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Histidine Triad-like Motif of the Rotavirus NSP2 Octamer Mediates both RTPase and NTPase Activities

Rodrigo Vasquez-Del Carpio[1], Fernando D. Gonzalez-Nilo[2] Gonzalo Riadi[2], Zenobia F. Taraporewala[1] and John T. Patton[1]*

[1]*Laboratory of Infectious Diseases, NIAID, National Institutes of Health, Bethesda MD 20892, USA*

[2]*Centro de Bioinformatica y Simulacion Molecular (CBSM) Universidad de Talca, 2 Norte 685, Casilla 721, Talca - Chile*

**Corresponding author*

Rotavirus NSP2 is an abundant non-structural RNA-binding protein essential for forming the viral factories that support replication of the double-stranded RNA genome. NSP2 exists as stable doughnut-shaped octamers within the infected cell, representing the tail-to-tail interaction of two tetramers. Extending diagonally across the surface of each octamer are four highly basic grooves that function as binding sites for single-stranded RNA. Between the N and C-terminal domains of each monomer is a deep electropositive cleft containing a catalytic site that hydrolyzes the γ-β phosphoanhydride bond of any NTP. The catalytic site has similarity to those of the histidine triad (HIT) family of nucleotide-binding proteins. Due to the close proximity of the grooves and clefts, we investigated the possibility that the RNA-binding activity of the groove promoted the insertion of the 5′-triphosphate moiety of the RNA into the cleft, and the subsequent hydrolysis of its γ-β phosphoanhydride bond. Our results show that NSP2 hydrolyzes the γP from RNAs and NTPs through Mg^{2+}-dependent activities that proceed with similar reaction velocities, that require the catalytic His225 residue, and that produce a phosphorylated intermediate. Competition assays indicate that although both substrates enter the active site, RNA is the preferred substrate due to its higher affinity for the octamer. The RNA triphosphatase (RTPase) activity of NSP2 may account for the absence of the 5′-terminal γP on the (−) strands of the double-stranded RNA genome segments. This is the first report of a HIT-like protein with a multifunctional catalytic site, capable of accommodating both NTPs and RNAs during γP hydrolysis.

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Introduction

Rotaviruses, members of the *Reoviridae* family, are the leading cause of acute gastroenteritis in young children throughout the world.[1] The rotavirus virion is a triple-layered icosahedral particle containing 11 segments of double-stranded (ds)RNA.[2,3] Loss of the outer-most layer during entry give rises to a transcriptionally active double-layered particle (DLP) that directs the synthesis of viral (+)-strand RNAs.[4] The (+)-strand RNAs are replicated to dsRNAs and packaged into pre-virion core particles within large inclusion bodies (viroplasms) that form in the cytoplasm of the infected cell.[5–7] Interactions between two abundant non-structural proteins, NSP2 and NSP5, are essential for the formation of such inclusions.[8–10] These proteins are

Present address: R. Vasquez-Del Carpio, Structural Biology Program, Department of Molecular Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029, USA.

Abbreviations used: ds, double-stranded; ss, single-stranded; DLP, double-layered particle; RdRP, RNA-dependent RNA polymerase; NTPase, nucleoside-triphosphate phosphohydrolase; PKCI, protein kinase C-interacting protein; HIT, histidine triad; RTPase, RNA triphosphatase; VLS, viroplasm-like structure; TAP, tobacco acid pyrophosphatase.

E-mail address of the corresponding author: jpatton@niaid.nih.gov

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also components of the replication intermediates that synthesize dsRNAs in viroplasms.[5,11,12] The association of NSP2 with such intermediates may be mediated by interaction with the viral RNA-dependent RNA polymerase (RdRP), VP1.[13]

NSP2 is a basic protein (M_r = 35,000) that self-assembles into stable doughnut-shaped octamers, formed by the tail-to-tail interaction of two tetramers (Figure 1).[14] The overall architecture of such octamers is highly conserved even among distantly related groups of rotaviruses.[15] The octamers possess single-stranded (ss)RNA-binding activity capable of destabilizing RNA–RNA duplexes by an ATP and Mg^{2+} independent mechanism.[16,17] In addition, the octamers have a Mg^{2+}-dependent nucleoside-triphosphate phosphohydrolase (NTPase) activity that cleaves the γ-β phosphoanhydride bond of any nucleoside triphosphate (NTP), yielding the products NDP and P_i.[16] Following cleavage, the γP is transferred to NSP2, generating a short-lived phosphorylated form of the protein.[16,18] The hydrolitic activity of NSP2 is essential for genome replication.[15] NSP2 octamers in the presence of NTPs undergo a conformational transition, shifting from a relaxed to a more condensed state as is typical of molecular motors.[19] Collectively, these properties have led to the suggestion that the NSP2 octamer may facilitate genome packaging and replication by relaxing secondary structures in viral template RNAs that impede polymerase function and by assisting in the translocation of viral RNAs into pre-virion cores.

The NSP2 monomer has two distinct domains (N and C-terminal), separated by an electropositive 25 Å deep cleft that contains residues involved in the binding and hydrolysis of NTPs. The location of the NTP-binding site was initially proposed based on the structural similarity of the C-terminal domain with the catalytic core of protein kinase C-interacting protein (PKCI), a prototypical member of the histidine triad (HIT) family of nucleotide-binding proteins.[14,20] Although lacking a precise signature HIT motif (HØHØHØØ, where is Ø is a hydrophobic residue), mutagenesis has indicated that conserved basic residues in the NSP2 cleft form a HIT-like motif (H^{221}-G-(K/H)-Ø-H^{225}-Ø-R-V) responsible for the binding and hydrolysis of NTPs.[18] More recently, data obtained by co-crystallization of NSP2 with nucleotide analogs has shown that His225 is the catalytic residue of the motif, becoming phosphorylated through the covalent attachment of the γP released during attack on the γ-β phosphoanhydride bond of the NTP.[21] In contrast, the catalytic histidine of HIT proteins traditionally undergoes nucleotidylation by way of an αP linkage formed during attack of the β–α phosphoanhydride bond of the nucleotide substrate.[20]

Extending diagonally across the NSP2 octamer surface are four highly basic grooves, 30 Å wide and 25 Å deep, that function as ssRNA-binding sites.[14,22] Each groove is lined by two 24-residue electropositive loops, originating between the two subdomains of the N terminus of each monomer (Figure 1). The location of these loops is such that they position the electropositive residues at the entrance of the catalytic clefts containing the HIT-like motif. Due to the close proximity of the grooves and clefts, binding of ssRNA in a groove may impact the passage of NTPs into or out of the cleft and, therefore, may influence the NTPase activity of the octamer. On the other hand, interaction of ssRNA with a groove may promote entry of the triphosphorylated 5′ end of the bound RNA into the cleft, an event that may be further advanced by the strongly electropositive characteristics of the cleft residues. Given that the interior dimensions of the cleft are sufficient to accommodate ssRNA,[14] we investigated the possibility that the NSP2 octamer could function as an RNA triphosphatase (RTPase), wherein those same residues involved in NTP

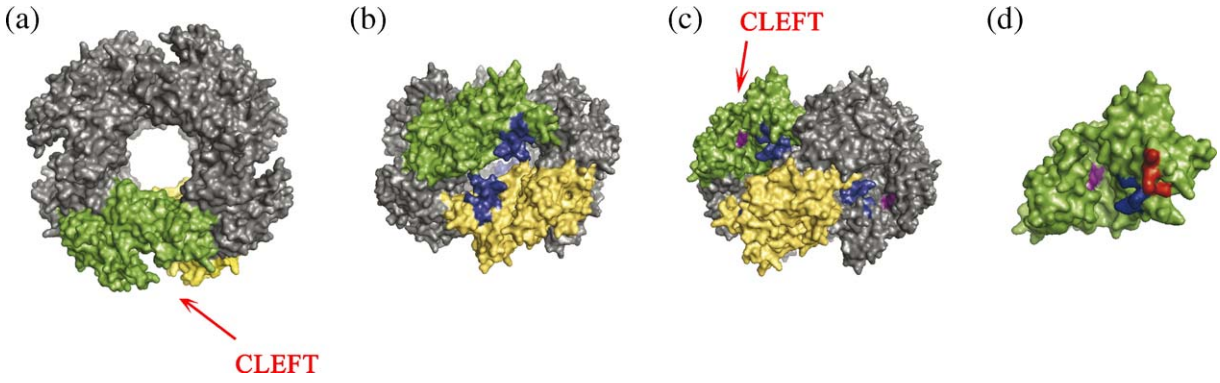

Figure 1. Structural proximity of the NTP-binding cleft and the RNA-binding grooves. Surface representation of the NSP2 octamer viewed along the 4-fold (a) and 2-fold axes ((b) and (c)). Two monomers have been identified in the octamer (green and yellow). The image in (b) represents a 90° rotation along the *x*-axis with respect to the image in (a). Residues 53 to 76 that form part of the electropositive loops, which line the RNA-binding grooves, are denoted in blue. (c) The image in (b) was rotated 45° clockwise along the *y*-axis to allow visualization into the cleft. Residues within the cleft that make up the active site for NTP hydrolysis are shown in purple. (d) Surface representation of one monomer as viewed in (c). The residues selected for glutamine mutagenesis are shown in red (K^{37}, K^{38}, K^{58}) and blue (K^{59}, R^{60}, R^{68}). Images were prepared using PyMOL [http//pymol.sourceforge.net/].