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Overexpression of the $E1_{192-283}$ and $E2_{384-649}$ Proteins of Hepatitis C Virus in GST Fusion Forms in *E. coli* and Their Immunogenicity

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=Abstract=

C 형 간염 바이러스의 외피당단백질 E1 및 E2의 융합단백질 GST-$E1_{192-283}$ 및 -$E2_{384-649}$의 대장균에서의 과량발현 및 면역원성 연구

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C 형 간염 바이러스 (Hepatitis C Virus, HCV) 는 두종류의 외피당단백질 $E1_{192-383}$ 및 $E2_{384-746}$를 갖고 있다. $E1_{192-383}$ 및 $E2_{384-740}$ 단백질은 glutathione S-transferae (GST) 융합단백질의 형태로 대장균에서 발현되지 않았으나, 이 단백질들의 C말단에 존재하는 소수성영역을 제거하였을 때 GST-$E1_{192-283}$융합단백질은 과량으로 가용성형태로 발현되었고 GST-$E2_{384-649}$ 융합단백질은 비가용성 형태로 발현되었다. 이 융합단백질들 각각은 HCV 양성환자의 혈청과 특이적으로 반응하였다. Thrombin으로 처리하여 얻은 정제된 $E1_{192-283}$단백질 및 융합형태의 GST-$E2_{384-649}$ 단백질 각각을 생쥐에 접종하였을 때 E1 및 E2 특이적인 항체가 생성되었다. 이 결과들은 $E1_{192-383}$ 및 $E2_{384-649}$융합단백질 C말단에 존재하는 소수성영역이 이 단백질들의 발현량 및 가용성에 영향을 주며 $E1_{192-283}$ 단백질 영역내에 HCV 양성환자의 혈청과 특이적으로 반응하는 epitope (s)이 존재한다는 것을 제시해 주고 있다.

Key Words: HCV envelope proteins, E1, E2, immunogenicity

INTRODUCTION

Hepatitis C virus (HCV) is known to be a major causative agent of parentally transmitted non-A, non-B hepatitis worldwide [1]. The HCV contains a positive-stranded RNA genome of 9.5 kb which encodes a large polyprotein precursor of 3011 amino acids. *In vitro* translation and cell culture expression studies established that the HCV polyprotein is processed by a cellular signalase to produce core, E1, and E2/p7 as structural proteins [2-5], and by viral proteases to produce NS2, NS3, NS4A, NS4B, NS 5A, and NS5B as nonstructural proteins [6-9].

Much effort has been given to express and characterize the HCV E1 and E2 envelope proteins in bacteria [10-11], insects [12-13], and mammalian cells [12-17]. These studies revealed that the E1 and E2 are heavily glycosylated proteins and can form a E1/E2 heterodimer mainly by noncovalent interaction [15-17]. Previously, a truncated HCV $E2_{384-643}$ protein was expressed as a fusion protein of maltose binding protein in bacteria [10]. The GST-HCV E1 fusion protein with a deletion of C-terminal region was expressed to a high level in *E.coli* and the fusion protein was found to be antigenic [11].

This study was performed to express high levels of truncated HCV E1 and E2 as GST fusion pro-

teins and to analyze their immunological properties. A simple procedure to obtain pure antigens in large quantities as soluble forms in bacteria may serve to study the immunogenicities of proteins.

MATERIALS AND METHODS

Construction of the full-length and truncated GST-E1 and GST-E2 fusion plasmid

The plasmid c740 encodes the gene for HCV structural proteins, core, E1, and E2 [2]. The cDNA encoding HCV E1 protein (Tyr-192 to Gly-383) was amplified from the plasmid c740 after polymerase chain reaction (PCR). A sense primer with a sequence of 5'-GCGAATTCCTATGTACGAGGTGCGCAACGT-3' containing an *Eco*RI site and an antisense primer with a sequence of 5'-GCGAATTCTTACCGTCAACGCCAGCAAA-3' containing an *Eco*RI site and a stop codon were used in the PCR. The amplified fragment digested with *Eco*RI was cloned into the corresponding site of pTZ19U plasmid by standard cloning procedures [18]. The *Eco*RI fragment of the resulting plasmid was recloned into the *Eco*RI site of pGEX-2T expression vector (Pharmacia, Piscataway, NJ). This construct, pGEX/E1, contains the full-length of HCV E1 gene fused to the glutathione S-transferase. To delete C-terminal portion of the E1 gene, the pGEX/E1 was digested with *Bam*HI. The resulting 270 bp of *Bam*HI fragment was cloned into pGEX-2T. The resulting plasmid, pGEX/$E1_{192-283}$, codes for the truncated E1 protein from Tyr-192 to Ser-283. This plasmid contains 7 extra amino acid residues (Gly-Ser-Pro-Gly-Ile-Pro-Met) between the thrombin cleavage site and the amino terminus of E1 protein, and 6 amino acids (Pro-Gly-Ile-His-Arg-Asp) derived from the vector at the carboxy terminus of the truncated E1 protein (Fig. 1).

The full-length of HCV E2 protein was constructed by a similar procedure as described above. A sense primer (5'-GCGGATCCATGCACACCCACGTGACAGGGG-3') containing a *Bam*HI site and antisense primer (5'-GCGAATTCTTACATCAGTAGCATCATCCAT-3') containing an *Eco*RI site and a stop codon were used to amplify His-384-Leu-740 from the plasmid c740. The resulting fragment was cloned into the *Bam*HI and *Eco*RI sites of pTZ19U. The *Bam*HI-*Eco*RI fragment of the resulting plasmid was recloned into pGEX-2T. The resulting plasmid, pGEX/E2, contains the putative full-length HCV E2 gene. The pGEX/E2 was partially digested with HaeII to eliminate a C-terminal portion of the E2 gene. The digested DNA was separated by electrophoresis on 1% agarose gel. A linear DNA fragment of about 6 kb was isolated by electroelution from the gel slice. After complete digestion with *Eco*RI, the resulting 5.7 kb DNA fragment was treated with 3' exonuclease and filled in with T4 DNA polymerase, and ligated by using T4 DNA ligase. The resulting plasmid, pGEX/$E2_{384-649}$, codes for the HCV E2 protein from His-384 to Gly-649. This plasmid contains 3 extra amino acids (Gly-Ser-Met) between the thrombin cleavage site and the amino terminus of E2 protein, and 5 amino acids (Val-Ile-His-Arg-Asp) derived from the vector at the carboxy terminus of the truncated E2 protein (Fig. 1).

Expression of the GST-E1 and GST-E2 fusion genes

E. coli DH5 was transformed with each fusion plasmid. The expression of the fusion genes in each transformant was directed by adding IPTG to 0.5 mM. To compare expression levels of the fusion genes, one ml of each bacterial culture was harvested at 4 hrs post-induction by centrifugation at $4,000 \times g$ for 15 min. The pellets were dissolved in 40 μl of Laemmli sample buffer [19] containing 8 M urea.

Five μl of each protein sample was boiled and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE).

Purification of the HCV $E1_{192-283}$ protein

Affinity purification and thrombin cleavage of the GST-$E1_{192-283}$ fusion protein was mainly carried out under the conditions previously reported [20]. One liter of bacterial culture was harvested at 4 hrs post-induction. The pellet was washed once with buffer A (137 mM NaCl, 0.3 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.2, 1 mM DTT, 1mM PMSF) and resuspended in 10 ml of buffer A. The cell

suspension was sonicated with Sonifier 450 (Branson) on ice. Triton X-100 was added to the sonicated cell lysate to 1% (v/v) of final concentration. The supernatant as a soluble fraction was obtained by centrifugation of the sonicated cell lysate at 12,000×g for 30 min at 4℃. The soluble fraction was loaded onto a GST-agarose (Sigma, St Louis, MA) column (bed volume 5 ml; diameter 1 cm) and the column was washed with 5 column volumes of buffer A. The elution buffer was 50 mM Tris-HCl buffer (pH 8.0) containing 5 mM reduced glutathione. The fractions containing the GST-$E1_{192-283}$ fusion protein were pooled and dialyzed against buffer A at 4℃. The dialyzed protein solution was concentrated to about 7 mg/ml by Amicon Centriflo 25 centrifugation according to the manufacturer's instructions. Seven mg of the purified GST-$E1_{192-283}$ fusion protein was digested with 3 units of human thrombin (Novagen, Madison, WI) in a reaction buffer (20mM Tris-HCl, pH 8.0,

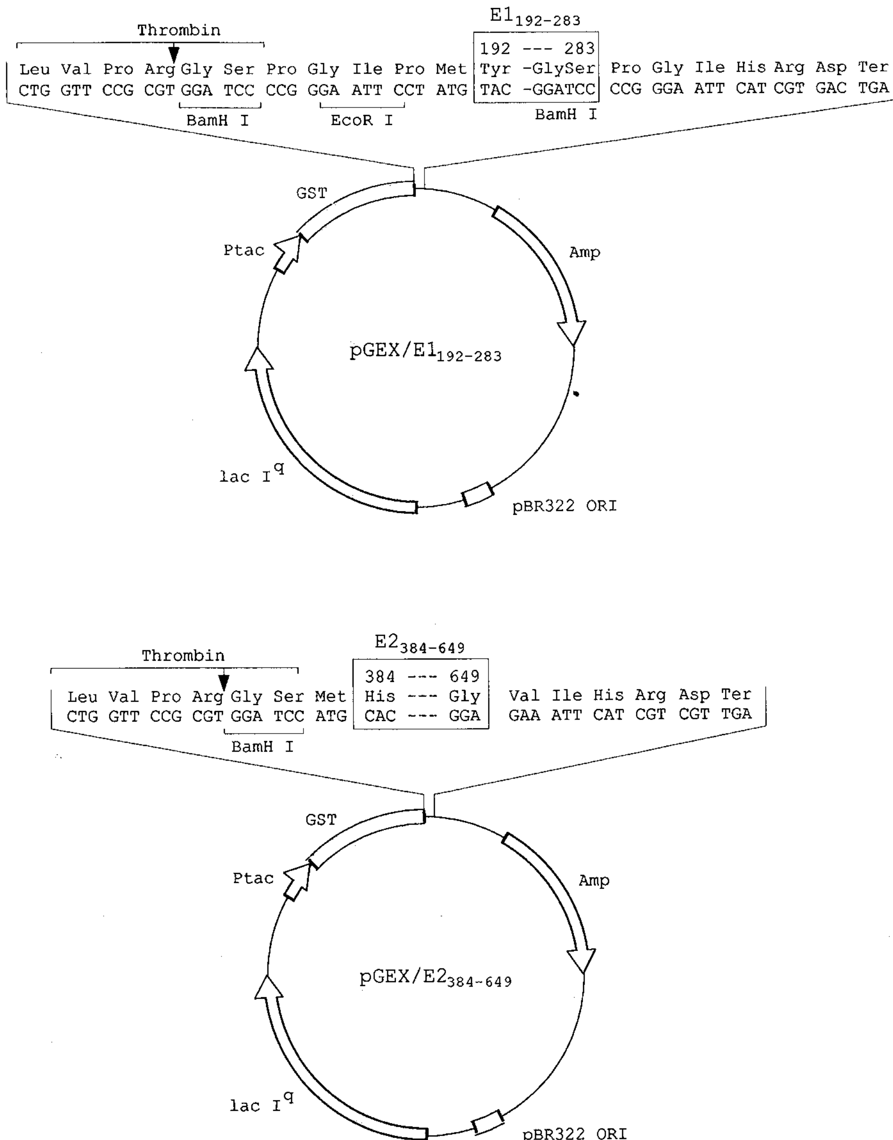

Fig. 1. Schematic representation of pGEX/$E1_{192-283}$ and /$E2_{384-649}$ which encode truncated GST-$E1_{192-283}$ and -$E2_{384-649}$ fusion proteins, respectively.

150 mM NaCl, 2.5 mM $CaCl_2$) for 36 hrs at 4℃. The digested protein solution which was diluted 10-fold with buffer A, was applied onto a GST-agarose column (bed volume 4 ml). One-point-two ml of each flowthrough fraction was collected. Proteins bound to the column were eluted as described above. The flowthrough fractions containing the HCV $E1_{192-283}$ protein were pooled and concentrated by lyophilization and stored at -20℃.

Immunization of mice with the purified HCV $E1_{192-283}$ or the GST-$E2_{384-649}$ fusion protein

Six-week-old female BALB/c mice were immunized intraperitoneally with either 10 μg of the purified HCV $E1_{192-283}$ or the GST-$E2_{384-649}$ fusion protein, which had been mixed with Freund's adjuvant. The GST-$E2_{384-649}$ fusion protein was electroeluted from the protein band of 57 kDa [21].

Mice were boosted three times by intraperitoneal injections of 20 μg of each antigen at 3, 5, and 7 weeks. Sera of the immunized mice were tested by Western blot analyses for their reactivity against either the GST-$E1_{192-283}$ or GST-$E2_{384-649}$ fusion proteins.

Immunoblotting

Proteins separated by SDS-PAGE were electroblotted onto nitrocellulose membranes [22]. The membranes were blocked with 10% non-fat dried milk in phosphate buffered saline (PBS) for 2 hrs at 37℃ and then washed once with PBS. The membranes were incubated with either mouse anti-$E1_{192-283}$ serum diluted 1:1,000 in PBS, mouse anti-GST-$E2_{384-649}$ serum diluted 1:1000 or rabbit anti-E2 antibody diluted 1:1,000 in PBS for 1-2 hrs at RT and washed three times for 20 min in PBS containing 0.05% tween 20 (PBST). An HCV positive serum from a chronic hepatitis patient was diluted 1:1,000 in PBS and then used in immunoblotting. The blots were incubated with either goat anti-mouse IgG, -rabbit IgG, or -human IgG conjugated with alkaline phosphatase or horseradish peroxidase (Sigma) at 1:1,000 dilution in PBS for 1 hr at RT and washed for 1-2 hrs in PBST with three changes. Reactive proteins were detected by adding the substrate solution containing nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Promega, Madison, WI) or by enhanced chemiluminescence (ECL:Amersham, Little Chalfont, Buckinghamshire).

RESULTS

Expression of the full-length and truncated GST-E1 and GST-E2 fusion proteins

pGEX/E1 and /E2 plasmids encoding the full-length E1 and E2 genes respectively, were constructed and their expressions were analyzed as described in Materials and Methods (Fig. 2). The pGEX containing the sequence of GST expressed an expected protein of 27 kDa (lane 2). However, neither pGEX/E1 nor pGEX/E2 expressed expected full-length proteins (lanes 3 and 4). A hydropathy plot of each full-length HCV E1 and E2 protein showed a hydrophobic transmembrane domain at their carboxy-terminal regions. In order to overexpress the GST-E1 and GST-E2 fusion proteins, a hydrophobic C-terminus of each protein was eliminated. The resulting pGEX/$E1_{192-283}$ and pGEX/$E2_{384-649}$ (Fig. 1) overexpressed the GST-$E1_{192-183}$ and GST-$E2_{384-649}$ fusion proteins, respectively (Fig. 2, lanes 5 and 6).

Next, we examined whether the expressed fusion proteins were soluble. Five ml of IPTG induced bacterial culture containing pGEX/$E1_{192-283}$ or pGEX/$E2_{384-649}$ was pelleted at 4 hrs post-induction. Each pellet was resuspended in 400 μl of buffer A. After sonication of the cell suspensions, the soluble and insoluble fractions were obtained by centrifugation as described in Materials and Methods. The insoluble fractions were dissolved in 50 μl of Laemmli sample buffer containing 8 M urea. Proteins in 5 μl of each insoluble fraction and 10 μl of each soluble fraction were analyzed by SDS-10% PAGE and visualized by staining with Coomassie blue (Fig. 3). The GST-$E1_{192-283}$ fusion protein was detected in both the soluble and insoluble fractions. The total amounts of protein in the sonicated cell lysate and the soluble fraction were determined [23]. More than 90% of the total protein in the sonicated cell lysate was found in the soluble fraction, indicating that most of the GST-$E1_{192-283}$ fusion pro-

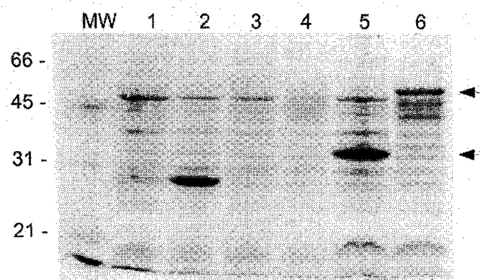

Fig. 2. Expression analysis of the full-length and truncated GST-E1 and -E2 fusion proteins in *E.coli.* Proteins in whole cell lysates were separated by SDS-PAGE and stained with Coomassie blue as described in Materials and Methods. Lanes 1 and 2; whole cell lysates of bacteria harboring pGEX without and with IPTG induction; lanes 3 and 4, whole cell lysates of bacteria containing the full-length GST-E1 and -E2 fusion genes with IPTG induction; lanes 5 and 6, whole cell lysates of bacteria harboring the GST-$E1_{192-283}$ and -$E2_{384-649}$ fusion genes with IPTG induction. Top and bottom arrows indicate the GST-$E2_{384-649}$ and -$E1_{192-283}$ fusion proteins, respectively. MW, molecular mass marker (in kDa).

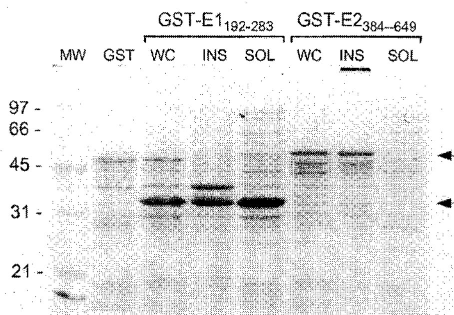

Fig. 3. The solubility of the GST-$E1_{192-283}$ and -$E2_{384-649}$ fusion proteins expressed in *E. coli.* Five ml of each bacterial culture producing GST-$E1_{192-283}$ or -$E2_{384-649}$ fusion proteins was harvested at 4 hrs post-induction by IPTG. The soluble (SOL) and insoluble (INS) fractions were prepared as described in the text. Each protein sample was separated by SDS-PAGE and visualized by staining with Coomassie blue. Lanes: Top and bottom arrows indicate GST-$E1_{192-283}$ and -$E2_{384-649}$ fusion proteins, respectively.

tein was in the soluble fraction. The GST-$E2_{384-649}$ fusion protein of 57 kDa was expressed mainly as an insoluble form (Fig. 3). A small portion of the fusion protein was expressed as a soluble form,

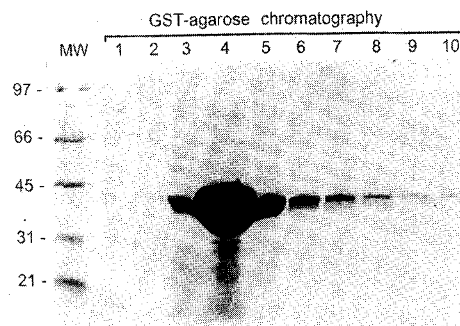

Fig. 4. A simple purification of the GST-$E1_{192-283}$ fusion protein by GST-agarose affinity chromatography. The soluble fraction obtained from one liter of bacterial culture harboring pGEX/$E1_{192-283}$ was applied onto a GST-agarose column and the proteins bound to the column were eluted as described in Materials and Methods. The proteins in the eluate were analyzed by SDS-PAGE and stained with Coomassie blue.

which could be purified by GST-agarose chromatography (data not shown).

Preparation of the HCV $E1_{192-283}$ protein

The soluble GST-$E1_{192-283}$ fusion protein fraction was applied onto a GST-agarose column and the proteins were eluted as described in Materials and Methods. The proteins were analyzed by SDS-PAGE and visualized by Coomassie blue staining (Fig. 4). The GST-$E1_{192-283}$ fusion protein was almost completely eluted with about 10 ml of the elution buffer. No GST-$E1_{192-283}$ fusion protein was found in the flowthrough fraction (data not shown). The fusion protein was purified to more than 90% homogeneity by this affinity chromatography step. This simple procedure for purification of the GST-$E1_{192-283}$ fusion protein usually yielded about 30~50 mg protein/liter of culture medium. To obtain the HCV $E1_{192-283}$ protein, seven mg of the purified fusion protein was treated with thrombin and the cleaved fusion protein solution was applied onto a GST-agarose column. Proteins in the flowthrough fraction and in the eluate were obtained as described in Materials and Methods and analyzed by SDS-PAGE and visualized by Coomassie blue staining. The HCV $E1_{192-283}$ protein was found mostly in the flowthrough fraction (Fig. 5A). The GST pro-

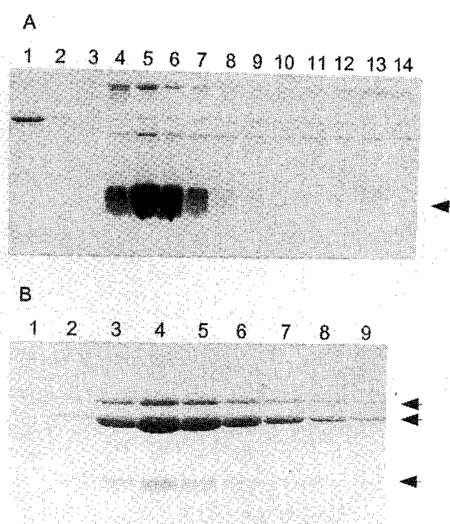

Fig. 5. A simple purification of the HCV $E1_{192-283}$ protein. The GST-$E1_{192-283}$ fusion protein digested by thrombin was applied onto a GST-agarose column. Proteins in the flowthrough fractions (A) and proteins bound to the column (B) were obtained as described in Materials and Methods. The proteins were analyzed by SDS-PAGE and stained with Coomassie blue. An arrow in panel A indicates the HCV $E1_{192-283}$ protein. Top, middle, and bottom arrows in panel B indicate the GST-$E1_{192-283}$ fusion, GST, and HCV $E1_{192-283}$ proteins, respectively.

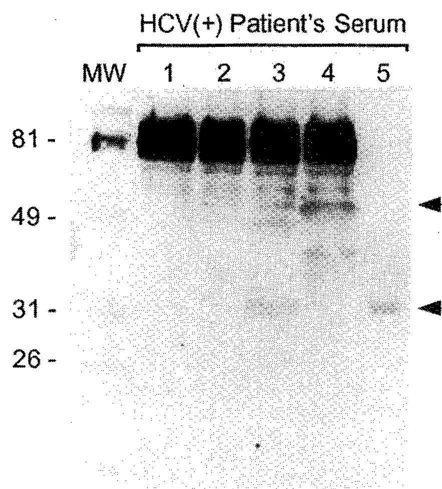

Fig. 6. The reactivity of the GST-$E1_{192-283}$ and -$E2_{384-649}$ fusion proteins with an HCV patient serum. Proteins in the whole cell lysates of bacteria harboring pGEX, pGEX/$E1_{192-283}$ or /$E2_{384-649}$ and the purified GST-$E1_{192-283}$ protein were separated by SDS-PAGE and transferred to nitrocellulose membrane. Immunoblotting was carried out with an HCV patient serum as described in Materials and Methods. Lanes 1 and 2, whole cell lysates of bacteria harboring pGEX with or without induction by IPTG; lanes 3 and 4, whole cell lysates of bacteria harboring GST-$E1_{192-283}$ or -$E2_{384-649}$ fusion genes with IPTG induction; lane 5, the purified GST-$E1_{192-283}$ protein.

tein generated by thrombin cleavage of the fusion protein and small amounts of the HCV $E1_{192-283}$ and uncleaved GST-$E1_{192-283}$ fusion proteins were found in the eluate (Fig. 5B). The results indicated that thrombin cleaved the GST-$E1_{192-283}$ fusion protein efficiently and the HCV $E1_{192-283}$ protein generated by thrombin cleavage was purified to more than 90% homogeneity by GST-agarose chromatography. This simple procedure for the protein purification yielded about 1 mg of the $E1_{192-283}$ protein, which was equivalent to 53% of the theoretical yield (1.9 mg), from approximatly 7 mg of the purified GST-$E1_{192-283}$ fusion protein.

The purified HCV $E1_{192-283}$ and GST-$E2_{384-649}$ fusion proteins are immunologically active

To know whether GST-$E1_{384-283}$ and -$E2_{384-649}$ fusion proteins expressed in bacteria were antigenic, immunoblotting was carried out with an HCV patient serum (Fig. 6). The patient's serum reacted specifically with the GST-$E1_{192-283}$ and -$E2_{384-649}$ proteins in the whole cell lysates as well as the purified GST-$E1_{192-283}$ protein (lanes 3, 4, and 5). The purified $E1_{192-283}$ protein was specifically recognized by the HCV patient serum in immunoblotting analysis (data not shown). The result indicated that the GST-$E1_{192-283}$ and -$E2_{384-649}$ fusion proteins contained antigenic epitopes which allowed specific reaction with HCV patient serum.

To know whether the purified HCV $E1_{192-283}$ and GST-$E2_{384-649}$ fusion proteins expressed in bacteria function as immunogens, mice were immunized with either the purified $E1_{192-283}$ or the GST-$E2_{384-649}$ fusion proteins as described in Materials and Methods. Sera obtained from mice at one week aft-

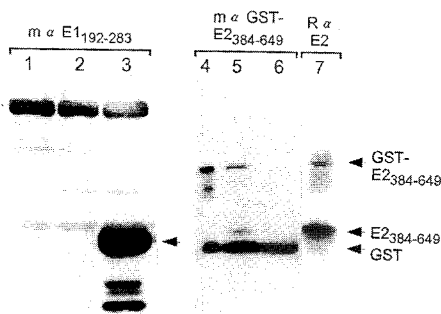

Fig. 7. Immunogenicity of the HCV $E1_{192\text{-}283}$ and GST-$E2_{384\text{-}649}$ proteins. Proteins in the whole cell lysates of bacteria harboring pGEX or pGEX/$E1_{192\text{-}283}$ as well as the purified GST-$E2_{384\text{-}649}$ protein with or without thrombin digestion were separated by SDS-PAGE and transferred to nitrocellulose membranes. Immunoblottings were carried out with either mice sera immunized with the purified HCV $E1_{192\text{-}283}$ or GST-$E_{2384\text{-}649}$ proteins, or rabbit anti-E2 antibodies (RαE2) as described in Materials and Methods. Lane 1, whole cell lysate of bacteria harboring pGEX; lanes 2 and 6, whole cell lysates of bacteria harboring pGEX with IPTG induction; Lane 3, whole cell lysate of bacteria harboring pGEX/$E1_{192\text{-}283}$ with IPTG induction; lane 4, the purified GST-$E2_{384\text{-}649}$ fusion protein; lanes 5 and 7, GST-$E2_{384\text{-}649}$ fusion protein with thrombin digestion.

er the final booster immunization were diluted as described in Materials and Methods and then used in immunoblottings. The serum of mouse immunized with the purified HCV $E1_{192\text{-}283}$ reacted specifically with the GST-$E1_{192\text{-}283}$ fusion protein and smaller proteins which may be degradation products of the fusion protein (Fig. 7, lane 3). The serum did not react specifically with the GST protein of 27 kDa (lane 2). The result indicated that the specific antibodies to the HCV $E1_{192\text{-}283}$ protein were generated in mice. The serum collected from the mouse immunized with the GST-$E2_{384\text{-}649}$ fusion protein reacted specifically with the purified GST-$E2_{384\text{-}649}$ protein (lane 4) and the HCV $E2_{384\text{-}649}$ as well as the GST proteins produced by thrombin digestion of the fusion protein (lane 5). The generation of the GST and $E2_{384\text{-}649}$ proteins by thrombin digestion of the purified GST-$E2_{384\text{-}649}$ protein was confirmed by rabbit anti-E2 specific antibody (lane 7). The result indicated that the GST-$E2_{384\text{-}649}$ fusion protein was able to generate specific antibodies to the GST as well as the HCV $E2_{384\text{-}649}$ protein in mice.

DISCUSSION

In this report we overexpressed the truncated HCV envelope E1 and E2 proteins as GST fusion form in bacteria and examined their immunological properties. The expression of the full-length of GST-E1 and GST-E2 fusion proteins was not detected. However, a deletion of 101 amino acids from the C-terminus from the GST-$E1_{192\text{-}383}$ fusion protein allowed the fusion protein to be expressed abundantly as a soluble form. Also, a deletion of 91 amino acids from 650 to 740 in the carboxy terminal portion of the full-length GST-E2 fusion protein resulted in an abundant expression of the fusion protein even if it was insoluble. Presumably elimination of hydrophobic C-terminus regions of the E1 and E2 proteins resulted in the overexpression of the fusion proteins. These results were consistent with previous data, suggesting that truncation of the putative membrane association region circumvents the difficulty of expressing E1 protein in *E. coli* [11]. A GST-HCV core fusion protein with a deletion of 68 amino acids from its C-terminus including a hydrophobic transmembrane region also resulted in overexpression of the fusion protein as a soluble form in bacteria [24]. The E1 and E2 proteins lacking C-terminal hydrophobic regions were secreted into the culture media in mammalian and insect cells [25]. Thus, it may be suggested that a hydrophobic C-terminal region of HCV structural proteins may interfere with producing the proteins. The GST-$E2_{384\text{-}649}$ fusion protein, which was expressed as an insoluble form, contained at least two internal hydrophobic domains, one from Gln-434 to Ala-449 and the other from Ala-490 to Gly-517 [25]. These domains may affect the solubility of the expressed protein. Thus, elimination of the internal hydrophobic domains or further truncation of the C-terminal portion may enchance the expression level and solubility of E2 protein.

An HCV patient serum recognized specifically the GST-$E1_{192\text{-}283}$ and GST-$E2_{384\text{-}649}$ fusion proteins

expressed in bacteria. The GST-$E2_{383-649}$ and purified $E1_{192-283}$ proteins generated specific antibodies in mice, respectively, indicating that the proteins contained antigenic epitopes, even if they were not glycosylated. A synthetic peptide from amino acid residues 201 to 223 in the E1 protein has been shown to be reactive to HCV patient sera [26]. HCV-specific cytotoxic T lymphocytes (CTL) for the E1 protein have been detected in persons with chronic hepatitis. A critical epitope for the CTL has been shown to be localized at amino acid residues 235 to 242 in the E1 protein [27]. Thus, the $E1_{192-283}$ protein purified in large quantity as a soluble form in this study, which contains B- and T-cell epitopes, may serve as an immunological agent for further determination of the role of E1 protein in HCV infection.

SUMMARY

The truncated $E1_{192-283}$ and $E2_{384-649}$ genes of hepatitis C virus (HCV) linked to the gene for glutathione S-transferase (GST) were constructed and their expressions were analyzed. The GST-$E1_{192-283}$ fusion gene overexpressed the fusion protein in *E. coli* as a soluble form, while the GST-$E1_{192-383}$ plasmid did not express expected fusion protein. The purified GST-$E1_{192-283}$ fusion protein was efficiently cleaved by thrombin. More than 90% pure HCV $E1_{192-283}$ protein was obtained by GST-agarose chromatography. The truncated GST-$E2_{384-649}$ fusion gene expressed the fusion protein mainly as an insoluble form, whereas the GST-$E2_{384-740}$ did not express the fusion protein. The truncated GST-$E1_{182-283}$ and GST-$E2_{384-649}$ fusion proteins reacted specifically with an HCV patient serum. In addition, mice immunized with either the purified $E1_{192-283}$ or GST-$E2_{384-649}$ proteins generated specific antibodies to each antigen. The results suggested that hydrophobic carboxyl portions of the E1 and E2 proteins might affect expression levels as well as the solubility of each fusion protein in bacteria. Also, the truncated E1 protein with Tyr-192 to Ser-283 contained antigenic epitope(s) which could be specifically recognized by an HCV patient serum.

REFERENCES

1. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M: Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244: 359-3621, 1989.
2. Hijikata M, Kato N, Ootsuyama Y, Nakagawa M, Shimotohno, K: Gene mapping of the putative structural region of the hepatitis C virus genome by in vitro processing analysis: Proc Natl Acad Sci USA 88: 5547-5551, 1991.
3. Selby MJ, Choo Q-L, Berger K, Kuo G, Glazer E, Eckart M, Lee C, Chien D, Kuo C, Houghton, M: Expression, identification and subcellular localization of the proteins encoded by the hepatitis C viral genome. J Gen Virol 74: 1103-1113, 1993.
4. Grakoui A, Wychowski C, Lin C, Feinstone SM, Rice CM: Expression and identification of hepatitis C virus polyprotein cleavage products. J Virol 67: 1385-1395, 1993.
5. Lin C, Lindenbach BD, Pragai BM, McCourt DW, Rice CM: Processing in the hepatitis C virus E2-NS2 region: identification of p7 and two distinct E2-specific products with different C termini. J Virol 68: 5063-5073, 1994.
6. Hijikata M, Mizushima H, Akagi T, Mori S, Kakiuchi N, Kato N, Tanaka T, Kimura K, Shimotohno K: Two distinct proteinase activities required for the processing of a putative nonstructural precursor protein of hepatitis C virus. J Virol 67: 4665-4675, 1993.
7. Grakoui A, McCourt DW, Wychowski C, Feinstone SM, Rice CM: Characterization of the hepatitis C virus-encoded serine proteinase: determination of proteinase-dependent polyprotein cleavage sites. J Virol 67: 2832-2843, 1993.
8. Bartenschlager R, Ahlborn-Laake L, Mous J, Jacobson H: Nonstructural protein 3 of the hepatitis C virus encodes a serine-type proteinase required for cleavage at the NS3/4 and NS4/5 junctions. J Virol 67: 3835-3844, 1993.
9. Tomei L, Failla C, Santolini E, De Francesco R,

La Monica N: NS3 is a serine protease required for processing of hepatitis C virus polyprotein. J Virol 67: 4017-4026, 1993.

10. Mita E, Hayashi N, Ueda K, Kasahara A, Fusamoto H, Takamizawa A, Matsubara K, Okayama H, Kamada T: Expression of MBP-HCV NS1/E2 fusion protein in E. coli and detection of anti-NS1/E2 antibody in type C chronic liver disease. Biochem Biophys Res Comm 183: 925-930, 1992.
11. Yan B-S, Liao L-Y, Leou K, Chang Y-C, Syu W-J: Truncating the putative membrane association region circumvents the difficulty of expressing hepatitis C virus protein E1 in Escherichia coli. J Virol Methods 49: 343-352, 1994.
12. Matsuura Y, Harada S, Suzuki R, Watanabe Y, Inoue Y, Saito I, Miyamura T: Expression of processed envelope protein of hepatitis C virus in mammalian and insect cells. J Virol 66: 1425-1431, 1992.
13. Lanford RE, Notvall L, Chavez D, White R, Frenzel G, Simonsen C, Kim J: Analysis of hepatitis C virus capsid, E1, and E2/NS1 proteins expressed in insect cells. Virology 197: 225-235, 1992.
14. Spaete RR, Alexander D, Rugroden ME, Choo Q-L, Berger K, Crawford K, Kuo C Leng S, Lee C, Ralston R, Thudium K, Tung JW, Kuo G, Houghton M: Charaterization of the hepatitis C virus E2/NS1 gene product expressed in mammalian cells. Virology 188: 819-830, 1992.
15. Ralston R, Thudium K, Berger K, Kuo C, Gervase B, Hall J, Selby M, Kuo G, Houghton M, Choo Q-L: Characterization of hepatitis C virus envelope glycoprotein complexes expressed by recombinant vaccinia viruses. J Virol 67: 6753-6761, 1993.
16. Dubuisson J, Hsu HH, Cheung RC, Greenberg HB, Russell DG, Rice CM: Formation and interacellular localization of hepatitis C virus envelope glycoprotein complexes expressed by recombinant vaccinia and sindbis viruses. J Virol 68: 6147-6160, 1994.
17. Deleersnyder V, Lellez A, Wychowski C, Blight K, Xu L, Hahn YS, Rice CM, Dubuisson J: Formation of native hepatitis C virus glycoprotein complexes. J Virol 71: 697-704, 1997.
18. Maniatis T, Fritsch EF, Sambrook J: Molecular Cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992.
19. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T 4. Nature (London) 227: 680-685, 1970.
20. Smith DB, Johnson KS: Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene 67: 31-40, 1988.
21. Harlow E, Lane D: Antibodies: a laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, p70, 1988.
22. Towbin H, Staehelin T, Cordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76: 4350-4354, 1993.
23. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254, 1976.
24. Seong YR, Lee EK, Choi S, Chon SK, Im D-S: Overexpression and simple purification of a truncated immunologically reactive GST-HCV core (1-123) fusion protein. J Virol Methods 59: 13-21, 1996.
25. Matsuura Y, Harada S, Suzuki R, Watanabe Y, Inoue Y, Saito I, Miyamura T: Expression of processed envelope protein of hepatitis C virus in mammalian and insect cells. J Virol 66: 1425-1431, 1992.
26. Ray R, Khanna A, Lagging M, Meyer K, Choo Q-L, Ralston R, Houghton M, Becherer PR: Peptide immunogen mimicry of putative E1 glycoprotein-specific epitopes in hepatitis C virus. J Virol 68: 4420-4426, 1994.
27. Koziel MJ, Dudley D, Wong JT, Dienstag J, Houghton M, Ralston R, Walkers BD: Intrahepatic cytotoxic T lymphocytes specific for hepatitis C virus in persons with chronic hepatitis. J Immunol 149: 3339-3344, 1992.