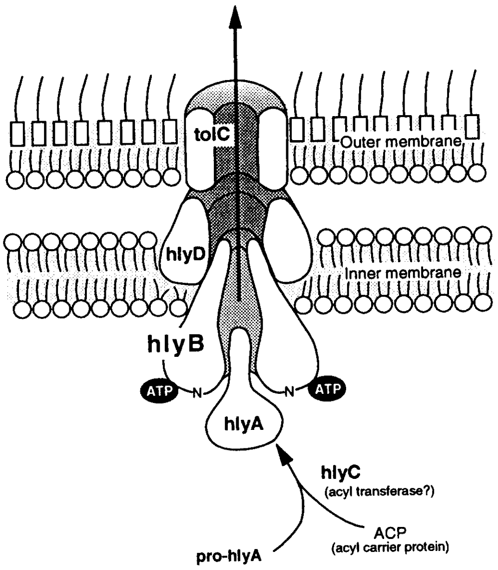

**Fig. 6.** A possible model of the transportation of EHEC-hlyA across the bacterial membranes. The model was constructed based on the data of α-hemolysin, a member of the ABC transporter superfamily[21,22]. N in hlyB represents the amino terminal sequence; in the case of EHEC-hly, the amino terminal sequence may play a role in the transportation (text).

cal to that of strain RIMD 0509952 (reported by Makino et al.[8]). The most unique feature of the EHEC O128-hly sequences found was that the O128-hlyB has only one methionine residue at the amino terminus, in sharp contrast to the O157-hlyB, which has two repeated methionine residues at the amino terminus; as a consequence, the O128-hlyB sequence was shorter than the O157-hlyB by one amino acid. α-hemolysin has also only one methionine residue at the amino terminus[18].

EHEC-hly and α-hemolysin are members of the RTX family, associated with the ATP-dependent hlyB/hlyD/tolC secretion system through which hemolysin (hlyA) is secreted across the bacterial membranes[21]. In this secretion system (as shown in Fig. 6), it is considered that the transmembrane domain of hlyB constitutes a pocket which binds with hlyA (the signal sequence at the carboxy terminus of hlyA), and the ATP-binding domain (located at the cytoplasm side) of hlyB generates energy for the transport[21].

EHEC belonging to serotype O157:H7 does not effectively secrete EHEC-hly in the culture supernatant; however, when the *hlyB* and *hlyD* genes of the α-hemolysin operon are supplied, the EHEC becomes able to secrete hemolytic activity5). There is a possibility that the amino terminal sequence of the EHEC O157-hlyB possessing two repeated methionine residues, found only in the serotype O157:H7 strains, interferes with the transportation of hlyA.

In the case of $\alpha$-hemolysin, it is proposed that the hlyA gene product (pro-hlyA) is activated through acylation at the two lysine residues (Lys-564 and Lys-690, with an internal span of 25 amino acids) by the function of hlyC22). In this study, the corresponding two acylation sites (A and B) were assigned with EHEC-hlyA (Lys-550 and Lys-675, with an internal span of 24 amino acids).

The precise role of EHEC-hly in pathogenesis of EHEC infections is still not known. However, accumulation of knowledge indicates that EHEC-hly seems to be associated with severe clinical disease in humans. EHEC-hly lyses erythrocytes resulted in release of heme and hemoglobin, which serve as a source of iron and stimulate the growth of EHEC23). In the case of EHEC belonging to serotype O111:H-, EHEC-hly$^+$ phenotype was observed in 88% of strains from patients with HUS but only in 22.2% of strains from patients with diarrhea, indicating that the presence of EHEC-hly increases the ability of EHEC O111 to cause extraintestinal complications in humans17). Antibodies to EHEC-hly have been detected in sera of patients with HUS5). If EHEC-hly acts as a contributor to the development of HUS, toxoid of EHEC-hly, in addition to Shiga toxoid, should also be developed by genetic engineering (e. g., site-directed mutagenesis) to prevent the disease.

**Acknowledgments.** This study was supported by a grant-in-aid for scientific research (B) from the Ministry of Education, Science, Sports and Culture.

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