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Detection of Glycoproteins (B and D) and Thymidine Kinase Genes of *Herpes simplex virus* Type 2 Strain G

Hyun Kang, Jong Kuk Park, Hong Sun Uh, Soo Young Kim and Hyung Hoan Lee*

Department of Biological Science, Konkuk University, Seoul 143-701, Korea

=Abstract=

*Bam*HI restriction pattern and genomic library of *Herpes simplex* virus type 2 (HSV-2) strain G were constructed, and locations of the glycoproteins gB and gD, and *tk* genes on the fragments were detected by Southern blot analysis. HSV-2 genomic DNAs were cleaved into twenty-seven fragments by *Bam*HI enzyme in the range of 0.72 to 15.08 (total 150.44 kb), which were cloned into the *Bam*HI site of pBluescript SK(+) to construct genome library of the HSV-2. The library was named by the order of the fragment size from smallest one to largest one. HSV-2 glycoprotein gD gene was located in pHLA2-21 and pHLA2-22 recombinant plasmids, gB gene in pHLA2-24 plasmid, and *tk* gene in pHLA2-11 clone by Southern blot analysis.

Key Words: *Herpes simplex* virus, Glycoprotein, Glycoproteins B and D genes, Thymidine kinase gene, TK protein

INTRODUCTION

Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) belong to the family *Herpesviridae* [20], contain double-stranded linear DNA molecules with 152 kilobase pairs which encode at least 70 polypeptides [5]. The DNA core is surrounded by a capsid containing 162 capsomers, which is enveloped [19]. The envelope of HSV and the plasma membrane of HSV-infected cells contain antigenically distinct glycoproteins [24]. Glycoproteins on the surfaces of virus are important for viral adsorption and entry into host cells. HSV glycoproteins designated gB, gC, gD, and gE, are encoded by the viral genomes and expressed on virion surface [24]. 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 [3, 15, 18, 22]. gD stimulates the production of high titers of virus-neutralizing activity and play an important role in the initial stages of viral infection [6, 8, 9]. gB and gD are essential for secondary interactions at the cell surface lead to virus entry into cells. HSV *tk* gene is an early gene, and may be a model of gene regulation in infected cells because TK transcription is regulated both positively and negatively by other viral products [7]. Viral TK phosphorylates deoxynucleoside as a substrate [2], and is far more effective than cellular TK in phosphorylating acyclovir which is a base analogue [20].

HSV-2 strain G [4] is a pathogene to human, and glycoproteins gB and gD, and *tk* genes of

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*; Corresponding author: 이형환, 서울 광진구 모진동 93-1 (우)143-701 건국대학교 이과대학 생물학과 Tel: 02-450-3426 Fax: 02-452-9715 Email: hhlee@kkucc.konkuk.ac.kr

the HSV are useful and important for development of vaccines or antiviral mechanism. Therefore, we undertook this research to locate the genes. We analyzed the HSV-2 strain G genome using restriction enzyme, constructed the genome library, and searched the locations of the genes on the restriction enzyme fragments. Further studies are necessary to characterize the genes and proteins, and used them for beneficial purposes.

MATERIALS AND METHODS

Virus, cell, plasmid, bacteria and media

Herpes simplex virus type 2 (HSV-2) strain G (ATCC VR-734, Rockville, MD) was propagated in Vero cells obtained from Korean Type Culture Collection to prepare viral DNAs. The cells were cultured at 37℃ in Eagle's minimal essential medium (MEM) (Gibco, Middletton, WI, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco), and 50 μg/ml gentamycin (Gibco). The virus was titrated for infectivity as described by Lee *et al.* [11]. Virus inoculum used was extracellular nonoccluded virus (NOV) derived from a cell culture medium [11, 14]. pBluescript SK(+) vector harboring in *Escherichia coli* XL1-Blue was used for cloning. *E. coli* was maintained in the LB-broth (Difco Laboratories, Detroit, MI, USA).

Probes

*Xho*I and *Kpn*I double-digested 3.3 kb DNA fragment of clone pHLA-21 containing HSV-1 gB gene sequence was used as probe for the detection of the HSV-2 gB gene, *Hind*III and *Nru*I double-digested 1.45 kb fragment of clone pHLA-17 containing the HSV-1 gD gene sequence was used as gD gene probe of HSV-2, and *Asp*I 1.59 kb fragment of clone pHLB-4 containing the HSV-1 *tk* gene sequence was used as *tk* gene probe of HSV-2 [10]. The *Bam*HI fragments of HSV-2 strain G were used as probe DNAs for self-identification of the *Bam*HI fragments (Figure 1).

Purification of viral DNA

Vero cells (5×10^6 cells per 25 cm^2 flask) were incubated at 37℃ for 1 h. 5 ml MEM medium containing 5% FBS was then added to the dishes and incubated at 37℃ for 72 h. The cells were inoculated with HSV-2 NOV at the multiplicity of infection (m.o.i.) of 5 p.f.u./cell, and then 5 ml of the media were added to the flask and incubated at 37℃ for 5 days. Nonoccluded virions and viral DNAs were purified with the procedure described by Lee and Lee [13].

Plasmid isolation

E. coli containing recombinant plasmids were cultured in LB broth (Difco) at 37℃. The plasmid DNA was then purified with the procedure described by Birnboim and Doly [1].

Restriction enzyme digestions and agarose gel electrophoresis

All restriction endonuclease, calf intestinal alkaline phosphatase and T4 DNA ligase were obtained from Boehringer Mannheim (Indianapolis, IN, USA) and Promega (Arlington Height, IL, USA). All restriction endonuclease digestions were performed according to the manufacturer's instructions. HSV-2 genomic DNA and vector DNAs were digested with *Bam*HI and electrophoresed on 0.6% agarose gel. The molecular sizes of each DNA fragment were determined by comparing their mobility with *Bst*EII or *Hind*III-digested phage λ DNA fragments. The reactions were ended by the addition of 1/10 volume of a stop solution [28]. Details of gel electrophoresis and visualization of the DNA fragments have been described by Lee *et al.* [12, 15].

Cloning and transformation

Cloning was carried out by mixing together 15 μl (0.2 μg) of inserting DNA, 20 μl (0.1 μg) of vector DNA, 5 μl of 5 mM ATP, 5 μl

of 10× T4 DNA ligase buffer, 2 μl (1.8 units/μl) of T4 DNA ligase and 3 μl of distilled water, and then reacting 50 μl of the solution for 18 h at 14℃ [15, 21]. The reaction condition was examined by 1.0% agarose gel electrophoresis [12]. The *E. coli* competent cells were prepared and transformed by the procedure described by Mandel and Higa [17].

Southern blot analysis

Viral genomic library preparations were completely digested with *Bam*HI, and then the probe DNAs were labelled *in vitro* by ECL (enhanced chemiluminescence) procedure [25], using 20 μl (300 ng) of cDNA, 20 μl of horse radish peroxidase and 20 μl of glutaraldehyde. The total mixture (60 μl) was incubated for 10 minutes at 37℃, and held on ice for 10~15 minutes. Viral DNA fragments on the gels were transferred on Hybond-N^+ filter (Amersham) and hybridized with the probe DNAs

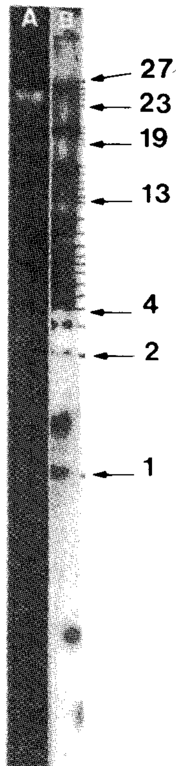

Figure 1. *Bam*HI restriction enzyme fragment pattern of HSV-2 DNA genome. Lane A represents the *Bam*HI fragments and lane B is the Southern hybridized-bands of the lane A with clones probes. The viral DNAs were digested with *Bam*HI, electrophoresed on 0.6% agarose gel, and then hybridized with each clone probe. The numbers at the side of the gel indicate the order of the restricted DNA fragments from smallest fragment 1 to largest one 27 (Table 1).

Table 1. Sizes and genome library of HSV-2 DNA digested with *Bam*HI

Order of fragments	Sizes (kb) of fragments	Name of recombinant (kb)
1	0.72	pHLA2-1 (3.68)
2	1.25	pHLA2-2 (4.21)
3	1.30	pHLA2-3 (4.26)
4	1.35	pHLA2-4 (4.31)
5	1.60	pHLA2-5 (4.56)
6	1.70	pHLA2-6 (4.66)
7	1.75	pHLA2-7 (4.71)
8	1.80	pHLA2-8 (4.76)
9	2.10	pHLA2-9 (5.06)
10	2.30	pHLA2-10 (5.26)
11	3.90	pHLA2-11 (6.86)
12	4.10	pHLA2-12 (7.06)
13	5.10	pHLA2-13 (8.06)
14	5.30	pHLA2-14 (8.26)
15	5.40	pHLA2-15 (8.36)
16	5.45	pHLA2-16 (8.41)
17	5.50	pHLA2-17 (8.46)
18	5.62	pHLA2-18 (8.58)
19	6.30	pHLA2-19 (9.26)
20	6.60	pHLA2-20 (9.56)
21	7.00	pHLA2-21 (9.96)
22	7.70	pHLA2-22 (10.66)
23	11.20	pHLA2-23 (14.16)
24	12.00	pHLA2-24 (14.96)
25	13.70	pHLA2-25 (16.66)
26	14.62	pHLA2-26 (17.58)
27	15.08	pHLA2-27 (18.04)
Total size	150.44 kb	

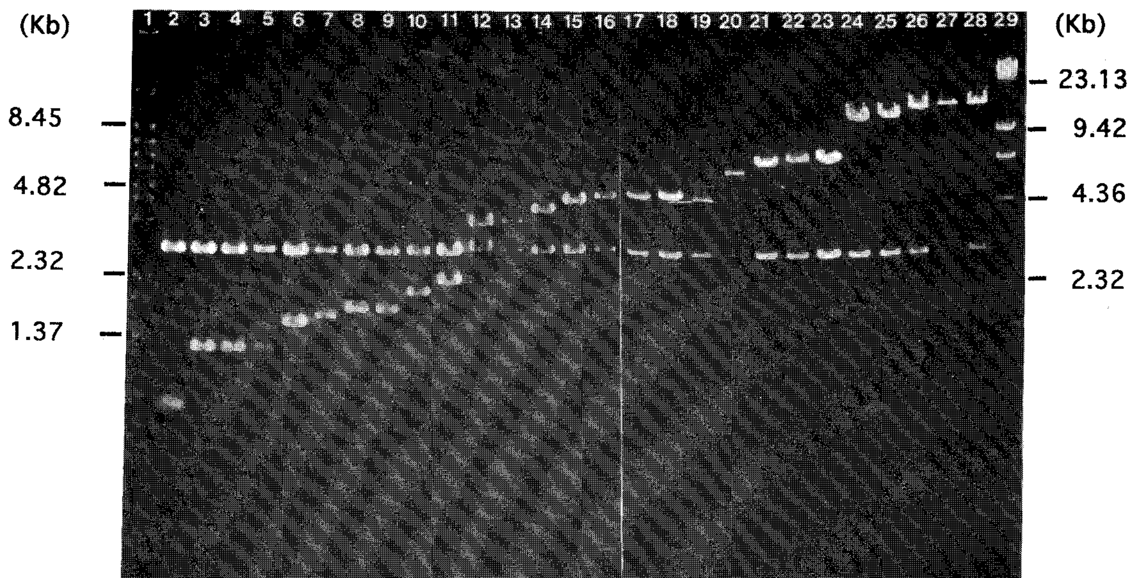

Figure 2. HSV-2 genome library of *Bam*HI fragments cloned in pBluescript SK(+) plasmid.The recombinant plasmids were redigested with *Bam*HI enzyme and electrophoresed. Linearized vector DNAs (2.96 kb) with *Bam*HI were arranged in the center of the gel, and upper and lower bands of the vector DNAs represent the *Bam*HI fragments inserted in pBluescript SK(+). Lanes 1, λ phage DNAs digested with *Bst*EII; 2, pHLA2-1; 3, pHLA2-2; 4, pHLA2-3; 5, pHLA2-4; 6, pHLA2-5; 7, pHLA2-6; 8, pHLA2-7; 9, pHLA2-8; 10, pHLA2-9; 11, pHLA2-10; 12, pHLA2-11; 13, pHLA2-12; 14, pHLA2-13; 15, pHLA2-14; 16, pHLA2-15; 17, pHLA2-16; 18, pHLA2-17; 19, pHLA2-18; 20, pHLA2-19; 21, pHLA2-20; 22, pHLA2-21; 23; pHLA2-22; 24, pHLA2-23; 25, pHLA2-24; 26, pHLA2-25; 27, pHLA2-26; 28, pHLA2-27 clones digested with *Bam*HI. Lane 29, λ phage DNAs digested with *Hind*III.

by the procedure of Southern [23].

RESULTS AND DISCUSSION

Construction of *Bam*HI genomic library of HSV-2 strain G

To construct genome library of HSV-2 DNA genome, the DNA genome was digested with *Bam*HI, and the resulting fragments were fractionated on 0.6% agarose gel (Figure 1A). The DNA genome was cleaved by *Bam*HI enzyme into twenty-seven fragments. The molecular sizes of the fragments were ranged from 0.72 to 15.08 kb, and the molecular size of the whole HSV-2 strain G DNA genome was estimated in approximately 150.44 kb. Each fragment was confirmed by Southern hybridization with the separated *Bam*HI fragments (Figure 1B). Each fragments were numerically numbered on the basis of molecular sizes from the smallest one to the largest one; 1 to 27 (Table 1). Total fragments were inserted into the *Bam*HI site of pBluescript SK(+) vector, and then each clone was named through an arabic numeral designation on the basis of molecular sizes such that the smallest insert fragment which was digested with *Bam*HI was named pHLA2-1, the second larger one, pHLA2-2 (Figure 2, Table 1). To confirm the cloned DNA fragments the recombinant plasmids were redigested with *Bam*HI restriction enzyme and then their electrophoretic mobilities in agarose gel were observed (Figure 2). In the Figures 2, 3, 4, and 5 the original *Bam*HI DNA fragments and linear form of their host vector DNAs (2.96 kb) at the center of the gel were observed. The linear forms of their host vector DNA fragments horizontally appeared on the center of the gel, however, the restriction fragments were present according to their sizes.

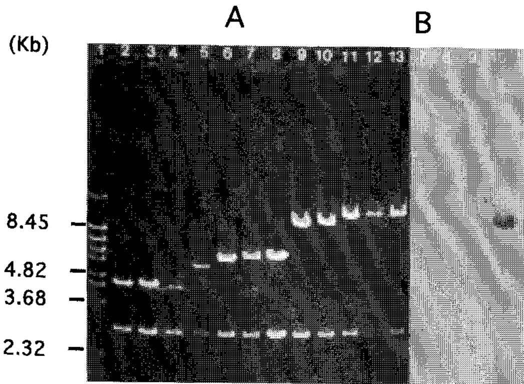

Figure 3. Detection of HSV-2 gB gene in the *Bam*HI fragments by Southern hybridization. Recombinant plasmids were digested with *Bam*HI, electrophoresed (A), and hybridized (B). In the center of the gel the linearized vectors are present, and the upper bands are the inserted DNA fragments. Lanes 1 in A photo, λ phage DNAs digested with *Bst*EII; 2-13, pHLA2-16 to pHLA2-27 clones DNAs digested with *Bam*HI. The gB gene probe was hybridized on thepHLA2-24 clone.

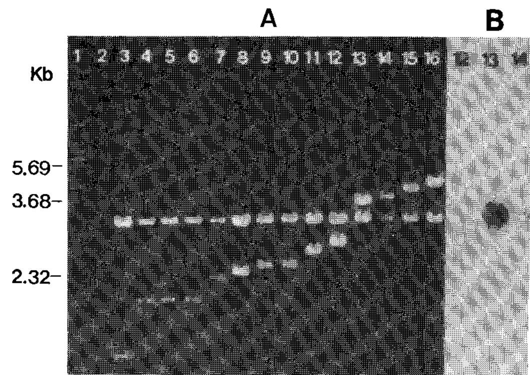

Figure 5. Detection of HSV-2 thymidine kinase gene in the *Bam*HI fragments by Southern hybridization. Recombinant plasmids were digested with *Bam*HI, electrophoresed (A) and hybridized (B). In the center of the gel linearized vectors are present, and the upper and lower bands are the inserted DNA fragments. Lane 1, λ phage DNAs digested with *Bst*EII. Lanes 3-16 in A photo, pHLA2-1 to pHLA2-14 clones DNAs digested with *Bam*HI. The *tk* gene probe was hybridized at the clone pHLA2-11.

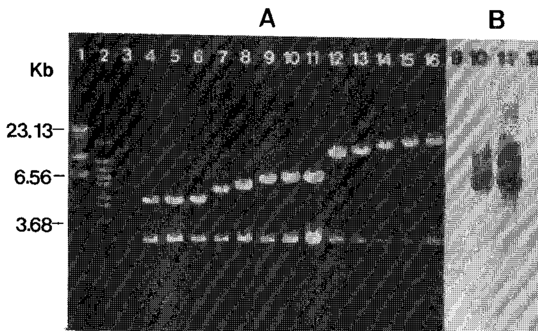

Figure 4. Detection of HSV-2 gD gene in the *Bam*HI fragments by Southern hybridization. Recombinant plasmids were digested with *Bam*HI, electrophoresed (A) and hybridized (B). In the center of the gel linearized vectors are present, and the upper bands are the inserted DNA fragments. Lanes 1 and 2, λ phage DNAs digested with *Hind*III and *Bst*EII, respectively. Lanes 4-16 in A photo, pHLA2-15 to pHLA2-27 clones DNAs digested with *Bam*HI. The gD gene probe was hybridized at the clones pHLA2-21 and pHLA2-22.

Detection of the DNA fragments containing gB, gD and *tk* genes

The *Bam*HI DNA fragments on the gel (Figure 2 and 3) were hybridized with probes to detect the glycoproteins gB and gD, and *tk* genes in the fragments. The gB gene probe was hybridized on the pHLA2-24 clone (Figure 3 lane 10), and the size of the inserted fragment was 12 kb (Table 1). The gD gene probe was hybridized at the clones pHLA2-21 and pHLA2-22 (Figure 4 lanes 10 and 11), and the sizes of the inserted fragment were 7.0 and 7.7 kb, respectively (Table 1). The *tk* gene probe was hybridized at the clone pHLA2-11 (Figure 5 lane 13), and the size of the inserted fragment was 3.9 kb (Table 1). The HSV-2 genome DNAs were cleaved into 27 fragments by *Bam*HI digestion and the locations of the three genes were detected by the Southern hybridization.

The construction of HSV-2 genomic library and preliminary researches now provide the materials required for the further studies on the genes of HSV-2.

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