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Short communication

Tomato spotted wilt virus glycoprotein G_C is cleaved at acidic pH

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Abstract

Tomato spotted wilt virus (TSWV) is a plant-infecting member of the family *Bunyaviridae*. TSWV encodes two envelope glycoproteins, G_N and G_C, which are required for virus infection of the arthropod vector. Other members of the *Bunyaviridae* enter host cells by pH-dependent endocytosis. During this process, the glycoproteins are exposed to conditions of acidic pH within endocytic vesicles causing the G_C protein to change conformation. This conformational change renders G_C more sensitive to protease cleavage. We subjected TSWV virions to varying pH conditions and determined that TSWV G_C, but not G_N, was cleaved under acidic pH conditions, and that this phenomenon did not occur at neutral or alkaline pH. This data provides evidence that G_C changes conformation at low pH which results in altered protease sensitivity. Furthermore, sequence analysis of G_C predicts the presence of internal hydrophobic domains, regions that are characteristic of fusion proteins. Like studies with other members of the *Bunyaviridae*, this study is the first step towards characterizing the nature of cell entry by TSWV.

Keywords: Envelope glycoprotein; *Frankliniella occidentalis*; *Tospovirus*

Tomato spotted wilt virus (TSWV) is the prototypic member of the genus *Tospovirus* in the family *Bunyaviridae*. The Tospoviruses are unique within the *Bunyaviridae* because they infect plant and insect hosts. All viruses in the *Bunyaviridae* have a tripartite, negative-strand RNA genome that codes for four structural proteins: a nucleocapsid (N) protein on the small (S) RNA, two membrane glycoproteins on the medium (M) RNA segment and a large (L) protein on the large RNA. The glycoproteins are derived from a polyprotein that is proteolytically processed to yield the two glycoproteins (GPs) designated G_N and G_C based on their position relative to the amino- and carboxyl-termini of the polyprotein.

The envelope GPs play important roles in the entry of TSWV into the insect midgut, the first site of infection (Ullman et al., 1992); however, the distinct role of G_C in virus entry has not been characterized. TSWV is transmitted to plants via thrips (Thysanoptera:Thripidae) that harbor the virus in a persistent-propagative manner. Both G_N and G_C are critical for infection of thrips, but they are not required for initial infection of plants (Nagata et al., 2000; Resende et al., 1991). Electron microscopy observations of larval thrips that fed on TSWV-infected plants suggest that TSWV virions fuse with midgut membranes during viral entry (Ullman et al., 2002). A soluble form of the G_N protein binds larval thrips midguts and inhibits TSWV acquisition indicating that G_N is involved in virus acquisition by thrips (Whitfield et al., 2004).

Virus entry into a host cell entails a complex series of events that culminate with the fusion of viral and host membrane. Many enveloped viruses enter cells by pH-dependent receptor-mediated endocytosis. Virus attachment is followed by the formation of a cellular compartment (e.g., endosome) and a subsequent change in pH within that compartment triggers a conformational change in the viral fusion protein allowing a fusion peptide or fusion loop to become exposed (Modis et al., 2004; Skehel and Wiley, 2000). The fusion peptide inserts into the target membrane and leads to the fusion of virion and host membranes and the subsequent release of the

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virion contents into the cytoplasm (reviewed in Sieczkarski and Whittaker, 2002).

Evidence indicates that members of the *Bunyaviridae* enter cells by pH-dependent receptor mediated endocytosis (Hacker and Hardy, 1997; Jin et al., 2002; Pekosz and Gonzalez-Scarano, 1996). Jin et al. (2002) found that Hantaan virus enters cells via pH-dependent endocytosis and that entry vesicles were composed of clathrin. Further support for pH-dependent entry comes from studies of the orthobunyaviruses. After exposure to acidic pH, La Crosse virus (LAC) and California encephalitis (CE) G_C undergoes a conformational change and is more susceptible to protease cleavage, consistent with endocytic entry (Gonzalez-Scarano, 1985; Hacker and Hardy, 1997; Pekosz and Gonzalez-Scarano, 1996). Furthermore, CE and LAC-infected cells form syncytia when the extracellular pH is lowered which indicates that virus entry is stimulated by low pH (Gonzalez-Scarano et al., 1984; Hacker and Hardy, 1997). When CE-infected cells were treated with G_C monoclonal antibody (MAb), virus fusion was inhibited without compromising virus attachment (Hacker and Hardy, 1997). These data provide strong evidence that members of the *Bunyaviridae* enter cells in pH-dependent process and the G_C protein plays a role in membrane fusion. Here, we provide the first evidence that the G_C protein of a plant-infecting member of the *Bunyaviridae* undergoes a pH-dependent cleavage consistent with a G_C conformational change at low pH.

Virus was maintained in *Datura stramonium* plants as described in Ullman et al. (1992, 1993) and TSWV virions were isolated using the method of Gonsalves and Trujillo (1986). To determine whether TSWV GPs undergo pH dependent cleavage, virions were incubated in 100 mM sodium phosphate buffer at pH 5.8, 6.1, 7.0, or 8.0 or citrate buffer (15 mM sodium citrate, 135 mM NaCl; Hacker and Hardy, 1997) at pH 5.8, 7.0, or 8.0. Samples were incubated at 27 or 37 °C for 3 h. Alternatively, in some experiments samples were also subjected to pH change for 15 min and then neutralized with 100 mM Tris–HCl, pH 7.0 before incubation for 3 h at 37 °C. Equal amounts of protein were separated on 10% polyacrylamide gels by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membrane, and incubated with G_N or G_C MAbs.

We used sequence analysis programs to identify structural features of the 1135 amino acid TSWV polyprotein. PeptideCutter (http://www.expasy.org/tools/peptidecutter/) was used to identify enzymes that cleave the polyprotein and positions of cleavage sites. The sequence analysis programs HMMTOP (Tusnády and Simon, 2001), Tmpred (Hofmann and Stoffel, 1993), SOSUI (http://sosui.proteome.bio.tuat.ac.jp/sosuiframe0.html), TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/) and PHDhtm (Rost et al., 1996) were used to predict hydrophobic and transmembrane domains of G_C.

A pH-dependent cleavage of G_C was observed by Western blot analysis. At neutral and alkaline pH, the G_C protein molecular mass was approximately 85 kDa (Fig. 1A and B), but at acidic pH we observed an 85 kDa protein and a 72 kDa protein that reacted with the G_C MAb. The 72 kDa band was more intense in the pH 5.8 treatments than in the pH 6.1 treatment indicating that the cleaved protein was the predominant form of G_C at the most acidic pH tested. We did not observe the expected 13 kDa cleavage product by Western blot (Fig. 1A) or Coomassie stained gels (data not shown). It is possible that the smaller cleavage product was susceptible to further enzymatic degradation and therefore no longer detectable. Two forms of G_C were also observed

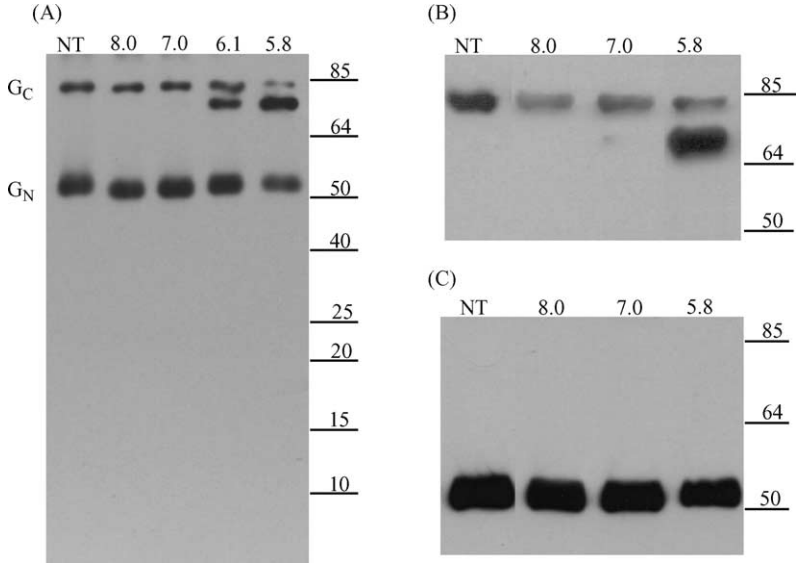

Fig. 1. G_C is cleaved at acidic pH. Purified TSWV virions were incubated for 2 h at 37 °C in 100 mM sodium phosphate buffer at pH 8.0, 7.0, 6.1, 5.8, or not treated (NT). Equal amounts of protein were added to each well of a 10% polyacrylamide gel. Proteins were separated by SDS-PAGE and transferred to nitrocellulose membrane. (A) Membrane probed with G_N and G_C monoclonal antibodies (MAb). (B) Membrane probed with G_C MAb. (C) Membrane probed with G_N MAb.