Genome Segment S8 of Grass Carp Hemorrhage Virus Encodes a Virion Protein

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Abstract
The complete nucleotide sequence of the genome segment S8 of grass carp hemorrhage virus (GCHV) was determined from cDNA corresponding to the viral genomic RNA. It is 1,287 nucleotides in length and contains a large open reading frame that could encode a protein of 409 amino acids with a predicted molecular mass of 44 kD. The S8 was expressed using the pET fusion protein vector and detected by Western blotting analysis using the chicken egg IgY against intact GCHV particles, indicating that S8 encodes a virion protein. Amino acid sequence comparisons revealed that the protein encoded by S8 is closely related to protein σ2 of mammalian reovirus, suggesting that the deduced protein of S8 is an inner capsid protein.

The nucleotide sequence data for GCHV S8 reported in this paper can be accessed in the GenBank databases under the accession number AF259053.
The strain 873 of GCHV was adapted for growth in CIK cell [7]. Virus was purified from a continuous sucrose gradient (30–60% sucrose) and centrifuged at 100,000 g for 2 h. Genomic dsRNA was extracted from the purified virus particles by proteinase K and phenol-chloroform extraction. The synthesis of the full-length cDNA of dsRNA was carried out with the method of Lambden et al. [8]. Amplified cDNA products were separated on agarose gel (fig. 1C) and the fragment approximately 1,300 bp, which corresponded in size to that calculated from the GCHV dsRNA molecular weight for S8 segment, was excised and purified by Glassmilk DNA purify kit (BioStar). The S8 cDNA was directly ligated into pGEM-T vector, and transformed into DH5α strain of Escherichia coli (Gibco BRL). The recombinant plasmid containing the full-length cDNA was identified according to the size of inserted segment by PCR and was purified using a plasmid DNA purification miniprep kit (Viogene). The nucleotide sequence of S8 was determined on an ABI 310 Genetic Analyzer (Perkin-Elmer). To verify the S8 cDNA, the genome dsRNAs were separated in a 1.0% agarose gel, transferred to a Nylon membrane (Hybond), and hybridized with the S8 cDNA probe labeled with digoxigenin-dUTP, followed by Northern blotting analysis using Dig High Primer Labeling and Detection Starter Kit (Boehringer-Mannheim) (fig. 1A, B).

The complete nucleotide and amino acid sequences of genome segment S8 of GCHV are shown in figure 2. The S8 sequence is 1,287 bp long and has a large open reading frame (ORF). The distribution of the four bases was found relatively rich in cytidine (21.3% A, 22.68% G, 23.66% U, 32.36% C). The ORF starts with the first AUG (at nucleotides 12–14) and terminates at nucleotides 1239–1241 with a UAA. The AUG appears to be a very favorable context for initiation of translation (AU-CAUG), according to the consensus sequence established by Kozak [9]. The terminal region of the GCHV S8 segment displays the nucleotide sequences 5’ GUAUUAU and CAUC 3’, which are conserved in genome segments of GCHV [unpubl. data]. In addition, segment-specific inverted repeats, GUGAUGGCA at 9–17 and UGCUCA-CAC at 1270–1277, were identified adjacent to the terminal sequences.

The ORF of S8 encodes a 409 amino acid protein of calculated molecular mass of 44 kD. To clarify the identifying of the protein, full-length segment of S8 was amplified with two primers (5’ AGGGATCCGCGGAATGTTATT 3’ and 5’ GGAAGCTTGGCGGTAAA-GTATGA 3’) from the pGEM-T vector and inserted into the BamH I and Hind III sites of pET-28a vector and expressed in Escherichia coli. Protein expression was performed according to the pET system manual provided by Novagen Corp. The extracted E. coli proteins were resolved by SDS-PAGE, transferred to NC membrane (Gelman); Western blotting was performed with immunoglobulin (IgY) against intact virus particles that isolated from the yolks of eggs of GCHV-immunized hens according to the method of Horikoshi et al. [10]. Peroxidase-labeled rabbit anti-chicken IgY (Promage) was used for final detection. The results are shown in figure 3, in which an approximately 48-kD band was identified. It is very similar to the predicted molecular mass of the fusion protein because the sizes of the S8 product and foreign sequence in pET28a vector are 44 and 4.5 kD, respectively. The band, 48 kD, was also detected by Western blotting, and no obvious additional bands could be detected in the preparation, identifying the IgY that reacted specifically with the protein expressed by S8. It could be deduced that GCHV S8 encodes a virion protein.

Since prior work has revealed that the largest three segments and S10 segment are similar to the segments of mammalian reovirus (MRV), a member of another genus, Orthoreovirus in the family Reoviridae [4], we compared the deduced protein of GCHV S8 with the reported proteins of MRV in computer using CLUSTALW programs. The protein encoded by S8 showed 18.54% identity with that of the inner capsid protein Û2 of MRV [11] in a 426-amino-acid alignment length though no long identical predicted amino acid sequences were found between them. More detailed comparisons revealed that it is more closely...
Fig. 2. Complete nucleotide sequence (presented in the cDNA form) of S8 RNA segment of GCHV. The conserved 5' and 3'-terminal nucleotide sequences are indicated with a gray background and the inverted repeats are underlined. The initiation and termination sites are boxed.

Fig. 3. Western blot analysis of expressed pET-S8 fusion protein. The extracted E. coli proteins were resolved by 10% SDS-PAGE and stained with Coomassie blue (lane A) or transferred to NC membrane and probed with IgY (lane C). An insert-minus plasmid control (lane B) was also probed with IgY (lane D).

Fig. 4. Secondary structure, charge, hydrophilic plot, and antigenic index of the protein encoded by GCHV S8. A = Alpha regions; B = beta regions; T = turn regions; F = flexible regions; P = positive charge; H = hydrophilic plot; G = antigenic index.

related to MRV σ2 protein. First, the secondary structure analysis of the amino acid sequence of the ORF [12] indicated that the protein included a carboxy-terminal region that is formed from α-helices and β-turns and a large amino-terminal region that is formed predominantly by β-strands and β-turns. A similar distribution of the secondary structure along the sequence was also observed in MRV σ2 [11], suggesting that the deduced proteins of GCHV S8 and MRV σ2 are structurally and functionally related. Second, the hydrophilic plots [13] showed very similar profiles among the deduced proteins of GCHV S8 and MRV σ2 (data not shown), suggesting that they may have an analogous molecular conformation. Third, the size of the predicted protein encoded by S8, 44 kD, is very similar to MRV σ2, 47 kD. These facts support the hypothesis that GCHV S8 may correspond to the MRV S2 gene, which encodes the inner capsid protein, MRV σ2 [11]. This implies that S8 encodes an analogous inner capsid protein of GCHV.

A search made against protein database using BLASTp algorithm [14] revealed that a region (amino acids 228–263) in deduced protein encoded by S8 has similarity with a region in the immunoglobulin gamma (amino acids 210–245) of cynomolgus monkey [15] with 38% identity. It does not appear to be an easily interpretable match. Whereas, interestingly, this short region is characterized
by the largest hydrophilic and highest antigenic region and contains three cysteine residues (fig. 4). Moreover, it is identified as the most flexible portion, which corresponds to the sequences predicted to predominantly form β-turns which alternate with β-sheets. The region may be an important determinant for protein-protein interaction or for host immune response to this protein. Moreover, positive charges are concentrated in this region, implying that it also could be involved in protein RNA interaction. Some suggestions are given by prior work on MRV Û2 showing that it can interact with MRV λ1 protein to form the inner capsid [16] and has affinity for reovirus dsRNA [11]. However, extensive evidence has to be presented to strengthen the above views. Our study serves as a preliminary to future investigations to learn more about the structure and function of protein encoded by GCHV S8.

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