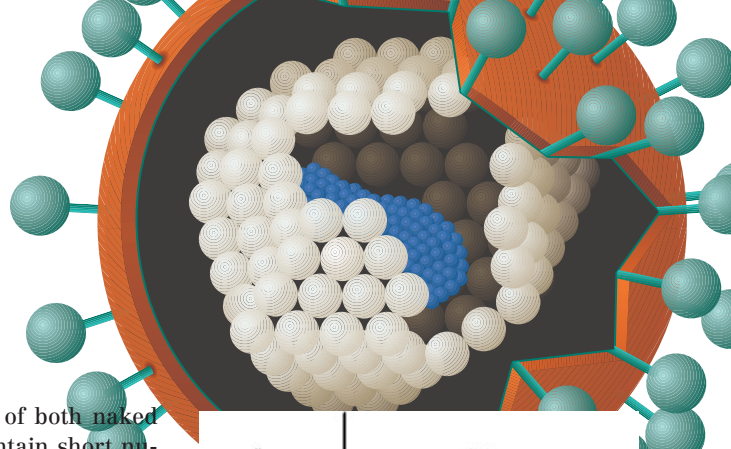


Viral Ion Channels



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Genomes of a number of both naked and enveloped viruses contain short nucleotide sequences of less than 300 base pairs in length, which encode short peptides, all containing a sequence of hydrophobic amino acids, (predicted to be a transmembrane (TM) α -helix) [1–3]. These peptides have been classified as viral ion channels, auxiliary proteins which are not essential for viral reproduction. Mutants, in which the genes are deleted, reproduce more slowly and the rate at which new virus particles are released is reduced. During its reproductive cycle, the virus encounters a number of cellular compartments all of which are confined by phospholipid bilayers. A variety of different intrinsic membrane proteins are employed by the host to regulate the concentration of ions across the bilayer, and these electrochemical gradients are then used to drive a variety of energetic processes. As part of the process of hijacking the host's metabolism, the virus needs to be able to control these gradients, and it is for this purpose that they produce ion channels.

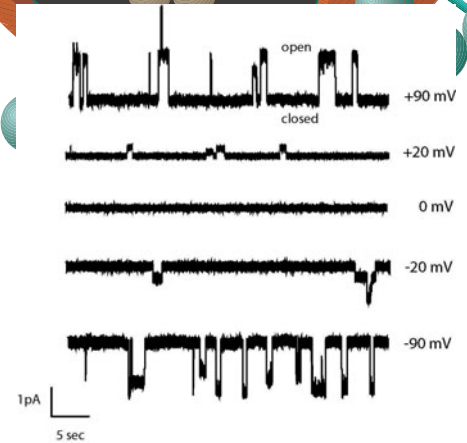


Fig. 1: Single channel recordings of a peptide corresponding to the transmembrane sequence of Vpu from HIV-1 and reconstituted into artificial lipid bilayers. The peptide is derived from solid phase peptide synthesis. The lipid is spanning an aperture with a diameter of approximately 150 nm separating two chambers filled with 300 mM KCl, buffered at pH 7.4 in which silver coated electrodes are recording. The various holding potentials are given in the figure. Conductance levels are found to be in the range of 10–25 pS. Channel openings are indicated by a rectangular-shaped increase in current. Data recorded by Andrea Sauerwein (Erasmus student).

Viral ion channels are auxiliary proteins produced by viruses, including HIV and Influenza. The proteins are short polypeptides, with typically no more than 100 amino acids, which oligomerise in lipid bilayers to form bundles enclosing an ion-conducting pore. These molecules have attracted considerable interest partly because of their medical relevance, but also because they can be used to model more complex mammalian proteins and they have potential applications in bionanotechnology.

Viruses are unable to replicate on their own and to do so must enter a host's cell and hijack its metabolic machinery. They can be separated into two categories: naked viruses consist of a protein coat (capsid) surrounding a short DNA or RNA genome; enveloped viruses wrap part of the host's cell surface membrane around their capsid and bud out of the host cell in vesicle-like bodies, instead of bursting out of the host as the amount of protein in the cytoplasm exceeds capacity.

IC	Virus	E/N	Length	G/P	Selectivity
3A	Polio virus	N	87		
2B	Polio/Coxsackie B3	N	99		
6K	Alpha virus	E	58-61		cation
E	SARS-CoV	E	76		cation
Kcv	PBCV-1	E	94		cation
M2	Influenza A	E	97	G	proton
NB	Influenza B	E	100	G	cation/proton
p7	HCV	E	63		cation
Vpu	HIV-1	E	81	P	cation

Tab. 1: Viral proteins to be detected as forming ion channels (IC), especially when reconstituted into lipid bilayers. G and P stand for glycosylated and phosphorylated, respectively. N and E denote 'naked' and 'enveloped' viruses.

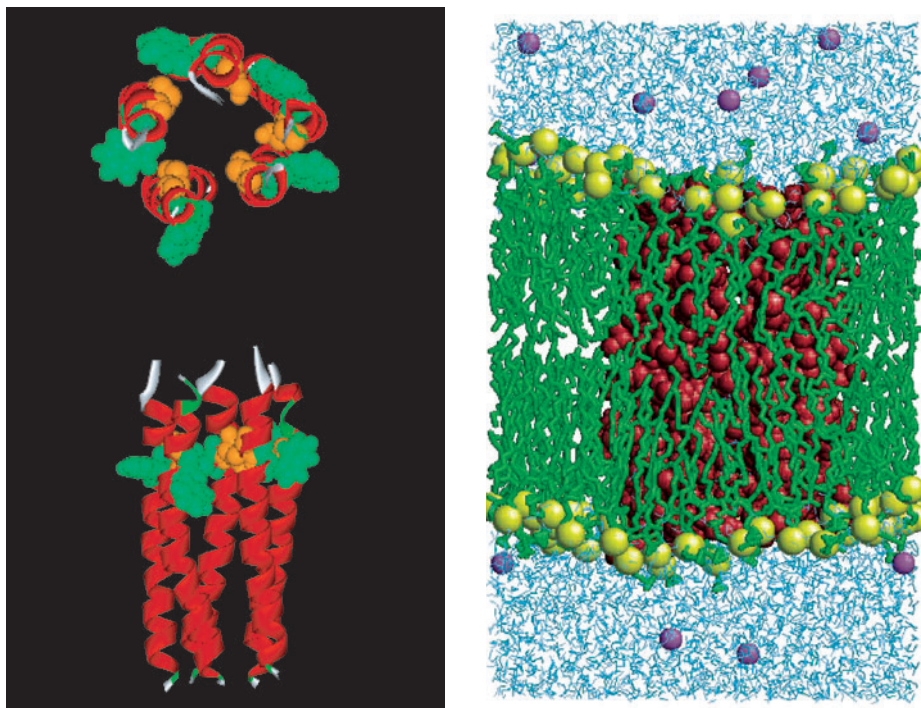


Fig. 2: A computational model of a pentameric assembly of the transmembrane helices (red) of Vpu from HIV-1. (A) in a top view down from the C terminal end with tryptophans (green) and serines (orange) highlighted. (B) side view of the model. The model is based on findings in other ion channels that hydrophilic amino acids face the lumen of the pore. (C) shows a 'MD simulation box' with the bundle (red) embedded in a fully hydrated (water molecules are represented by small blue triangles) lipid bilayer (stick representation of the hydrophobic chains in green, the phosphorous headgroups (yellow) are enlarged to indicate the so-called lipid headgroup region). The spheres in magenta are Na^+ , placed to counterbalance any negative charge derived from the charged amino acid side chains. The picture shows a snapshot after 1 ns of MD simulation.

The influenza virus (an enveloped virus) enters the cell via the endosomal pathway. V-type ATPases, (proton pumps), in the endosomal membrane begin to acidify the vesicle, whose internal pH falls to 5.5. This low pH is required by peptidase enzymes which are transported into the vesicle. The M2 protein, a 97 amino acid protein which occurs in the viral membrane, senses the acidic environment of the endosome and oligomerises to form a proton-conducting pore which allows H^+ to enter the virus. This causes a conformational change in a second viral protein – hemagglutinin, allowing the viral membrane to fuse with the endosomal membrane and releasing the virus into the cytoplasm. A change in the protonation state of residue His-37 of M2 has been found to be the trigger for channel opening.

Relatively little structural information is available for M2, although the single transmembrane portion has been proved to be an amphipathic α -helix. Vpu from Human Immunodeficiency Virus type-1 (HIV-1) is somewhat easier to study, because it is not glycosylated and has only two phosphorylation sites and its structure has therefore been more extensively

characterised. It is 81 amino acids long, also has a single transmembrane α -helix, (which can be synthesised by solid phase peptide synthesis), and an intracellular cytoplasmic domain which consists of two further α -helices. It forms homo-oligomeric bundles in the membranes of the host cell. The cytoplasmic domain down-regulates the expression of the CD4 protein on the surface of the infected Th cell and inhibits the formation of the CD4-gp160 complex, (gp160 is a precursor of the viral capsid proteins gp120 and gp41, and antigen presentation by CD4 is essential for immune system activation). The transmembrane domain enhances viral particle release by infected cells by homo-oligomerising to form weakly cation-selective ion channels. This is thought to alter the electrochemical gradient across cell membranes, allowing the virus to bud out of the cell. A large amount of structural data has been gathered for Vpu, which has been used in computational approaches which address the mechanisms of ion transduction and modulator-protein interactions on an atomic scale.

Limited structural information is available for other viral ion channels: p7 from the Hepatitis C virus (HCV) and 2B

from Polio Virus (PV) have two transmembrane helices; a more complex topology has been proposed for the Kcv channel from the plant virus PBCV-1, (Kcv and the bacterial K^+ channel KcsA have high sequence homology). Other viral proteins have been designated as ion channels based on the channel activity detected when reconstituted into artificial lipid bilayers (Tab. 1).

Investigative Techniques

One of the important tools for channel characterisation is Bilayer Recording. The protein under investigation is reconstituted into an artificial lipid bilayer, spanning a small aperture which connects two buffer-filled chambers (Fig. 1). Silver electrodes in each chamber are used to apply a potential difference of up to ± 150 mV across the membrane and the resulting flux of ions through the channels is measured. Individual channels have a typical conductance in the range 20–40 pS and are generally weakly cation selective. Experimental data produced by Bilayer Recording can be used to further refine computer-generated models.

A range of spectroscopic techniques are available to study viral ion channels individually or as oligomers including Circular Dichroism and Fourier Transform Infra Red spectroscopy [4]. Owing to the small size of the proteins, it is extremely difficult to obtain protein crystals suitable for X-ray diffraction; however solution state NMR structures for the cytoplasmic portion of Vpu and solid state NMR structures for the transmembrane helix have been determined separately and combined to produce computer models for use in molecular dynamics (MD) and drug binding simulations. MD simulations consider the individual movement of all molecules, (including water and phospholipids), at the atomic level (Fig. 2). Ligand docking simulations can be used to screen potential channel blockers and modulators and can generate binding and inhibition constants.

Viral ion channels are the focus of considerable research attention: partly because increasing viral resistance to drugs which target major globular proteins, has stimulated the need to find new drug targets. Amantidine, the earliest antiviral drug and its derivatives, target the M2 protein of Influenza A, blocking the pore. Other drugs based on the inhibitor di-

ureticum amiloride may be used in the future to inhibit the channel activity of Vpu and to slow down the release of the HIV-1 virus from the cell surface. Recently amino sugars have shown promising results inhibiting p7 from the Hepatitis C virus in reconstituted bilayers.

Outlook

Information about new viral ion channels is constantly emerging, driven by genome sequencing and bioinformatics. The proposed protein sequences need to be verified experimentally as being able to form channels and the characteristics of these channels need to be proven. The individual roles of these channels in the life cycle of each specific virus also need to be identified.

The investigations on viral ion channels may be of benefit in several fields. First of all sensors based on lipid membranes in which these proteins are embedded will be used for drug screening and consequently large scale production of these proteins is in high demand. It has been shown that it is the transmembrane part of the M2 channel which is solely required for drug-protein interaction, similar to Vpu. Automated solid phase peptide synthesis may deliver large quantities for production lines before expression methods are developed to produce the whole protein in large quantities. Based on their short length, viral ion channels may serve as models for the larger host channels and a thorough investigation of their mechanism of function will shed light on the minimalist architecture required for channel function. This knowledge may then improve their applicability in more complex bio-devices.

References

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