Cloning and Expression of *Herpes simplex* virus type 1 gH and type 2 gB genes in *Escherichia coli*

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대장균에서 *Herpes simplex* virus type 1 gH와 type 2 gB genes의 클로닝과 발현
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Abstract: *Herpes simplex* virus type 1 glycoprotein H (gH) and type 2 gB genes were cloned and expressed in *Escherichia coli* cells. The gH gene (2.5 kb) of the pQE30-gH1 clone was very lowly expressed, however, a part (662 bp BamH1 fragment) of the gH gene in the pQE31-gH62 clone was highly expressed approximately 24 kDa protein by the induction of IPTG, which was detected by SDS-PAGE analysis. The gB gene (3.47 kb) of the pQE-gB2 clone was lowly expressed by IPTG induction. The viral glycoprotein gene use an expression device of a eukaryotic cell, but *E. coli* system is deficient to post-translational modification machinery. It was thought that the glycoprotein gene expression in *E. coli* system is very difficult. The results indicate that pQE vector expression system may provide a useful alternative approach to expression system for the production of glycoprotein.

INTRODUCTION

*Herpes simplex* virus types 1 (HSV-1) and 2 (HSV-2) belong to the family *Herpesviridae*, contain double-stranded linear DNA molecules with 152 kilobase pairs [7, 23], which are surrounded by a capsid and then are enveloped [22]. The envelope of HSV and the plasma membrane of HSV-infected cells contain antigenically distinct glycoproteins [29]. Glycoproteins encoded by HSV represent on the virion envelope and infected cell surfaces. Thus so far eleven glycoproteins have been identified in HSV and they are referred to by alphabetical designations gB, gC, gD, gE, gG, gH to gM [3, 21, 23, 28, 29]. They are important for viral adsorption and entry into host cells. gB is the only glycoprotein that is known to be required for viral growth and probably has a role in viral entry and cell fusion [4, 20, 27]. gH appears to play a role in entry, egress, and cell-cell spread [8, 9, 10, 11, 29]. This glycoprotein is characterized by an apparent molecular weight of the 110,000, and the gene has been mapped and sequenced [9]. gB and gH are essential for secondary

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interactions at the cell surface lead to virus entry into cells. HSV is a pathogen to human [5], and glycoproteins gB and gH genes of the HSV are useful and important for development of vaccines or antiviral mechanism. Therefore, we undertook the production of the eukaryotic viral glycoproteins in prokaryotic cell systems. We analyzed the productions of HSV-1 gH and HSV-2 gB in *Escherichia coli*. Further studies are necessary to characterize the genes and proteins, and used them for beneficial purposes.

**MATERIALS AND METHODS**

**Plasmids, bacterial strains and medium**

Plasmids pQE30, pQE31 and pQE32 (Qiagen Inc.) were used for expression in prokaryotic cells. pBluescript SK(+) plasmid (Stratagene Co.) was used for cloning. pHLA2-24 [13] and pHLB-4 plasmids [14] were used as source of the genes gB and gH, respectively. *E. coli* XL1-Blue (Stratagene Co.) was used as a host for cloning vector and maintained in the LB-broth (Difco laboratory, Detroit, MI, USA). *E. coli* M15 (Research Institute for Genetic Engineering, Konkuk University) was used as expression host for gH gene clones and maintained in the modified LB broth containing 25μg/ml kanamycin.

**Reagents and restriction enzymes**

All restriction endonucleases, T4 DNA ligase and calf intestinal alkaline phosphatase (CIP) were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Boehringer Mannheim (Indianapolis, In, USA), and New England Biolab (Beverly, MA, U.S.A). Another reagent was purchased from Difco (Detroit, MI, USA), Gibco (Gaithersburg, MD, USA) and Promega Biotec (Madison, WI, USA).

**Purification of plasmid DNA**

*E. coli* containing recombinant plasmids was cultured in LB broth at 37°C and then the plasmid DNA was purified by the procedure described by Birnboim and Doly [1] and then the plasmid DNA was repurified with the WizardTM Plus Minipreps DNA Purification System (Promega).

**Oligonucleotide Probe**

Oligonucleotide sequence (primer: 5’-TAATA GATTCAATTGTGAGCGG-3’) for sequencing was synthesized from Korea Biotech Inc., (Daejon, Korea).

**Restriction enzyme digestions and PAGE**

All restriction endonuclease digestions were performed according to the manufacturer’s instructions. The digested DNA was electrophoresed on 0.8% agarose gel and the molecular weight of each DNA fragment was determined by comparing its mobility with *HindIII* or *BstEII*-digested phage λ DNA fragments. Details of gel electrophoresis and visualization of the DNA fragments have been described by Lee *et al.*, [16, 17, 25]. DNA fragments in agarose gel were purified by the procedure described by Lee *et al.*, [18].

**Cloning and transformation in *E. coli* cells**

Cloning was carried out by mixing together 15μl (0.2μg) of inserting DNA, 20μl (0.1μg) of vector DNA, 5 μl of 5 mM ATP, 5 μl of 10x T4 DNA ligase buffer, 2 μl (1.8 units/μl) of T4 DNA ligase and 3 μl of distilled water, and then the total 50 μl mixture was reacted at 14°C for 18 h. The reaction condition was examined by 1.0 % agarose gel electrophoresis [15, 16, 17, 25]. DNA from low melting agarose gel was eluted by a slight modification of the procedure described by Lee *et al.*, [15]. The *E. coli* competent cells were prepared and trans-formed by the Mandel and Higa method [19].

**Construction of the expression clones**

pQE31-gH662 clone: The pHcgH plasmid containing HSV-1 gH gene, which was constructed by using pHLB-4 plasmid, was cleaved out with *BglII* to create 2.5kb gH gene DNA fragment and then recovered by GeneClean III kit (Bio101, Inc). The 2.5 kb DNA fragment was ligated into the *BamHI* site of the pQE30 vector by T4 DNA ligase, transformed in *E. coli* strain M15, and named as pQE30-gH1 plasmid. The pQE30-gH1 plasmid was digested with *BamHI* to create a 662 base pairs of the gH gene DNA fragment and recovered by GeneClean III kit (Bio101, Inc). Then the 662 base pairs was inserted into the *BamHI* site of