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Protein Expression and Purification 49 (2006) 95–101
Protein Expression & Purification
www.elsevier.com/locate/yprep

Purification and application of bacterially expressed chimeric protein E1E2 of hepatitis C virus

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Received 22 January 2006, and in revised form 15 February 2006
Available online 15 March 2006

Abstract

E1 and E2 glycoproteins are structural components of hepatitis C virus (HCV) virion. They are involved in cellular receptors interaction, neutralising antibodies elicitation, and viral morphogenesis. They are considered as major candidates for anti-HCV vaccine. In this report, we first expressed tandem E1E2 as well as C-terminally truncated E1 fragment and C-terminally truncated E2 fragment, respectively, in *Escherichia coli* cells and the proteins were purified to homogenesis. All the purified proteins can react specifically with patient sera. Both purified chimeric protein E1E2 and protein E2 can interact with a putative cellular receptor CD81, while purified protein E1 cannot interact with CD81. The sera of rabbit immunized with the E1E2 inhibited the binding of E2 protein to the major extracellular loop of human CD81 and reacted with both proteins E1 and E2, respectively. Anti-E1 and E2 antibodies can be generated simultaneously in the rabbit immunized with the E1E2, and the titers of antibodies were 63 or 56% higher than the titers induced by E1 or E2 alone, respectively. The results suggest that E1 and E2 can enhance their immunogenicity each other in chimeric protein E1E2 and the *E. coli*-derived chimeric protein E1E2 and corresponding antisera can be used as an useful tools in anti-HCV vaccine research.

Keywords: Hepatitis C virus; E1; E2; Immunization; Virus–receptor interaction; CD81

Hepatitis C virus (HCV)[1] infection is an important public-health problem, and more than 180 million people are infected worldwide [1]. Most infected patients do not clear the virus, which results in chronic hepatitis that can lead to liver cirrhosis and hepatocellular carcinoma (HCC) [1]. HCV is a single-stranded RNA(+) virus belonging to the *Flaviviridae* family, encoding a polyprotein precursor which is cleaved by both host cellular and viral proteases into structural (core, E1, E2, and p7) and non structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins [2]. Since there is no effective vaccine and current therapy has significant limitations, information on the early events of infection, virus binding, and entry, is needed for vaccine and therapeutic drug development [3,4]. It is emergent to develop effective prophylactic and therapeutic measures against HCV to fight back this global public-health threat.

Hepatitis C virus E1 and E2 glycoproteins are type I integral transmembrane (TM) proteins [5,6]. E2 interacts with E1 to form a stable non-covalently linked heterodimer or heterogeneous disulfide-linked aggregates [7,8], which are believed to result from a non productive folding pathway. Immunolocalization studies and glycans analyses have shown that HCV glycoproteins are located in the endoplasmic reticulum (ER) [9,10]. Deletion of the transmembrane domains of E1 and E2 has shown that these truncated forms of HCV glycoproteins are secreted into the extracellular medium [11,12]. Evaluation of HCV envelope protein-based vaccines requires an effective method for antigen detection and an inexpensive supply of large quantities of antigens. Although envelope glycoproteins expressed in mammalian cell systems in theory would best

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[1] *Abbreviations used:* HCV, hepatitis C virus; HCC, hepatocellular carcinoma; TM, transmembrane; TBS, Tris buffer saline; HRP, horseradish peroxidase; PBS, phosphate buffer saline; LEL, large extracellular loop; VLP, virus-like particles.

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doi:10.1016/j.pep.2006.02.013

reflect properties of envelope proteins present on HCV virion membrane, low yield as well as difficulty in purification and scaling up makes such systems unsuitable for large-scale applications [13]. Bacterial expression systems, compared to other higher organism expression systems, usually offer higher yield at considerably lower cost. So, bacterially expressed truncated E1 and E2, respectively, has been previously reported [14,15]. Previous research work of HCV vaccine used truncated E2 alone as surrogate for viral particles. However, truncated E2 may not fully mimic the surface of HCV virions because the virus encodes two envelope glycoproteins that associate with each other as E1E2 heterodimers. These two envelope glycoproteins are involved in cellular receptors interaction, neutralising antibodies elicitation, and viral morphogenesis. Immunized with E1 and E2 together may be better mimic the immunological response of native HCV, get better immunological effect, and provide better protection.

In this work, C-terminally truncated tandem E1E2 as well as C-terminally truncated E1 fragment and C-terminally truncated E2 fragment were expressed in *Escherichia coli* cells and the proteins were purified using immobilized-metal affinity chromatography. The sera of rabbit immunized with the E1E2 inhibited the binding of E2 protein to human CD81 and reacted with proteins E1 and E2, effectively. Anti-E1 and -E2 antibodies can be generated in the rabbit immunized with the E1E2, and the titers of antibodies were much higher than the titers induced by E1 or E2 alone. Our results suggest that E1 and E2 can enhance their immunogenicity each other in chimeric protein E1E2.

Materials and methods

Construction of recombinant expression plasmids

The recombinant plasmids were constructed by inserting the corresponding DNA fragments into the prokaryotic expression vector pET-His 2.9 (Jingmei Biotech). The DNA fragments were amplified by PCR from pBRTM/HCV1-3011 plasmid (genotype 1a, a generous gift from Dr. Charles M. Rice, University of Washington) using primers in Table 1. DNA sequences coding for E1 amino acid (aa) 192–341 was amplified by PCR from pBRTM/HCV1-3011 plasmid using 1a and 1b primers. DNA sequences coding for E2 amino acid (aa) 384–694 was also amplified by PCR from pBRTM/HCV1-3011 plasmid using 2a and 2b primers. DNA sequences coding for E1 and E2 were T-A cloned into a pMD18-T vector (Takara, Dalian). The ligation products were transformed into DH5α competent cells. The transformed cells were plated on LB plates containing ampicillin, X-Gal and IPTG, which allowed for color selection of colonies. Randomly selected individual white clones were grown for 8 h and then used for plasmid extraction. pMD18-T/E1 was cloned into pET-His 2.9 between the *Bgl*II/*Bam*HI and *Hin*dIII sites to create pETHis-E1. And plasmid pMD18-T/E2 was also cloned into pET-His 2.9 between the *Bam*HI and *Hin*dIII sites to create pETHis-E2.

Table 1
Primers used in this study

Primer	Oligonucleotides	
1a	5′ *AGATCT*GTGCCCGCTTCAGCC3′	(*Bgl*II)
1b	5′ *AAGCTT***CTA**GTCCATGATGGCTTG3′	(*Hin*dIII)
1c	5′ *CTGCAG*GTCCATGATGGCTTG 3′	(*Pst*I)
2a	5′ *GGATCC*GCGGAAACCCACGTC3′	(*Bam*HI)
2b	5′ *AAGCTT***CTA**CACGTCCACAATGTT 3′	(*Hin*dIII)
2c	5′ *CTGCAG*GCGGAAACCCACGTC 3′	(*Pst*I)

Italic nucleotides represent the restriction enzymes used. Bold nucleotides CTA represent the stop codon.

For construction the E1E2 chimeric gene, DNA sequences coding for E1 amino acid (aa) 192–341 was amplified by PCR from pBRTM/HCV1-3011 plasmid using 1a and 1c primers. DNA sequences coding for E2 amino acid (aa) 384–694 was also amplified by PCR from pBRTM/HCV1-3011 plasmid using 2c and 2b primers. After select the correct clone as the same step described above, pMD18-T/E2 694 (with *Pst*I restriction site) was cloned into pMD18-T/E1 341 (with *Pst*I restriction site) between the *Pst*I and *Hin*dIII sites to create pMD18-T/E1E2. Plasmid pMD18-T/E1E2 was cloned into pETHis 2.9 between the *Bgl*II/*Bam*HI and *Hin*dIII sites to create pETHis-E1E2.

Expression and purification of chimeric protein E1E2

The pETHis-E1E2 plasmid was expressed in *E. coli* strain BL21 (DE3) following induction 1 mM of IPTG. Cells were grown in Luria broth to a density of 0.4 A_{600} prior to adding IPTG and then grown further to a density of about 1.0 A_{600} at 37 °C. We tried a wide temperature range (+15 to +37 °C) for the expression. Unfortunately, in all these cases the polypeptide was found predominantly in inclusion bodies (data not shown). Cells were harvested by centrifugation and the pellet was suspended in binding buffer (20 mM Tris–HCl, pH 8.0, 500 mM NaCl, 5 mM imidazole, 1 mM NaF, 1 mM PMSF, and 1 mM DTT), sonicated and centrifuged at 12,000*g* at 4 °C for 30 min. The pellet was resuspended in urea buffer (6 M urea, 20 mM Tris–HCl, pH 8.0, 500 mM NaCl, 5 mM Imidazole, 1 mM NaF, 1 mM PMSF, and 1 mM DTT), then the suspension was centrifuged at 12,000*g* at 4 °C for 30 min, and the supernatant was applied to Ni–NTA affinity column (Qiagen) equilibrated in urea buffer. The Ni–NTA affinity column then washed with gradient washing buffers (6 M urea, 20 mM Tris–HCl, pH 8.0, 500 mM NaCl, 5, 20, 40, 80, and 100 mM Imidazole). Purified E1E2 protein was eluted with elution buffer (6 M urea, 20 mM Tris–HCl, pH 8.0, 500 mM NaCl, and 200 mM Imidazole) and dialyzed with dialytic buffer (20 mM Tris–HCl, pH 8.0, 500 mM NaCl, 0.1% Triton X-100, and 0.2% BSA). Purified E1 and E2 proteins were harvested by treating

with the aforementioned step. Only freshly purified proteins were used for further applications.

Expression and purification of human GST–CD81

Vector construction, *E. coli* expression, and purification of human GST–CD81 have been described [16].

Western blot analysis

E1, E2, and recombinant E1E2 proteins were separated by SDS–PAGE using a 12% polyacrylamide gel and were transferred to nitrocellulose membrane, as described by Sambrook and Russell [17]. The blots were first blocked with 5% non-fat milk in Tris buffer saline(TBS) containing 1 g/L Tween-20 and then probed with human HCV positive sera, rabbit anti-E1E2 antisera or the first antibody against HCV E2 protein (a generous gift from Dr. Jean Dubuisson, institut de Biologie de Lille & Institut Pasteur de Lille) for 1 h at 37 °C. After extensive washes, appropriate secondary antibodies conjugated with horseradish peroxidase (HRP) were applied onto the blots for at least 1 h at 37 °C. Blots were washed five times with TBS containing 1 g/L Tween-20. Reagents for enhanced chemiluminescence were applied to the blots and the light signals were detected by X-ray film.

Far Western blot analysis

Purified E1, E2, and E1E2 proteins were separated by SDS–PAGE using a 12% polyacrylamide gel and were transferred to nitrocellulose membrane, as described by Sambrook and Russell [17]. After incubation of the membrane in 5% non-fat milk in TBS containing 1 g/L Tween 20 for 1 h at room temperature, the membrane was incubated with 10 μg/ml GST or GST–CD81 for 1 h at room temperature. After extensive washing, Western blot analysis using antibody against the GST was performed. As a control, all three proteins were analyzed by Western blot using human HCV positive sera.

E2 binding inhibition assay by the rabbit sera

The E1E2-immunized or non-immunized rabbit sera at serial dilutions were preincubated with 1 μg of the purified E2 proteins in dialytic buffer (20 mM Tris–HCl, pH 8.0, 500 mM NaCl, 0.1% Triton X-100, and 0.2% BSA) for 2 h at RT. One hundred nanograms of human GST–CD81 proteins bound to GST–agarose beads in dialytic buffer for 2 h at RT. The beads were then washed with dialytic buffer. The human GST–CD81 proteins bound to the beads were added to the preincubation mixtures. The mixtures were incubated for 12 h and then the beads were washed with dialytic buffer four times. Proteins bound to the beads were separated on 12% SDS–PAGE. A Western blot was carried out by using mouse anti-E2 antibody. The blot was reprobed with goat anti-GST monoclonal antibody.

Animal immunization and antisera preparation

New Zealand rabbits were kept in conventional conditions and were handled in compliance with College of Life Sciences, Wuhan University (Wuhan, China) guidelines for animal care and use. Rabbits were immunized subcutaneously multiple sites on the back with 0.1 mg of purified recombinant E1, E2, E1 + E2 (mixed with equimolecular E1 and E2), and E1E2 proteins with 20 mg/ml $Al(OH)_3$, respectively. A booster injection was given two weeks later and enhanced injections were given at one week later with the same quantity of antigen with 20 mg/ml $Al(OH)_3$. Blood was drawn from the rabbit ear vein every week. Three weeks after final boosting, total blood was collected through the carotid artery and sera were prepared using standard procedure [17].

ELISA

Fifty nanogram purified E1, E2, E1 + E2, and E1E2 proteins, dissolved in coating buffer (0.016 M Na_2CO_3, 0.034 M $NaHCO_3$, pH 9.6), were added to a 96-well microplate and incubated at 4 °C overnight. Wells were then blocked with 4% non-fat milk, 0.5% BSA in phosphate buffer saline (PBS), incubated for 1 h at RT, washed with PBS and dried at 37 °C for 1 h. For detection by chemiluminescent method, sera were serially diluted in sample buffer (1:10) then incubated at 37 °C for 30 min. Each well was washed then incubated with peroxidase conjugated goat anti-rabbit antibody at 37 °C for 30 min. The wells were washed again with PBS containing 0.05% Tween 20. The peroxidase reaction was visualized by using *O*-phenylenediamine as a substrate, after 10 min at 37 °C the reaction was stopped by adding 50 μl/well of 1 N H_2SO4 and absorbance was read at 492 nm. The serum samples were run in triplicate.

Results

Expression and purification of recombinant E1, E2, and E1E2 proteins

C-terminally truncated E1, C-terminally truncated E2, and chimeric E1E2 sequences from subtype 1a isolate were cloned into N-terminal hexa-histidine fusion expression vector pET-His 2.9. All the constructed plasmids were confirmed by restricted digestion and sequencing. BL21 (DE3) cells transformed with each recombinant plasmid and their expression were induced with IPTG and analyzed on SDS–PAGE. All the expressed proteins were found predominantly in inclusion bodies. The expressed proteins E1, E2, and E1E2 were about 30, 50, and 60%, respectively, in induced products by densitometric scanning. Complete solubilisation of the inclusion bodies were achieved in urea buffer (6 M urea, 20 mM Tris–HCl, pH 8.0, 500 mM NaCl, 5 mM Imidazole, 1 mM NaF, 1 mM PMSF, and 1 mM DTT), then these proteins were purified on Ni^{2+}–NTA

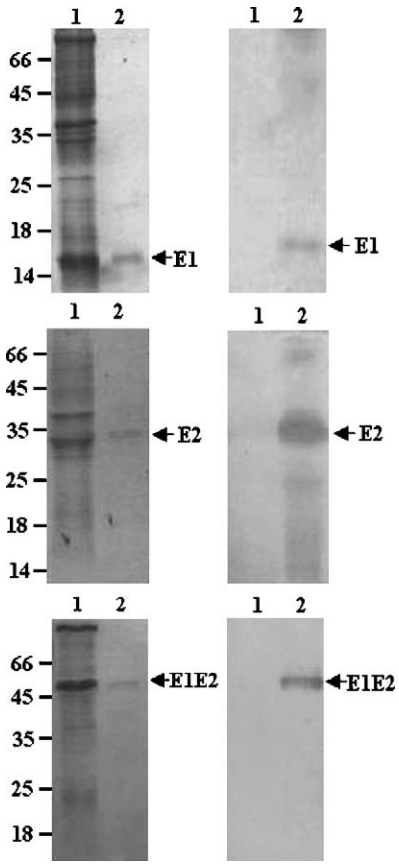

Fig. 1. SDS–PAGE and Western blot analysis of recombinant HCV envelope proteins. Left: SDS–PAGE of HCV envelope proteins. Lane 1, lysates of expressing recombinant HCV envelope proteins; lane 2, purified proteins obtained by column chromatography. Right, analysis of HCV envelope proteins by Western blot using human HCV positive sera. Lane 1, lysates of the induced BL21 (DE3) pET-His 2.9; lane 2, lysates of expressing recombinant HCV envelope proteins. Recombinant HCV envelope proteins positions are indicated by arrow. Molecular weight markers are shown at the left.

resin under denaturing conditions. Purified proteins were eluted in elution buffer (6 M urea, 20 mM Tris–HCl, pH 8.0, 500 mM NaCl, and 200 mM Imidazole) and dialyzed with dialytic buffer (20 mM Tris–HCl, pH 8.0, 500 mM NaCl, 0.1% Triton X-100, and 0.2% BSA). After SDS–PAGE and Coomassie brilliant blue staining, purified E1, E2, and E1E2 displayed a single band with molecular weight of 16, 34, and 52 kDa, respectively. The finally obtained E1, E2, and E1E2 were purified to homogeneity. Results of Western blot indicated that purified E1, E2, and E1E2 proteins were recognised by human HCV positive sera specifically and reacted strongly. Purified E2 and E1E2 proteins were also recognised by E2 monoclonal antibodies (data not shown). These results were shown in Fig. 1.

Interaction of E2 and E1E2 with human GST–CD81

To certificate the purified E2 and E1E2 used in this study is indeed functional, purified E2 and E1E2 were used to interact with human GST–CD81 shown by far Western blot analysis, while purified E1 protein cannot interact with human GST–CD81 (Fig. 2B). Furthermore, there was no any visible blot in the result of far Western blot of HCV envelope proteins with GST vector protein (Fig. 2C). When the purified E1, E2, and E1E2 were examined by reprobing the blot with human HCV positive sera, there was no remarkable difference in the position and amount of all three proteins (Fig. 2A). These results indicated that purified chimeric protein E1E2 and protein E2 can interact with a putative cellular receptor CD81, while purified protein E1 cannot interact with CD81 and neither E2 nor E1E2 fusion reacts with the GST vector protein.

Immunogenicity of E1, E2, and E1E2 proteins in rabbit

Rabbits were immunized with the purified proteins E1, E2, E1+E2, and E1E2 and boosted twice at two and three weeks after primary immunization, respectively, as described under Materials and methods. The immunized and non-immunized rabbits were bled at every week after first immunization. Total blood was collected through the carotid artery and sera were prepared at six weeks after

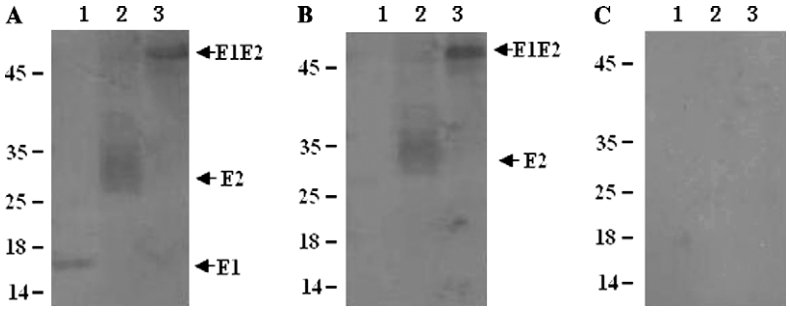

Fig. 2. Study on interaction of recombinant HCV E2 and E1E2 proteins with human GST–CD81. Purified proteins E1, E2, and E1E2 were subjected to far Western blot, the bound GST–CD81 was detected by anti-GST antibody. (A) Analysis of HCV envelope proteins by Western blot using human HCV positive sera. Lane 1, sample of E1; lane 2, sample of E2; lane 3, sample of E1E2. (B) Far Western blot of HCV envelope proteins with GST–CD81. Lane 1, sample of E1; lane 2, sample of E2; lane 3, sample of E1E2. (C) Far Western blot of HCV envelope proteins with GST. Lane 1, sample of E1; lane 2, sample of E2; lane 3, sample of E1E2. Recombinant HCV envelope proteins positions are indicated by arrow. Molecular weight markers are shown at the left.

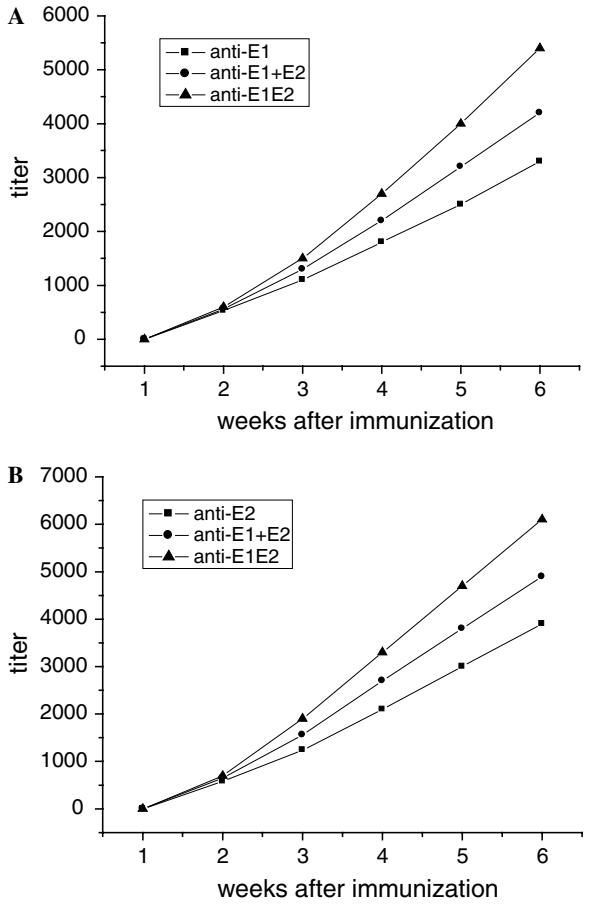

Fig. 3. Comparative antibody titers of anti-E1 raising after immunization (A) and anti-E2 raising after immunization (B). (A) Titers of anti-E1 induced by E1 immunization alone shown in square, titers of anti-E1 induced by E1 + E2 immunization shown in rotundity, titers of anti-E1 induced by E1E2 immunization shown in triangle. (B) Titers of anti-E2 induced by E2 immunization alone shown in square, titers of anti-E2 induced by E1 + E2 immunization shown in rotundity, titers of anti-E2 induced by E1E2 immunization shown in triangle.

first immunization. ELISA was carried out to determine induction of antibodies in the rabbit sera. The end-point titers of antibodies were determined by ELISA to be $1{:}3.3\times10^3$, $1{:}3.9\times10^3$, $1{:}5.9\times10^3$, and $1{:}6.4\times10^3$, respectively. The E1E2 antigens elicited the highest titers of antibodies, while the E1 antigens showed the lowest immunogenicity.

To determine whether the immunized rabbit sera contain anti-E1, -E2, -E1E2 or both anti-E1 and -E2 antibodies, reactivity of the E1E2-immunized rabbit sera with the E1, E2, and E1E2 proteins was investigated by ELISA. Anti-E1 and -E2 antibodies can be generated simultaneously in the rabbit immunized with the E1E2, and the titers of antibodies were 63 or 56% higher than the titers induced by E1 or E2 alone, respectively. Anti-E1 + E2 and anti-E1E2 end-point anti-E1 titers were determined by ELISA to be $1{:}4.2\times10^3$ and $1{:}5.4\times10^3$ (Fig. 3A). Anti-E1 + E2 and anti-E1E2 end-point anti-E2 titers were determined by ELISA to be $1{:}4.9\times10^3$ and $1{:}6.1\times10^3$ (Fig. 3B).

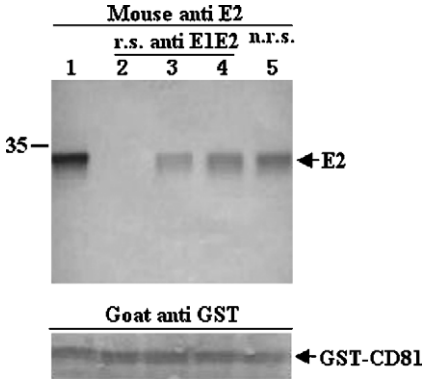

Fig. 4. Rabbit anti-E1E2 sera inhibit the binding of E2 protein to human GST–CD81. The sera of rabbit immunized with the recE1E2 proteins inhibit the binding of the E2 protein to of human CD81. One microgram of the E1E2 proteins were preincubated with an E1E2-immunized rabbit serum (r.s. anti-E1E2) in 2-fold serial dilutions from 200 to 800 (lanes 2–4) and non-immunized rabbit serum (n.r.s.) in 1:200 dilutions (lane 5), or without rabbit serum (lane 1). One microgram of GST–CD81 (lanes 1–5) proteins prebound to GST–agarose beads was added to each reaction mixture. After incubation for 2 h, the beads were washed. Proteins bound to the beads were analyzed by a Western blot using mouse anti-E2 antibody (upper panel). The blot was reprobed with goat anti-GST antibody (lower panel). Protein bands were detected by ECL. E2 and GST–CD81 positions are indicated by arrow. Molecular weight markers are shown at the left.

Rabbit anti-E1E2 antisera inhibit the binding of E2 protein to human GST–CD81

As the E1E2-immunized rabbit sera contained mainly anti-E2 antibodies (Fig. 3B), we examined whether the E1E2-immunized rabbit sera inhibit binding of E2 protein to major extracellular domain of human CD81. The purified E2 proteins was preincubated with the E1E2-immunized and non-immunized rabbit sera at serial dilutions, respectively. Equal amounts of the purified human GST–CD81 proteins were bound to GST–agarose beads and the bead-suspensions were then added to the preincubation mixtures. Proteins bound to human GST–CD81 proteins were analyzed by a Western blot using rabbit anti-E2 antibody (Fig. 4). E2 protein bound to the human GST–CD81 (lane 1), indicating that E2 protein bound to the GST–CD81 specifically. The E1E2-immunized rabbit sera at 1:200 dilutions inhibited completely the binding of E2 protein to GST–CD81 (lane 2), while non-immunized rabbit sera did not (lane 5). When the GST–CD81 proteins bound to the beads were examined by reprobing the blot with mouse anti-GST antibody, there was no remarkable difference in the amounts of the GST–CD81 proteins bound to the beads (Fig. 4, lower panel). Thus, the results indicate that the E1E2-immunized rabbit sera inhibit the specific binding of the E2 protein to the LEL domain of human CD81.

Discussion

Hepatitis C virus encodes two envelope glycoproteins, E1 and E2, which are released from HCV polyprotein by

signal peptidase cleavage and have separable functional properties. These proteins play important roles in viral attachment and entry into target cells as well as virus-host immune interactions [13,18–20]. It has been reported that the C-terminal hydrophobic region (aa 341–383) of E1 hinders its expression in *E. coli*, possibly through interference with normal bacterial membrane functions [21–23]. Truncation of the hydrophobic region resulted in high levels expression of recombinant E1 proteins [14].The similar effect was also observed when expression of E2 in bacterial cell [15]. Expression of E1 or E2 has been studied separately before, with regard to these two envelope proteins associate with each other as E1E2 heterodimers on the surface of HCV virions and the conformation of these two proteins probably have been changed, plays important roles in viral attachment on cell membrane, penetration into target cells and virus-host immune interactions. We expressed chimeric protein E1E2 to study the character and immunogenicity of this protein.

In this work, we first expressed tandem E1E2 as well as C-terminally truncated E1 fragment and C-terminally truncated E2 fragment, respectively, in *E. coli* cells. The chimeric protein E1E2 showed higher level expression than separately expressed E1 or E2 protein. Both purified chimeric protein E1E2 and protein E2 can interact with a putative cellular receptor CD81 indicated that binding of E2 to CD81 had not been influenced in chimeric protein E1E2. The antibodies of rabbit immunized with chimeric protein E1E2 showed higher titers than immunized with protein E1 or E2 alone and specifically reacted with proteins E1 and E2, showed that anti-E1 and -E2 antibodies can be generated simultaneity in the rabbit immunized with chimeric protein E1E2. The titers of antibodies were much higher than the titers induced by purified protein E1 or E2 alone. These results suggest that chimeric protein E1E2 has stronger immunogenicity than protein E1 and E2. One compelling reason is its higher molecular weight. There is a correlation between the size of a macromolecule and its immunogenicity. The most active immunogens tend to have a molecular mass of 100 kDa. Generally, substances with a molecular mass less than 5–10 kDa are poor immunogens. Another reason is epitopes splicing. The titers of anti-E1E2 against proteins E1 and E2 were higher than the titers of anti-E1+E2 against proteins E1 and E2, respectively. This result suggests that with the epitopes of E1 and E2 together, immunogenicity of chimeric protein E1E2 is not simple addition of immunogenicity of proteins E1 and E2, but enhance each other. It is likely that the inclusion of HCV E1, which may change the conformation of E2, enhance immunogenicity of chimeric protein E1E2. And the inclusion of HCV E2 also may change the conformation of E1, enhance immunogenicity of chimeric protein E1E2.

As shown in Results, anti-E2 antibodies generated in the rabbit immunized with chimeric protein E1E2 can inhibit the binding of E2 protein to human GST–CD81 and inhibited completely at 1:200 dilutions. CD81 is a member of the tetraspanin membrane protein superfamily, characterized by the presence of four transmembrane domains, three intracellular loops, and two extracellular domains, which in the case of CD81 are referred to as the small extracellular loop and the large extracellular loop (LEL). Both truncated E2 and virus-like particles (VLP) containing E2 interact with the LEL of the human cell surface protein CD81, suggesting that CD81 is a receptor for HCV [16,24,25]. With regard to E2 glycosylation, it has been demonstrated that enzymatically de-glycosylated form of E2 binds CD81 with affinity similar to that of core-glycosylated form, while highly glycosylated protein has substantially reduced affinity to CD81 [26]. Bacterially expressed truncated E2 can also interact with CD81 demonstrated that the non-glycosylated recE2 retains functionality of the native E2 glycoprotein [15]. Recently report shows that bacterially derived E1 proteins share identical epitopes with mammalian E1 glycoproteins, and that these epitopes can be presented to the host immune system in vivo [14]. Previous report showed that glycosylated forms of E1E2 efficiently binded to CD81 whereas truncated E2 was a week binder, suggesting that truncated E2 was probably not the best tool with which to study cellular interactions [27]. But in the present work, chimeric protein E1E2 bind effectively CD81 suggests at least some conformational similarities between the non-glycosylated and glycosylated forms of E1E2. Furthermore, rabbit antisera against *E. coli*-derived chimeric protein E1E2 specifically reacted with *Pichia pastoris*-expressed chimeric E1E2 glycoproteins (Weijia Cai, in preparation) in Western blot (data not shown). These results demonstrate that these prokaryotically expressed chimeric protein E1E2 share similar epitopes with eukaryotically expressed chimeric E1E2 glycoproteins. Recently, research work shows the dissociation of hCD81 LEL-GST from E1E2 was four times longer than from E2 and while E2 binds to target cells, E1 may act to stabilize the envelope protein complex and to facilitate membrane interactions and cell entry [28]. This chimeric protein E1E2 retains functionality of the native E1E2 glycoprotein, can be purified in adequate amounts, therefore, can be used as a better surrogate model than bacterially expressed truncated E1 or bacterially expressed truncated E2.

In summary, we first expressed tandem E1E2 in *E. coli* cells and produced large quantities of *E. coli*-derived C-terminally truncated proteins E1, E2, and E1E2 with high purity, obtained highly specific rabbit antisera against these proteins, and demonstrated the applicability of these sera for the detection of these proteins expressed in prokaryotic system. Anti-E1 and -E2 antibodies can be generated in the rabbit immunized with the E1E2, and the titers of antibodies were much higher than the titers induced by E1 and E2, respectively, and anti-E1E2 can detect chimeric protein E1E2 expressed in both prokaryotic and eukaryotic systems. These results indicate that *E. coli*-derived chimeric protein E1E2 are immunologically similar to yeast-derived chimeric protein E1E2 glycoproteins and E1E2 antigens

and corresponding antisera reported here can serve as useful tools in the development of E1E2-based HCV vaccine as well as other E1E2-related studies.

Acknowledgments

The authors thank Dr. Charles M. Rice (University of Washington) for providing pBRTM/HCV1-3011, Dr. Shoshana Levy (Stanford University) for providing CD81 plasmid, and Dr. Jean Dubuisson (institut de Biologie de Lille & Institut Pasteur de Lille) for providing HCV E2 monoclonal antibody. The authors thank Jinrong Gao and Huaping Li for helpful suggestions, and also thank Huaping Li for her technical help. This work was supported by grants from Bureau of Science and Technology of Wuhan, China (No. 20026002091).

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