

Abstract

# Characterization of Glycoproteins from Insect-Specific Goutanap and Negev Viruses <sup>†</sup>

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**Abstract:** *Negev viruses* (NVs) are a recently discovered taxon of enveloped, positive sense, single-stranded RNA viruses, infecting blood-sucking insects. While classical arthropod-borne (arbo)viruses like dengue and Chikungunya infect both insects and vertebrates, NVs are restricted to insects and do not have any known vertebrate host and are thus classified as insect-restricted viruses. Previous works have predicted a structure consisting of three ORFs, the first with homologous regions to RNA-dependent RNA polymerase, helicase, and methyl transferases in plant viruses. On the contrary, *ORF2* and *ORF3* do not have homologs and are predicted to encode membrane glycoproteins. Their structures, functions, and significance remain vague. We focus on the characterization of the viral proteins, structural organization of the virion, and the principles of their interaction with the host cell. We purified the virion particles of Negev virus produced in mosquito cells and identified its structural components. In addition, we cloned and overexpressed *ORF2* and *ORF3* of *Negev viruses*. Furthermore, we defined and successfully produced and purified recombinant *ORF2*. Subsequent characterization using gel filtration, ion exchange, and MALS techniques revealed that the *ORF2* of *Negev viruses* exhibit different higher order assembly patterns: dimerization and multimerization in a concentration- and pH-dependent manner that correspond to their biological role. We combine biochemical, structural and cell biology techniques to unravel mechanisms of Negev virus interaction with the host cell.

**Keywords:** Negev viruses; viral entry and fusion; viral envelope glycoproteins, insect restricted viruses



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