

CONFIRMATION OF THE GENOME POSITION AND MASS OF THE VIRAL PROTEIN, VP3, OF *SOLENOPSIS INVICTA* VIRUS 1 (PICORNAVIRALES: DICISTROVIRIDAE) INFECTING THE RED IMPORTED FIRE ANT (HYMENOPTERA: FORMICIDAE)

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Solenopsis invicta virus 1 (SINV-1) is the first virus to be discovered and characterized in any ant species (Valles et al. 2004). The virus exhibits a host range limited to fire ants in the *Solenopsis* genus (Valles et al. 2007). The single-stranded RNA genome is composed of 8,026 nucleotides containing 2 large open reading frames (ORFs) in the sense orientation with an untranslated region (UTR) at each end and between the two ORFs. The 5'-proximal ORF encodes for the non-structural proteins, helicase, protease, and RNA-dependent RNA polymerase (RdRp) and the 3'-proximal ORF for the structural (or capsid) proteins. ORF 2 was originally reported to commence at nucleotide position 4,390 at a canonical AUG start codon. However, it was later revealed empirically to actually start at codon GCU (genome position 4423-4425) which encodes for an alanine (Valles and Hashimoto 2008). SDS-PAGE analysis of purified SINV-1 particles yields 3 major (VP1, VP2, VP3) and one minor (VP4) capsid proteins. N-terminal analysis has revealed

the margins of the capsid proteins and the corresponding scissile bonds within the structural polyprotein (Valles and Hashimoto 2008). Amino acid residues at these junctions were consistent with other dicistroviruses and unclassified picorna-like insect-infecting viruses (Liljas et al. 2002). The objective of this short communication was to confirm production and genome location of VP3 within the SINV-1 structural polyprotein using Western analysis. Polyclonal antibodies were synthesized from a recombinant peptide sequence obtained from the predicted amino acid sequence of VP3 (i.e., SRGGYRYKFFADDN). The peptide sequence is located at amino acid positions 997-1010 of the structural polyprotein (Fig. 1A). Polyclonal antibodies were raised in a rabbit host by GenScript USA, Inc. (Piscataway, NJ) according to the company's standard protocols.

Ants used for purification of virus were collected by excavating nests from locations in Gainesville, FL. Colonies were examined by RT-PCR methods to identify ants exclusively infected with

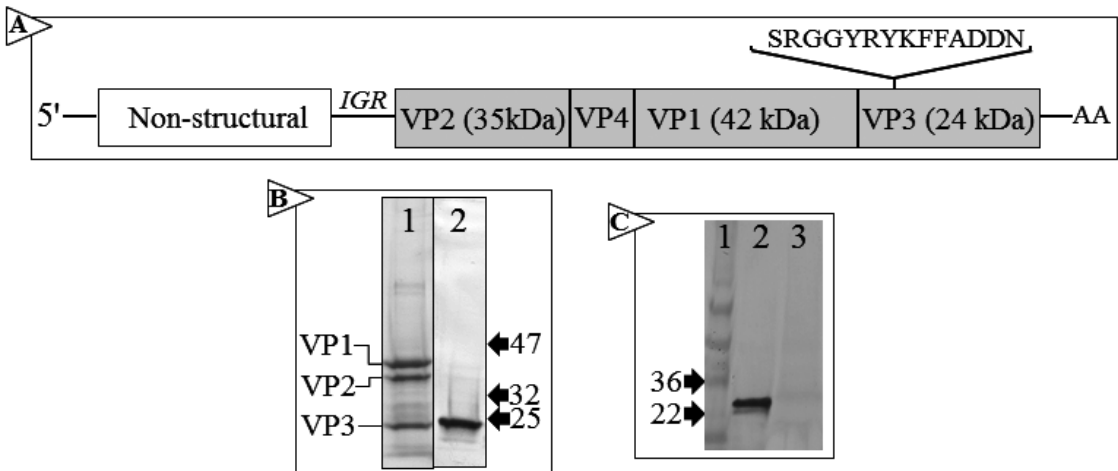


Fig. 1. (A) Diagrammatic representation of the SINV-1 genome. ORF 2 (3'-proximal) encodes the structural (or capsid) proteins. A short peptide sequence fragment was synthesized from the predicted sequence of ORF 2 at the approximate position shown and used to generate rabbit polyclonal antibodies. (B) Purified particles of SINV-1 were denatured and separated by SDS-PAGE (lane 1). Electroblooded proteins from lane 1 were probed with the polyclonal antibody preparation (lane 2). Molecular mass markers are provided on the right side (kDa). (C) Homogenates of alimentary canals dissected from SINV-1-infected (lane 1) and -uninfected (lane 2) *S. invicta* workers were also separated by SDS-PAGE and probed with the antibody preparation. Molecular mass markers are provided on the left side (kDa).

SINV-1 (Valles et al. 2009). SINV-1 purification was accomplished by differential and isopycnic centrifugation as described previously (Valles and Hashimoto 2009). Briefly, 30 g of ant workers from a SINV-1-infected fire ant colony were homogenized for 2 minutes in a Waring blender in 200 ml of NTB buffer (10 mM Tris, pH 7.25, 0.1 mM NaCl). The homogenate was filtered through cheesecloth then extracted with 2 volumes of chloroform for 30 minutes with gentle shaking. The mixture was centrifuged for 10 minutes at 2,000 rpm to separate the phases. The supernatant was removed and applied to a 1.2/1.5 g/ml CsCl step gradient and centrifuged at $195,000 \times g$ for 2 hours. The interface was recovered by pipette, brought to 1.35 g/ml CsCl and centrifuged for 17 hours at $330,000 \times g$. A band was observed at 1.33 g/ml and recovered. The solution was diluted in NTB buffer and centrifuged at $330,000 \times g$ for 2 hours. The pellet was resuspended in 0.5 ml of NTB.

Western blotting was accomplished by separating proteins by SDS-PAGE (10% polyacrylamide) and subsequently electroblotted onto a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA) for 2 hours at 350 mA. Western analysis was conducted with SINV-1 VP3 polyclonal antibodies at an 8,000-fold dilution. Briefly, the electroblotted PVDF membrane was blocked in TBS (tris buffered saline; 20 mM Tris-HCl, 500 mM NaCl, pH 7.5) + 1% BSA (bovine serum albumin) for 1 hour. Primary antibody (SINV-1 VP3) was added to the TBS + 1% BSA solution for 2 hrs at room temperature with shaking (40 rpm). The membrane was rinsed twice with TTBS (TBS + 0.05% Tween 20), probed with secondary antibody (10,000-fold dilution), goat anti-rabbit conjugated with alkaline phosphatase [Sigma, St. Louis, MO] for 1 hour, and rinsed twice with TTBS. The membrane was incubated for several minutes with BCIP (5-bromo-4-chloro-3-indolyl-phosphate) and NBT (nitro blue tetrazolium) for the colorimetric detection of alkaline phosphatase activity. Once bands were detected on the blot, the reaction was terminated by rinsing the membrane with deionized water 3 times.

Polyclonal antibodies generated from rabbits in response to challenge from the peptide synthesized from the predicted sequence of ORF 2 of SINV-1 recognized a single protein band from purified preparations of SINV-1 with a molecular mass of 24 kDa (Fig. 1B). Three prominent bands of anticipated mass are observed from purified SINV-1 particles (VP1, VP2, and VP3). While our previous work established the N-termini of each of these proteins, recognition of VP3 by antibodies generated from the predicted sequence of this capsid protein provides further confirmation of its synthesis, genome position, and mass. Furthermore, VP3 antibodies

also recognized a 24 kDa protein in alimentary canals taken from SINV-1-infected *S. invicta* workers but not uninfected *S. invicta* (Fig. 1C). Detection of VP3 with the antibody preparation using whole worker ants was observed, however, the response was less clear compared with alimentary canal preparations. We suspect that a component liberated from whole ants is responsible for altering the sensitivity of the detection reaction. We expected to also detect the unprocessed ORF 2 polyprotein (molecular mass of 126 kDa). However, a protein of this mass was not observed. Epitope availability, antibody detection limitations, and inactive periods of viral replication may explain failure to detect the unprocessed polyprotein. In conclusion, we provide further empirical evidence for the production of VP3 from SINV-1 ORF 2, as well as confirmation of the genome position of this capsid protein.

SUMMARY

A predicted peptide sequence corresponding to the capsid protein VP3 of SINV-1 was used to prepare a polyclonal antibody preparation and probe SDS-PAGE separated proteins from SINV-1 particles and *S. invicta* ants. The SINV-1 VP3 antibody preparation recognized a 24 kDa protein from denatured SINV-1 particles and from SINV-1-infected worker ants confirming VP3 synthesis, genome position, and mass.

ACKNOWLEDGMENTS

We gratefully acknowledge Drs. D. Oi and M-Y. Choi (USDA-ARS) for providing helpful reviews of the manuscript. Trade, firm, or corporation names in this publication are for convenience of the reader and do not constitute official endorsement or approval by the USDA-ARS.

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