Enhancement of sapovirus recombinant capsid protein expression in insect cells

Grant S. Hansman*, Tomoichiro Oka, Kazuhiro Katayama, Naokazu Takeda

Department of Virology II, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashi-murayama, Tokyo 208-0011, Japan

Received 13 April 2006; accepted 13 June 2006

Available online 23 June 2006

Edited by Hans-Dieter Klenk

© 2006 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Baculovirus; Virus-like particles; Capsid protein; Western blotting

1. Introduction

The family Caliciviridae is made up of four genera, Sapovirus, Norovirus, Lagovirus, and Vestivirus, which contain Sapporo virus (SaV), Norwalk virus (NoV), Rabbit hemorrhagic disease virus, and Feline calicivirus, respectively. Human SaV and NoV strains are agents of gastroenteritis. SaV strains were recently divided into five genogroups (GI–GV), of which GI, GII, GIV, and GV strains infect humans, while GIII strains infect porcine species [1]. The SaV GII, GIV, and GV genomes are each predicted to contain three main open reading frames (ORFs), whereas SaV GII and GIII have two ORFs. SaV ORF1 encodes for non-structural proteins and the major capsid protein (VP1). SaV ORF2 (VP2) and ORF3 (VP3) encoded proteins of yet unknown functions.

Human SaV and NoV strains are non-cultivable, but expression of the recombinant VP1 (rVP1) in a baculovirus expression system with insect cells results in the self-assembly of virus-like particles (VLPs) that are morphologically similar to the native viruses. However, the SaV rVP1 expression levels are considerably low. We have found that inclusions of short foreign nucleotide sequences inserted directly upstream from the predicted rVP1 AUG start codon lead to increased yield of VLPs. This method allowed us to express a SaV rVP1, which could not have been expressed to measurable or practical levels otherwise.

2. Materials and methods

SaV GI Mc114 strain (GenBank Accession Number, AY237422) was isolated from a male infant seven months of age from the McCormic Hospital, Chiang Mai, Thailand on the 7th May 2001 [8]. SaV GIV SW278 strain (Accession Number DQ125333) was isolated from an outbreak of gastroenteritis in Sweden, in 2004 [9]. SaV Mc114 rVP1 was designed with the 21-nucleotide-lobster sequence directly upstream from the VPI AUG start codon (termed Mc114 lob21 construct) as shown in Table 1. RNA extraction and RT-PCR were performed as previously described [3] and the PCR-amplified fragment was cloned into the Gateway Expression System (Invitrogen, Carlsbad, Calif.) as previously described [3], except for a slightly different donor vector (pDONR221). The sequence was verified as previously described [3]. A recombinant bacmid was transfected into Sf9 cells (Riken Cell Bank, Japan) and the recombinant baculovirus was collected as previously described [3]. EM, Western blotting, and antigen ELISA were performed as described previously [3,10,11].

The time-course expression of the Mc114 lob21 construct was compared to a construct without the additional upstream sequence (Mc114 Wt construct; [4]). The expression of the rVP1 constructs were analyzed by infecting recombinant baculoviruses at a MOI of 10 in 2.7 x 10⁶ confluent Tn5 cells in 1.0 ml of Ex-Cell 405 medium followed by incubation at 26 °C. The total culture medium was harvested 1, 2, 3, 4, 5, and 6 dpi. The culture medium was centrifuged for 5 min at 3000 x g, and further centrifuged for 40 min at 10000 x g. The VLPs in the culture medium were further concentrated by ultracentrifugation for 2 h at 50000 rpm at 4 °C (Beckman TLA-55 rotor), and then resuspended in 20 μl of Grace’s medium and stored at 4 °C. For Mc114 Western blotting, we used rabbit and guinea pig antisera that was raised against Mc114 VLPs [3,10,11]. Mc114 VP1 had a predicted molecular weight of approximately 60 kDa.
3. Results

EM confirmed that both Mc114 constructs, Mc114 lob21 and Mc114 Wt, formed VLPs morphologically similar to the native SaV with 38 nm in diameter (Fig. 1A). The antigen ELISA indicated that the Mc114 lob21 yield of VLPs was greater (between 1.5 and 3.0 \( \times \) ) than that of the Mc114 Wt at 4, 5, and 6 dpi (Fig. 1B). The differences in expression levels between these two constructs was also evident when we performed CsCl ultracentrifugation as described previously[12]. A viral band was visible with the Mc114 lob21 construct, but no viral band was visible with the Mc114 Wt construct (data not shown). These results indicated that the 21-nucleotides upstream from the SaV GI Mc114 VP1 AUG start codon increased the yield of VLPs.

Following the Mc114 lob21 results, we investigated whether a construct with an even shorter sequence directly upstream from the VP1 AUG start codon could form VLPs and whether this increased the expression levels and yield of VLPs. We decided to use the three nucleotides from the 3' end of the 21-nucleotide lobster sequence, i.e., nucleotides ACC (SW278 lob3 construct) and the entire 21-nucleotides of the lobster sequence (SW278 lob21 construct). The underlined sequence represents the putative VP1 sequences.

Table 1
Primer names and sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5'-3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mc114 lob21</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCACAATGCCATAAAAAACCGCCACC</td>
</tr>
<tr>
<td>Mc114 Wt</td>
<td>ATGGAGGGCAATGGCTCCAACTCA</td>
</tr>
<tr>
<td>SW278 lob3</td>
<td>GGGAGAACGTGTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCAACTGGAATGGCCAGCACCTGG</td>
</tr>
<tr>
<td>SW278 Wt</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGGAGGGTAATGGCCTACCCCAGGCTGGA</td>
</tr>
<tr>
<td>SW278 lob21</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCACAATGCCATAAAAAACCGCCACC</td>
</tr>
</tbody>
</table>

The bold represents the lobster sequences. For Mc114 we used the entire 21-nucleotides of the lobster sequence (Mc114 lob21 construct). For SW278 we used three nucleotides from the 3' end of the 21-nucleotide lobster sequence, i.e., nucleotides ACC (SW278 lob3 construct) and the entire 21-nucleotides of the lobster sequence (SW278 lob21 construct). The underlined sequence represents the putative VP1 sequences.

Fig. 1. Analysis of Mc114 expression in insect cells. (A) EM analysis of Mc114 lob21 VLPs. The bars represent 100 nm. (B) Antigen ELISA of the time-course expression of Mc114 lob21 and Mc114 Wt using hyperimmune rabbit and guinea pig antisera raised against Mc114 VLPs [4].
SW278 Wt VLPs were purified when 20 flasks (75 cm²) were used (data not shown). The purified SW278 lob3 VLPs was used to develop hyperimmune antiserum as previously described [12], and then the rVP1 time-course expression of the SW278 lob3 and SW278 Wt constructs were compared by Western blotting. SW278 VP1 had a predicted molecular weight of approximately 60 kDa. The 60-kDa SW278 lob3 rVP1 was clearly detected at 5 dpi, whereas a very faint 60-kDa SW278 Wt rVP1 was detected at 5 dpi (Fig. 2B). The expression level of rVP1 increased at 6 dpi with both constructs, however the rVP1 band intensity was greater with the SW278 lob3 construct. We estimated the SW278 lob3 had approximately 1.6× and 2.3× rVP1 increase at 5 dpi and 6 dpi, respectively [13]. These results indicated that the three nucleotides upstream from the SaV GIV SW278 VP1 AUG start codon increased the expression levels and yield of VLPs. Interestingly, we found that a construct that included the entire lobster 21-nucleotides (SW278 lob21 construct) formed VLPs, but the expression level was very low, i.e., less than 1 μg of VLPs when 20 flasks (75 cm²) were used (data not shown).

4. Discussion

Only three other expression studies of human SaV VLP formation have been reported using insect cells [14–16]. In one of those studies, the native upstream sequence of 73 nt from the predicted VP1 AUG codon was a crucial element for VLP formation [14], whereas one of the other two reports included the native 39 nucleotides upstream [15]. Porcine enteric calcivirus of SaV GIII also expressed VLPs in insect cells using a construct that contained nine foreign nucleotides upstream of the VP1 AUG codon, i.e., GTGTTCTGATGGGA (underlined) [17]. Jiang suggested that an upstream sequence could be responsible for initiating transcription or translation; alternatively, this element could serve as a spacer between the polyhedrin promoter and the VP1 gene, or as a stabilizer of the RNA after transcription [6,14]. Interestingly, Jiang also tried to express two other constructs that included the native 9 and 29 nucleotides upstream, respectively, but those also failed to express rVP1 or form VLPs [14]. We also expressed Mc114 rVP1 constructs that included the native 84 and 39 upstream nucleotide sequences, though they formed VLPs, they did not have increased the expression levels when compared to the Mc114 Wt construct (unpublished data). As mentioned earlier, the Kozak sequence, i.e., nucleotides ACCAUGG, is known to improve expression levels [7]. However, the Mc114 Wt construct included the Kozak sequence in the sense primer for the Gateway expression system (ACCAUGG, where the underlined AUG represented Mc114 VP1 start codon) (Table 1). Likewise, SW278 Wt also had the same Kozak sequence in the sense primer (Table 1). When we included the lobster 21-nucleotides in the Mc114 construct, we observed increased expression levels and yield of VLPs (Fig. 1), likewise when we included the ACC sequence upstream for the SW278 construct, we also observed increased expression levels and yield of VLPs (Fig. 2).

In conclusion, these results indicated that upstream sequences have increased the expression levels for SaV GI and GIV rVP1 by approximately 3× and 2.5×, respectively, in insect cells. Without the ACC foreign upstream sequence, we were unable to produce sufficient quantities of SW278 VLPs for development of hyperimmune antiserum. Since the SaV expression levels in insect cells were quite low and some strains cannot be expressed to detectable levels, we recommend that either the lob21 and/or the lob3 upstream sequences be incorporated into difficult to express constructs.

Acknowledgements: This work was supported in part by a grant for Research on Emerging and Re-emerging Infectious Diseases, Research on Food Safety from the Ministry of Health, Labor and Welfare of Japan, and a grant for Research on Health Science Focusing on Drug Innovation from The Japan Health Science Foundation.

References


