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Solution structure of the porcine sapovirus VPg core reveals a stable three-helical bundle with a conserved surface patch

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ABSTRACT

Viral protein genome-linked (VPg) proteins play a critical role in the life cycle of vertebrate and plant positive-sense RNA viruses by acting as a protein primer for genome replication and as a protein cap for translation initiation. Here we report the solution structure of the porcine sapovirus VPg core (VPg^C) determined by multi-dimensional NMR spectroscopy. The structure of VPg^C is composed of three α-helices stabilized by several conserved hydrophobic residues that form a helical bundle core similar to that of feline calicivirus VPg. The putative nucleotide acceptor Tyr956 within the first helix of the core is completely exposed to solvent accessible surface to facilitate nucleotidylation by viral RNA polymerase. Comparison of VPg structures suggests that the surface for nucleotidylation site is highly conserved among the *Caliciviridae* family, whereas the backbone core structures are different. These structural features suggest that caliciviruses share common mechanisms of VPg-dependent viral replication and translation.

1. Introduction

The *Caliciviridae* family is a member of single-stranded and positive-sense (+) RNA viruses and can be divided into at least four genera based on genome organization and genetic analysis: *Vesivirus*, *Lagovirus*, *Norovirus*, and *Sapovirus* [1,2]. Caliciviruses in a number of organisms cause a wide range of diseases in vertebrates. For instance, human norovirus (HuNV) causes acute gastroenteritis in humans [3], while the feline calicivirus (FCV) is related to upper respiratory infections in cats [4]. In particular, sapoviruses (SaVs) are a major cause of gastroenteritis worldwide in both humans and animals [5,6]. Although SaV infections have raised public health concerns of potential cross-species transmission [7], studies on the molecular mechanisms of viral infection and replication have been hindered by the lack of a cell culture system for human SaV. Therefore, a cultivable porcine sapovirus (PSV) system has been used to study the viral infectious cycle and molecular mechanisms of other SaVs including human SaV [8].

Viral protein genome-linked (VPg) proteins have been identified as a protein primer during RNA synthesis in many virus families including *Caliciviridae* and *Picornaviridae* [9,10]. VPg proteins are covalently linked to the 5′-end of the viral genome or sub-genome of (+) RNA viruses by a phosphodiester bond between the hydroxyl group of a serine or tyrosine residue in VPg and the 5′-end of uridine or guanine of the viral RNA [11,12]. Studies of the VPg proteins from various plant and vertebrate (+) RNA viruses revealed their critical roles in viral replication and translation [13,14]. In caliciviruses, the viral RNA polymerase can nucleotidylate the VPg protein [15], and the nucleotidylated VPg is extended to produce RNA-linked VPg in a template-dependent or -independent manner [16,17], indicating that caliciviral VPgs act as a protein primer for the genome synthesis of (+) RNA viruses. The nucleotide is linked to a conserved tyrosine residue: Tyr24, Tyr26 or/and Tyr117, Tyr27, and Tyr21 in the VPg of FCV, murine norovirus (MNV), HuNV, and rabbit hemorrhage disease virus (RHDV), respectively [15–19].

VPg proteins of caliciviruses are also involved in the initial stage of virus infection and viral protein synthesis. The (+) RNA viruses are immediately translated upon infection with the viral genome acting as an mRNA template. In this stage, the VPg covalently linked

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to the viral genome binds to host translation initiation factors (IFs) and functions as a proteinaceous cap substitute. The VPg proteins from FCV and MNV can directly interact with the cap-binding protein eIF4E [20,21]. The FCV VPg-eIF4E interaction is essential for FCV translation, but the interaction between MNV VPg and eIF4E is dispensable for viral translation initiation [20]. We recently demonstrated that the PSV VPg is essential for viral translation and infectivity by directly forming the VPg-eIF4E complex [22], whereas the interaction between VPg and eIF4G is required for MNV translation initiation [23]. These observations suggested that the caliciviral VPg proteins have diverse and complex roles in viral translation initiation and infection.

VPg proteins vary in size and sequence among viruses, indicating large differences in their respective structures. For example, the calicivirus VPgs are 13–15 kDa proteins that form a stable helical core structure [24]. On the other hand, the picornavirus VPgs are short peptides of 22–24 amino acids that have no distinct structure and are ordered upon interaction with RNA genome [14]. Recently, the structures of two VPg proteins from FCV and MNV that are closely related to PSV were determined using solution NMR spectroscopy [24]. Although the structured cores of the FCV and MNV VPgs are distinct, especially in terms of sequence length, they have a common compact helical core flanked by unstructured N- and C-terminal regions. The nucleotidylation Tyr residues are exposed to solvent and are located in a conserved position in the first helix of the core.

Our previous study clearly showed that the PSV VPg-eIF4E interaction is required for PSV translation, indicating that PSV is functionally similar to FCV [22]. However, structures of PSV VPgs have not been reported, which hinders understanding of the molecular mechanism of PSV VPg-mediated viral infection and proliferation. In this study, we determined the solution structure of the PSV VPg core (VPg^C).

2. Materials and methods

2.1. Cloning, expression, and purification of VPg

The genes encoding PSV VPgs (residues Ala935–Glu1048, Ala935–Gly1022, Ala945–Gly1022, and Ala948–Ser1006) were amplified from the PSV genome by polymerase chain reaction (PCR) (Fig. 1A). The PCR product was cloned into pP_{RO}EX-HTc (Invitrogen, Life Technologies). These expression constructs express 25 extra amino acids at the N-terminus including six continuous His residues and the Tobacco etch virus (TEV) cleavage sequence. The recombinant plasmids were transformed into the *Escherichia coli* B834(DE3) strain, which was grown in Luria–Bertani medium containing 100 μg mL^{-1} ampicillin at 310 K. After induction by the addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside, the culture medium was maintained for a further 8 h at 310 K. Cells were harvested by centrifugation and re-suspended in buffer A (20 mM Tris–HCl at pH 7.5 and 500 mM

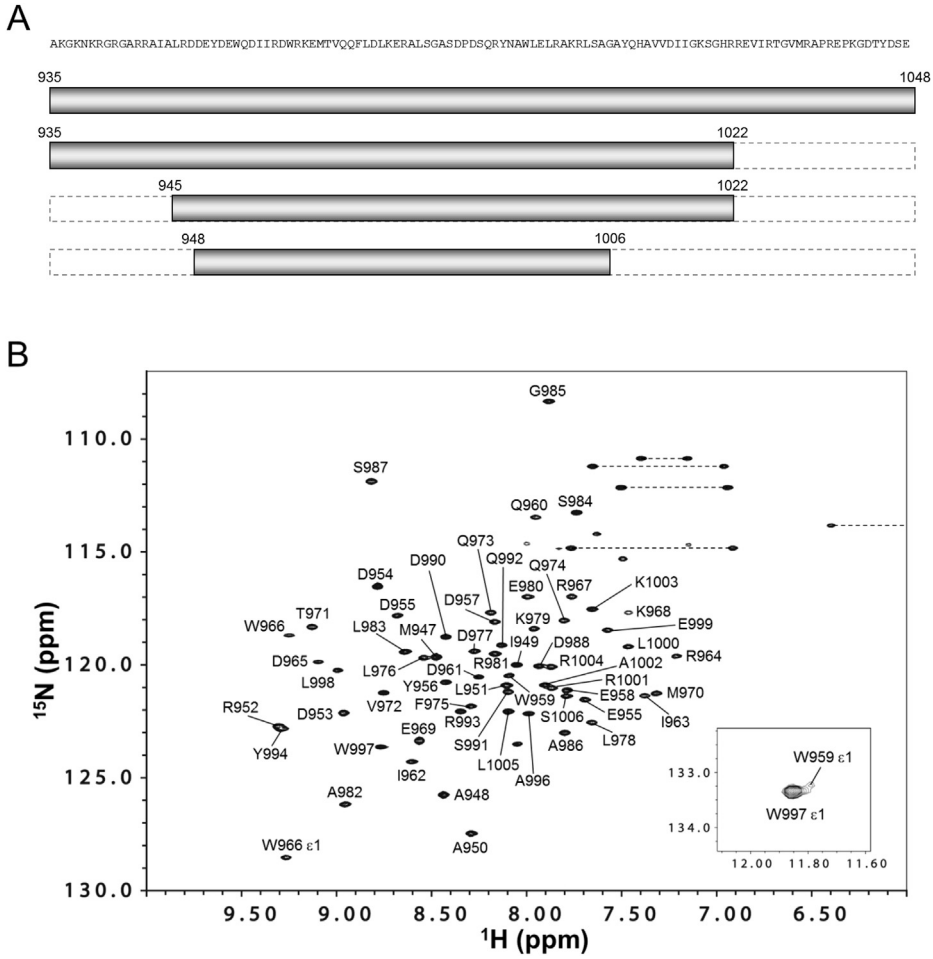

Fig. 1. PSV VPg protein constructs (A) and ^1H–^{15}N HSQC spectrum of PSV VPg^C (Ala948–Ser1006) (B). Residue numbers are labeled on the cross peaks. Cross peaks from the two tryptophan side chains of PSV VPg are shown in the inset.