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Relating Structure and Function of Viral Membrane-spanning Miniproteins

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Abstract

Many viruses express small hydrophobic membrane proteins. These proteins are often referred to as viroporins because they exhibit ion channel activity. However, the channel activity has not been definitively associated with a biological function in all cases. More generally, protein-protein and protein-phospholipid interactions have been associated with specific biological activities of these proteins. As research has progressed there is a decreased emphasis on potential roles of the channel activity, and increased research on multiple other biological functions. This being the case, it may be more appropriate to refer to them as “viral membrane-spanning miniproteins”. Structural studies are illustrated with Vpu from HIV-1 and p7 from HCV.

Introduction

Small viral membrane proteins perform multiple biological functions, which are generally associated with much larger proteins. Contributing factors may be the need to take optimal advantage of the limited sequence information encoded in compact viral genomes, and that these proteins are associated with lipid bilayers with different compositions and properties in various organelles, which can influence their structures, interactions, and functions. Here, we utilize two well-characterized examples, virus protein “u” (Vpu) of the human immunodeficiency virus (HIV-1) [1] and p7 of hepatitis C virus (HCV) [2], to describe the current state of the structural biology of this class of proteins. Neither of these proteins has been crystallized, therefore nearly all of the experimental structural information available is derived from nuclear magnetic resonance (NMR) spectroscopy. We contrast the properties of these proteins with those of the best studied example; the M2 protein of Influenza, which has a well-defined channel function. The structure of its trans-membrane channel domain has been extensively characterized by NMR spectroscopy, X-ray crystallography, and other physical methods [3,4].

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Despite the differences in their titles, DiMaio's review entitled "Viral Miniproteins" [5] and that by Nieva, Madan, and Carrasco [6] entitled "Viroporins: Structure and Biological Functions" are concerned with the same class of proteins. Viroporins are strongly associated with having channel activity. DiMaio, in a sense, breaks new ground by providing a more general view of these proteins. This is well justified because these proteins have multiple essential activities; even though in many cases they do display channel activity, it is not always clear if and how it is associated with biological functions. Strebel's review [7] describes functional implications of much of the available structural information on Vpu, including questions about the role of its channel activity. The review by Steinmann and Pietschmann provides essential background on p7 [8].

NMR of membrane proteins

Solution NMR is applicable to relatively small membrane proteins in model membrane environments, such as mixed organic solvents, detergent micelles, isotropic bicelles, and recently nanodiscs. Despite their limitations, these environments have been used because they enable the proteins to reorient rapidly enough in solution to yield high-resolution NMR spectra. Caution must be exercised in the interpretation of the results as these non-native environments can affect the properties of the proteins. For example, the commonly used solvent 50%TFE/50%water has a propensity to stabilize helices while destabilizing or distorting tertiary and quaternary structure. It may very well be the case that the same sensitivity of the structure to the lipid environment provides a range of functions [9] and makes it difficult to sort out the correct biological structure from the various types of NMR samples that have been studied [10].

Membrane proteins can also be reconstituted into phospholipids. When associated with bilayers, membrane proteins are effectively 'immobilized' on the timescales of the chemical shift and dipole-dipole nuclear spin interactions. Consequently, solid-state NMR methods are required to obtain high-resolution NMR spectra. Under physiological conditions, membrane proteins undergo rotational diffusion about the bilayer normal, which enables the application of rotationally aligned solid-state NMR [11]. This is complementary to other solid-state NMR approaches that measure distances and angles in unoriented samples with the application of magic angle spinning (MAS) [12,13] as well as mechanically or magnetically aligned samples for oriented sample solid-state NMR [14].

Vpu from HIV-1

HIV-1 has a single-stranded positive-sense RNA genome of 9.8 kb that is processed to form the structural, regulatory, and envelope proteins characteristic of a retrovirus. In addition it has four accessory proteins, one of which, Vpu, is a small 81-residue membrane protein [1].

The initial structural studies of Vpu were performed by solution NMR in TFE/water on a series of synthetic peptides having between 15 and 50 amino acids [15–19] with sequences corresponded to overlapping sections of the protein. General ideas about the overall architecture of the protein emerged from these studies. More detailed studies required the use of expressed polypeptides because of the opportunities for isotopic labeling [20–24]. From the combination of these studies, a picture of Vpu emerged as having an N-terminal

hydrophobic trans-membrane helix, which oligomerizes to form channels, and a C-terminal cytoplasmic domain with a helix-loop-helix arrangement. Significantly, the loop contains two conserved serine residues that are phosphorylated.

The combination of expressed polypeptides and phospholipid bilayer samples provides a near-native environment. Marassi et al [25] studied expressed full-length and truncated constructs of Vpu in phospholipid bilayers by oriented sample solid state NMR, which showed that the protein has two distinct domains. To define the minimum folding units, Ma et al [26] prepared three truncated forms of Vpu and compared their structural and functional properties to those of the full-length protein. Park et al [27] determined the three-dimensional structure of the channel-forming trans-membrane domain (VpuTM). Substitution of alanine at position 18 by a histidine (A18H) has been shown to render HIV-1 infections susceptible to rimantadine [28]. The structure of A18H VpuTM was determined, and compared to that of wild-type Vpu TM [29]. Rotationally aligned studies complement solution NMR studies in yielding a structure for the Vpu monomer (Zhang et al, unpublished results) (Figure 1). This structure is distinguished by having three helical segments, one of which is a hydrophobic membrane-spanning helix that can form a channel; the other two are part of an amphipathic surface domain. Notably, all of the helices can interact with other proteins contributing to the biological functions of Vpu.

In a complementary approach using unoriented samples, Sharpe et al [30] described solid-state magic angle spinning experiments on the N-terminal half of Vpu. Their data indicated that the transmembrane alpha helix extends beyond the hydrophobic core of the bilayer. Lu et al [31] described measurements that provided new constraints on the oligomerization state of Vpu. Their data indicated that a variety of oligomers coexist in phospholipid bilayers. This is also consistent with at least one of Vpu's activities being associated with its forming channels. Do et al applied magic angle spinning solid-state NMR [32] to expressed full-length Vpu and a construct consisting of residues 372–433 of CD4.

The binding between Vpu and β-TrCP does not require phosphorylation of the serines in the interhelical loop of the cytoplasmic domain of Vpu, however activation of the degradation pathway does. Coadou et al [33–37] used relatively short peptides to elucidate [35] the basis of β-TrCP recognition. The β-TrCP-bound structure of phosphorylated Vpu was found to be similar to the structure of the free peptide in solution and to the structure recognized by its antibody. Gharabi-Benarous [36] investigated a 22-amino acid peptide that mimics the phosphorylated Vpu antigen. Phosphorylation of Vpu at sites Ser52 and Ser56 on the DSGXXS motif is required for the interaction of Vpu with the ubiquitin ligase SCF (β-TrCP) that triggers CD4 degradation by the proteasome. The peptide residues forming this bend are recognized by a monoclonal antibody. Evrard-Todeschi et al [37] examined the binding and conformation of phosphopeptides to β-TrCP.

Vpu antagonizes the interferon-induced restriction factor BST-2 (tetherin) by a protein-protein interaction associated with the helical trans-membrane domains of each protein. Guatelli and Opella [38] have studied the mechanism of how Vpu antagonizes BST-2. They found that this results from an intermolecular interaction that occurs between the single trans-membrane domain in each protein. The antagonism of BST-2 involves a sequence of

three alanines and a tryptophan spaced at four residue intervals within the Vpu helix. Responsiveness to Vpu involves bulky hydrophobic residues in the C-terminal region of the BST-2 trans-membrane helix that likely fit between the alanines on the interactive face of Vpu. As a result, Vpu and BST-2 form an anti-parallel, lipid-embedded helix-helix interface. Changes in human BST-2 that mimic sequences found in nonhuman primate orthologs unresponsive to Vpu change the tilt angle of the trans-membrane helix in the lipid bilayer without abrogating its intrinsic ability to interact with Vpu. This provides a mechanism by which HIV-1 evades a key aspect of innate immunity and the species specificity of Vpu using an anti-parallel helix-helix packing model.

A tryptophan residue near the Vpu C-terminus is important for enhancing virion release. Vpu proteins with the W76G mutation degraded and down regulated BST-2 from the cell surface, yet they inefficiently stimulated virion release. In further NMR studies [39] it was found that the cytoplasmic domain of Vpu specifically interacts with lipids. Paramagnetic relaxation enhancement (PRE) studies show that W76 inserts into the lipid bilayer. These results are consistent with a model whereby W76 anchors the C-terminus of Vpu's cytoplasmic tail to the plasma membrane, enabling the movement of Vpu-bound BST-2 away from viral assembly sites.

p7 from HCV

HCV is an enveloped virus with a single-strand RNA genome of approximately 9.6 kb, which is translated into a single 3000-amino acid polyprotein that is cleaved to yield ten proteins [40]. Notably, the nucleotide sequence for the 63-residue membrane protein, p7, is sandwiched between those that code for the three structural protein that constitute the virion and the six nonstructural proteins that constitute the replication complex and perform other functions. p7 has been shown to be essential for efficient virus particle assembly and release [41], but not RNA replication [42]. p7 is generally categorized as a viroporin because it oligomerizes to form ion channels. It has been proposed that it is activated at low pH and dissipates acidification of a secretory compartment [8,40].

Griffin et al. described the oligomerization state of p7 as a hexamer [43]. Clarke et al. published a study on a FLAG-tagged p7 construct [44] where they conducted cross-linking studies and observed a predominately heptameric complex. Subsequently, Luik et al. [45] showed that the p7 subtype JFH-1 reconstituted in DHPC detergent micelles gave up to 6 bands after cross-linking in the SDS-PAGE analysis, suggesting a hexameric oligomer. Chandler et al have performed simulations that yield stable hexameric and heptameric channels [46].

Saint et al [47] synthesized a polypeptide with the sequence of the 63-residue p7 protein. They were able to obtain well-resolved NMR spectra in TFE/water but not detergent micelles. Montserrat et al [48] determined the secondary structure elements of the monomeric form of p7 in TFE/water, suggesting the presence of a helix-loop-helix 'hairpin' structure.

Cook et al performed initial NMR studies on expressed p7 in phospholipid bilayers and micelles [49,50] and obtained a similar helix-loop-helix arrangement of the secondary

structure of the protein. The protein contained helical segments tilted approximately 10° and 25° relative to the bilayer normal. In another study, they [51] described a series of solution NMR experiments that showed p7 has a range of dynamic properties as well as stable structural segments. The 63-amino acid protein has a remarkably complex structure made up of seven identifiable sections, four of which are helical segments with different tilt angles and dynamics. More definitively, Cook et al [52] determined the three-dimensional structure of p7 in DHPC micelles by solution NMR and in DMPC bilayers by solid-state NMR (Figure 1).

OuYang et al [53,54] determined the structure of p7 in DPC micelles by solution NMR. Their structure is an unusual hexameric assembly, where the individual p7 monomers not only interact with their immediate neighbors but also reach more distant neighbors. Their structure contains three rather than two helical segments and differs from the prior structures in other ways. The substantial differences in structure contrast with the similarities of the primary sequences and especially the corresponding hydropathy plots. Further research is required to sort out how the differences in structure are consistent with the similarities in function [55].

The structure of p7 has been determined in five different conditions, yielding in each case a different structure for the protein. This is typical for membrane proteins, because of their sensitivity to the solvent or lipid environment. The structure of p7 has been determined four times by solution NMR, twice in organic solvents (TFE/water [48] and methanol [56]) and twice in detergent micelles (DHPC [52] and DPC [53]). Recently, the structure of p7 shown in Figure 1 has been determined in the near-native environment of DMPC bilayers by solid-state NMR (Cook et al, unpublished results). Resolution of these differences will require the use of a common membrane environment to sort out the influence of the environment on the structure and dynamics of the protein. The structures of the monomers will play a crucial role in identifying whether there is a biologically functional form of the oligomeric channels. At present, there is less evidence of other functions of p7.

Conclusions

Although M2 of Influenza is the best characterized viroporin, structurally only the properties of the trans-membrane channel-forming domain are known with certainty. Progress is being made on the full-length protein [57,58]. In contrast, Cook et al [59] compared the properties of p7 and Vpu. The purified proteins were studied in the model membrane environments of micelles by solution NMR spectroscopy and in phospholipid bilayers by solid-state NMR spectroscopy. The resulting structural findings enabled comparisons to be made between the two proteins, demonstrating that they have quite different architectures. Most notably, Vpu has one trans-membrane helix and p7 has two trans-membrane helices. In addition there are significant differences in the structures and dynamics of their internal loop and terminal regions. It is difficult to classify these two proteins in the same structural family, since their structures are so different. However, there are some similarities in their functions, and, like M2 of Influenza, they are small viral membrane proteins.

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Highlights

- Many viruses express small membrane proteins.
- Although generally known as viroporins these the role of channel activity is generally undefined.
- The structures of these proteins are affected by the membrane environment.
- They have functions in the viral infection and release processes.

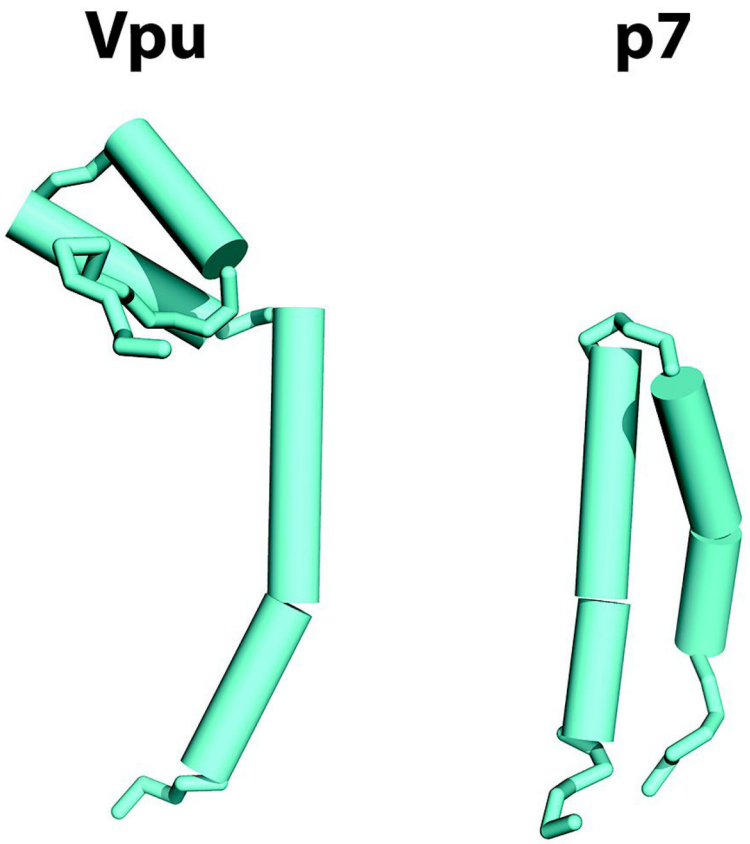

Figure 1.
Structural models of Vpu (left) and p7 (right) derived from NMR data (unpublished results).