Self-Assembly of Viral Ion Channel Forming Proteins

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Introduction

Some viral genomes encode small proteins which are assumed to form ion-conducting pores (1). These proteins are involved in the entry/exit pathways of the viruses. For some of the viruses these proteins are essential for their life cycle, for others they are just tools to enhance replication.

One of these proteins, M2 from influenza A, is already a drug-target. For other channel proteins drug development is still in its infancy. Knowledge about the mechanism of function of these proteins is only gradually emerging. It comprises essentially two steps: (i) the assembly of the proteins to form finally (ii) an ion- or substrate-conducting channel.

Vpu from HIV-1 is chosen as a test case in a combined computational and experimental approach to address these steps. Vpu is a 82 amino acid type I integral membrane protein specific for HIV-1 (2,3). Vpu supports HIV escape from the host cell by the following mechanisms: (i) Vpu is directing the HIV receptor CD4 to the ubiquitin-dependent proteasome degradation pathway in the endoplasmic reticulum (4 -9). The protein cytoplasmic domain is interacting with CD4 (7, 9-12); (ii) at the site of the plasma membrane Vpu is assumed to form ion-conducting channels with a positive effect on the budding process. This is supposed to be due to the oligomerisation of the protein (13) and consequently the ability to form a channel through which ions can pass (14,15). This

proposal emerges from experiments with full length protein and peptides corresponding to the transmembrane (TM) domain, both of which exhibit channel activity when reconstituted into artificial lipid bilayers (12,16); (iii) more recently evidence has emerged for Vpu interacting with a potassium channel (TASK-1) of the host cell, thereby blocking its channel activity and thus indirectly affecting the release of progeny virions (17).

From spectroscopic studies (reviewed in (1,18)) Vpu is known to possess a single helical transmembrane domain followed by a large cytoplasmic domain consisting of two to three α-helical subdomains depending of the experimental conditions.

Experimental Procedures

Expression and peptide synthesis:

Vpu_{1-81} was expressed as a fusion protein with glutathione-*S*-transferase using the pGex-vpu plasmid provided by Prof. P. Gage, Australia National University, Canberra, Aus and described in detail elsewhere (16, 19). Vpu_{1-32} with the sequence MQPIPIVAIV[10] ALVVAIIIAI[20] VVWSIVIIEY[30] RK was synthesised on a Pioneer Synthesizer from Applied Biosystems Instruments using Fmoc chemistry (16,19).

Channel recordings:

Protein and peptide were reconstituted in a membrane 1:4 mixture of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1,2-dioleoyl-*sn*-glycero-3-phospho-choline (DOPC) (Avanti Polar Lipids, Alabaster, US) (16,19). For channel recordings, a Delrin cup chamber system was used with an aperture diameter of 150 μm. Aliquots of 10 μl of Vpu_{1-81} or Vpu_{1-32}, dissolved in TFE at 1.0 mg/ml, were pipetted into the aqueous subphase in the *cis*-chamber (ground). The current response was recorded using a MultiClamp 700A system from Axon Instruments (Union City, US) and data were filtered with a Bessel-8-pole low-pass-filter at 100 Hz.

Computational modeling:

Pentameric bundles of Vpu_{8-26} were constructed by creating symmetric copies of the monomeric units around the central pore axis. Degrees of freedom like rotational angle around the helix axis, tilt angles and interhelical distances were changed systematically, whilst the potential energy of each conformer was recorded using the Engh-Huber force

field. This procedure was implemented in MOE (www.chemcomp.com) using self-made SVL scripts (J. Krüger and W. B. Fischer, manuscript in preparation).

Results and Discussion

Based on our working hypothesis that the monomeric Vpu units diffuse within the membrane and have to assembly prior to be able to conduct ions, a large emphasis is put on how these proteins interact with each other to form a bundle. This hypothesis leans on the proposed two-stage model for membrane protein folding and oligomerisation (20). Since experiments show that the full length protein and the peptide based on the TM part of Vpu exhibit almost identical channel characteristics such as similar conductance levels of ~16 and ~17 pS, respectively (Figure 1) all *in silico* studies are performed with just the TM domain.

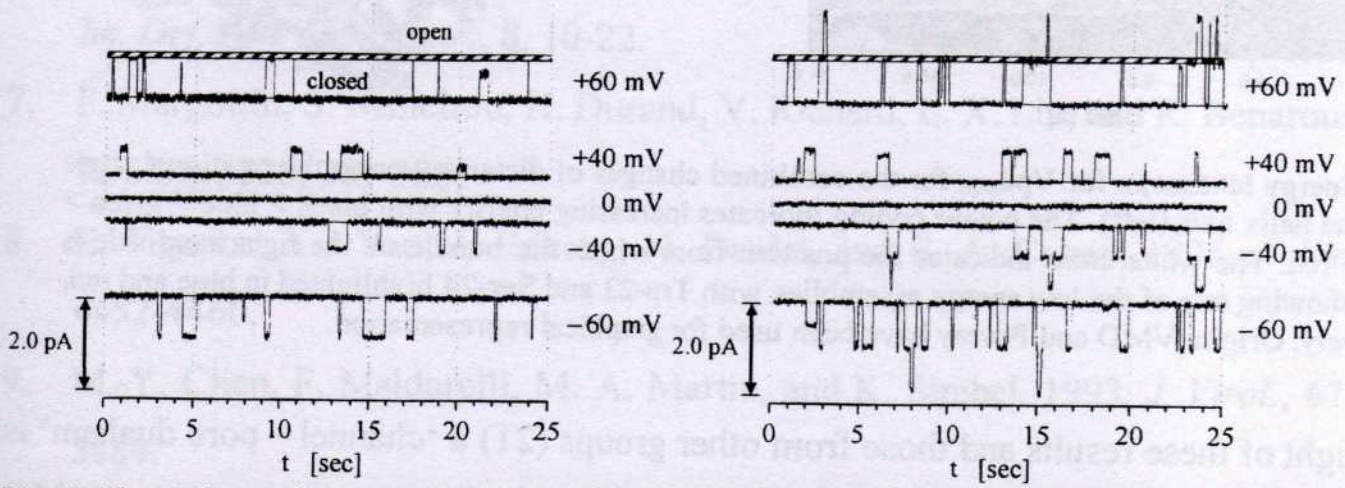

Fig. 1: Channel recordings at various holding potentials for the full length protein (left) and a peptide Vpu_{1-32} corresponding to the first 32 amino acids of Vpu including the TM domain. Each chamber was filled with buffer containing 300 mM KCl, 5 mM K^+-HEPES at pH = 7.0. The shaded areas indicate the main conductance state.

In a first step solely the TM domain, Vpu_{8-26} have been taken for screening protein – protein interaction thereby altering three degrees of freedom successively: rotational and tilt angel, as well as inter-helical distance. The result is a fine-grained energy landscape which suggests low energy models of the pentameric bundle (Figure 2). These bundles match those models which have been generated so far by simply assuming that hydrophilic residues within the TM domain face into the pore and aromatic residues, such as tryptophan are aligned so that they face the protein – lipid interface. Experimental studies with peptides corresponding to the TM domain of Vpu_{1-32} have been used to address the cation specificity reported for Vpu (14,19). Our own experimental data

further show that conductance follows the Eisenman I series which suggests Vpu to be a weakly-selective ion channel (19). Parallel to these experiments steered MD simulations have been applied to pull the physiological relevant ions K^+, Na^+ and Cl^- through the pentameric bundle. Preliminary results are in accordance with the experimental findings albeit discrimination between K^+ over Na^+ cannot be deduced from the data at this stage (in preparation).

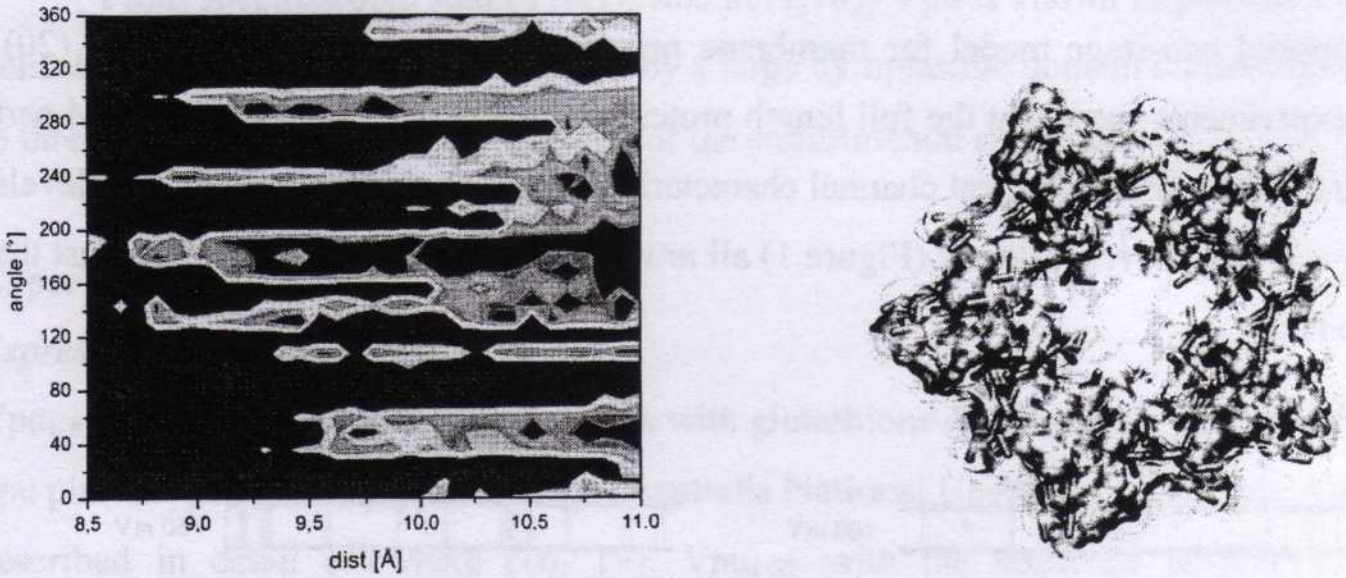

Fig. 2: Energy landscape for Vpu_{8-26} for the combined changes of distance versus the rotational angle around the helix axis (left). The colour coding indicates increasing energy with black > blue > green > yellow > red. The white cross indicates the position from which the bundle on the right hand side is chosen, showing one of the low energy assemblies with Trp-23 and Ser-24 highlighted in blue and red, respectively. Origin, VMD and Povray have been used for graphical representation.

In the light of these results and those from other groups (21) a 'channel – pore dualism' is suggested: depending in the environmental conditions, Vpu may act either as a weakly-selective ion channel or just a pore which may also conduct substrates.

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