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Biochemical and structural studies of the oligomerization domain of the Nipah virus phosphoprotein: Evidence for an elongated coiled-coil homotrimer

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ABSTRACT

Nipah virus (NiV) is a recently emerged severe human pathogen that belongs to the *Henipavirus* genus within the *Paramyxoviridae* family. The NiV genome is encapsidated by the nucleoprotein (N) within a helical nucleocapsid that is the substrate used by the polymerase for transcription and replication. The polymerase is recruited onto the nucleocapsid via its cofactor, the phosphoprotein (P). The NiV P protein has a modular organization, with alternating disordered and ordered domains. Among these latter, is the P multimerization domain (PMD) that was predicted to adopt a coiled-coil conformation. Using both biochemical and biophysical approaches, we show that NiV PMD forms a highly stable and elongated coiled-coil trimer, a finding in striking contrast with respect to the PMDs of *Paramyxoviridae* members investigated so far that were all found to tetramerize. The present results therefore represent the first report of a paramyxoviral P protein forming trimers.

Introduction

The Nipah (NiV) and Hendra (HeV) viruses are recently emerged, severe human pathogens within the *Paramyxoviridae* family (Eaton et al., 2007). A few distinctive properties, including the spectacularly larger size of their phosphoproteins (P), set them aside from other paramyxoviruses and led to their classification within the *Henipavirus* genus of the *Paramyxoviridae* family (Wang et al., 2000). The newly identified Cedar virus has been also classified within this genus (Marsh et al., 2012). The *Henipavirus* genome is encapsidated by the nucleoprotein (N) within a helical nucleocapsid. This helical N:RNA complex, rather than naked RNA, serves as substrate for both transcription and replication. By analogy with other paramyxoviruses, the viral polymerase is thought to consist of the L protein and the P protein. The P protein is an essential polymerase cofactor as it tethers L onto the nucleocapsid template. Beyond its role in recruiting the L protein, P is also thought to serve as a chaperone for N, in that its association prevents illegitimate self-assembly of N (see (Albertini et al., 2005; Blocquel et al., 2012a; Lamb and Parks, 2007; Roux, 2005) for reviews on paramyxovirus transcription and replication). *Henipavirus* N and P proteins were shown to interact with each other (Habchi et al., 2011), being able to form both homologous and heterologous N–P complexes (Blocquel et al., 2012b; Chan et al., 2004; Omi-Furutani et al., 2010).

So far, structural and molecular information on *Henipavirus* proteins is scarce. Indeed high-resolution structural data are limited to their surface proteins, where crystallographic studies led to the determination of the 3D structure of *Henipavirus* fusion and attachment proteins (Bowden et al., 2008a, 2008b, 2010;

Abbreviations: NiV, Nipah virus; HeV, Hendra virus; N, nucleoprotein; L, large protein; P, phosphoprotein; PNT, P N-terminal domain; PCT, P C-terminal domain; PMD, P multimerization domain; XD, X domain of P; MeV, measles virus; SeV, Sendai virus; MuV, mumps virus; RV, rabies virus; VSV, vesicular stomatitis virus; RDV, Rinderpest virus; RSV, respiratory syncytial virus; NMR, nuclear magnetic resonance; PCR, polymerase chain reaction; OD, optical density; IMAC, immobilized metal affinity chromatography; GF, gel filtration; SDS-PAGE, sodium dodecyl sulphate polyacrylamide electrophoresis; R_S, Stokes radius; R_g, radius of gyration; MALDI-TOF, matrix-assisted laser desorption ionization/time of flight; CD, circular dichroism; TFE, 2,2,2-trifluoroethanol; MRE, mean ellipticity values per residue; SAB, Suberic acid bis (*N*-hydroxy-succinimide ester); SAXS, small angle X-ray scattering; T_m, melting temperature

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Lou et al., 2006). The only available molecular data on *Henipavirus* N and P proteins come from our previous studies (Blocquel et al., 2012b; Habchi et al., 2011, 2010). Those studies showed that the N and P proteins possess a modular organization consisting of both ordered and intrinsically disordered regions, where these latter are functional regions that lack highly populated secondary and tertiary structure under physiological conditions in the absence of a partner (for reviews on IDPs, see (Chouard, 2011; Dunker et al., 2008a, 2008b; Turoverov et al., 2010; Uversky, 2010, 2013; Uversky and Dunker, 2010)). *Henipavirus* N proteins were shown to consist of a structured region (N_{CORE}) and of an intrinsically disordered C-terminal region (N_{TAIL}, aa 400–532). Likewise, the P proteins contain an exceptionally long N-terminal disordered region (PNT, aa 1–406 in NiV) (Habchi et al., 2010) and a C-terminal moiety (PCT, aa 407–709 in NiV). While PNT is common to both P and V proteins, with this latter originating from co-transcriptional editing (addition of an extra G nucleotide) of the mRNA of NiV P, PCT is unique to the P protein. PCT has a modular organization being composed of alternating disordered and ordered regions: it embraces a predicted disordered region overlapping the V ORF (aa 407–456 in NiV) referred to as spacer, a structured region (aa 470–578) referred to PMD (for P multimerization domain), a disordered linker (aa 579–659 in NiV) and a globular region (aa 660–709) referred to as X domain (XD) (Fig. 1) (Habchi et al., 2010). Like in the closely related measles (MeV) and Sendai (SeV) viruses (Belle et al., 2008; Bischak et al., 2010; Blanchard et al., 2004; Bourhis et al., 2004, 2005; Gely et al., 2010; Houben et al., 2007; Jensen et al., 2010; Johansson et al., 2003; Kavalenka et al., 2010; Longhi et al., 2003; Morin et al., 2006; Ringkjøbing Jensen et al., 2011), *Henipavirus* X domains adopt an α-helical conformation and were shown to trigger α-helical folding of *Henipavirus* N_{TAIL} (Habchi et al., 2011; Martinho et al., 2012).

The structure of MeV XD has been solved both in the crystal and in solution and was shown to consist of a triple α-helical bundle (Bernard et al., 2009; Johansson et al., 2003). High-resolution structural data are also available for the X domains of the closely related SeV and mumps virus (MuV), the structure of which has been solved by nuclear magnetic resonance (NMR) and X-ray crystallography, respectively (Blanchard et al., 2004; Kingston et al., 2008). Structural data are also available for the C-terminal P domains from *Rhabdoviridae* members, namely rabies virus (RV) (Mavrakis et al., 2004), vesicular stomatitis virus (VSV) (Ribeiro et al., 2008) and Mokola virus (Assenberg et al., 2010). Interestingly, comparison of the P nucleocapsid-binding domains solved so far suggests that the nucleocapsid binding domains are structurally conserved among *Paramyxoviridae* and *Rhabdoviridae* P in spite of low sequence conservation (Delmas et al., 2010).

In *Paramyxovirinae,* sequence analyses predict a coiled-coil region within PMD (Habchi et al., 2010; Karlin et al., 2003) (Fig. 1). In agreement, X-ray crystallography studies showed that SeV, MeV and MuV PMDs are coiled-coil tetramers (Communie et al., 2013; Cox et al., 2013; Tarbouriech et al., 2000b). The tetrameric coiled-coil organization of PMD has also been experimentally confirmed in the case of Rinderpest (RDV) (Rahaman et al., 2004) and respiratory syncytial virus (RSV) (Llorente et al., 2006, 2008). By contrast, the corresponding PMDs of RV and VSV were shown to form dimers with a different structural arrangement (Ding et al., 2006; Ivanov et al., 2010).

The actual oligomeric state of *Henipavirus* P is unknown. As a first step towards the characterization of *Henipavirus* P proteins, we report the expression, purification and characterization of NiV PMD. Using a combination of biochemical and biophysical approaches, we show that PMD forms a homotrimeric coiled-coil structure with an overall elongated shape.

Results and discussion

Cloning, expression and purification of PMD

The P gene fragment encoding PMD was cloned into the pDEST14 plasmid (Invitrogen) to yield a C-terminal histidine-tagged recombinant product, the expression of which is under the control of the T7 promoter. Most PMD was recovered from the soluble fraction of the bacterial lysate (not shown). The protein

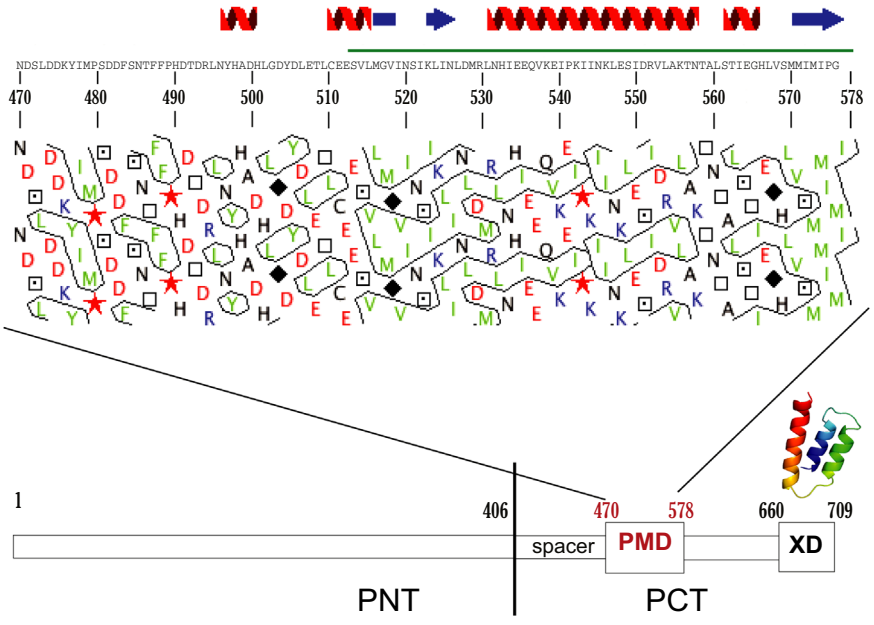

Fig. 1. Domain organization of NiV P. Domain organization of P showing that it is composed of two moieties, PNT (aa 1–406) and PCT (aa 407–709). PCT consists of a disordered region (aa 407–469) referred to as "spacer", a structured region (aa 470–478) referred to PMD for P multimerization domain, a disordered linker (aa 579–659) and a globular region (aa 660–709) referred to as X domain (XD). The HCA plot of PMD, displayed on the top, points out the presence of a region encompassing residues (underlined by a green bar) with the typical texture of a coiled-coil region (see (Ferron et al., 2005)). The ribbon representation of the structural model of NiV XD (Habchi et al., 2011) is shown. The structure was drawn using Pymol (DeLano, 2002).