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The cellular and subcellular membranes encounter an important playground for the activity of membrane proteins encoded by viruses. Viral membrane proteins, similar to their host companions, can be integral or attached to the membrane. They are involved in directing the cellular and viral reproduction, the fusion and budding processes. This review focuses especially on those integral viral membrane proteins which form channels or pores, the classification to be so, modeling by in silico methods and potential drug candidates. The sequence of an isolate of Vpu from HIV-1 is aligned with host ion channels and a toxin. The focus is on the alignment of the transmembrane domains. The results of the alignment are mapped onto the 3D structures of the respective channels and toxin. The results of the mapping support the idea of a 'channel–pore dualism' for Vpu.

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1. Introduction

The particular role of the channel forming proteins in the cellular life cycle of the virus is proposed to impose leakage upon the lipid membranes of the host [1]. The leakage is an essential part of the viral infection [2,3] and leads to an alteration of ionic conditions within the infected cells, which needs to be distinct from those for the uninfected host [4]. For some viruses these proteins are essential [5] and for others they are just 'only' intensifiers [6]. The development of escape mutants under the currently existing antiviral therapy which is directed mostly against other proteins than channel proteins makes those of cause highly attractive target sites for the ever pressing need of novel antiviral and also especially antiretroviral drug candidates. Membrane proteins in general are more conserved than soluble proteins, a fact which relies on the structural motif of the transmembrane domains (TMDs) allowing for less mutational flexibility without causing disease [7].

Sequence alignment of Vpu from HIV-1 with a toxin and a series of ion channels of the host is reported to assess structural and functional motifs of Vpu. This protocol may serve as an additional tool for the analysis of other viral channel proteins enabling their classification.

1.1. Viral channel forming proteins

Viral membrane proteins can be subdivided into four classes: (i) large proteins which are involved in viral entry and reported as...
fusion proteins, and mostly small to medium sized proteins which are reported as (ii) viral channel or pore forming proteins [8,9], (iii) membrane attached [10] or (iv) membrane penetrating proteins [11].

There are only a handful of proteins for which there is a consensus that they form channels (for reviews see [8,9,12,13]). Mentioning the proteins in brief, one of the proteins is M2 from influenza A [14,15]. It is now established that M2 is a proton channel [16–18]. It is now also suggested that the mode of action rather correlates with the one of a proton transporter [19]. Other channels from influenza B are reported to conduct protons, such as BM2 [20], or ions, such as NB [21]. Protein p7 from HCV [22,23], 2B from Polio virus [24], 3a [25] and E protein [26] from SARS-CoV are potential channel proteins. Plant viruses also encode a complement of a potassium channel, Kcv [27,28]. Vpu from HIV-1 is heavily debated whether it is forming a channel or whether this is just one of its roles in the cellular life cycle. Possibly on the boarder-line of being a channel with a stable more or less defined diameter is Vpr from HIV-1. Vpr has been shown to exhibit channel activity [29,30]. It is also considered that the protein harbors the capability to traverse through the membrane [31].

For some of the channels (see also [32]) the correlation between channel and the role in the life cycle is fairly established, and for others it still needs to be established. In general, change of electrochemical or substrate gradients can lead to an alteration of conformation or folds of other proteins which then trigger secondary events such as fusion (see the role of M2) or ion channel activity. The latter may affect Na⁺, K⁺, or Ca²⁺ regulation in the infected cell.

Two names are used in the literature when reporting about these proteins: viroporins [8] and ‘viral ion channels’ [9,33]. The first classification is based on experiments which indicate that viral infection impose leakage upon cell membranes which allow otherwise impermeable compounds to permeate [34]. More recent experiments were done with Polio virus indicating that upon 2B deletion permeability of infected HeLa cells to outside compounds is reduced [1]. Permeability essays reveal that a cut-off in size for the permeating substrates exists [35,36]. The second classification is based on proteins reconstituted into artificial lipid bilayers [37], a technique which has been widely used in verification of channel activity and selectivity [22,33,38–41].

1.2. Channel–pore dualism

In the classical sense an ion channel is a membrane protein, enabling passive diffusion of ions across the lipid membrane [42]. Ion channels show more or less precise ion selectivity and gating and can be selectively blocked by small molecules. The mode of action can be triggered either by voltage gradients of small molecule ligands or mechanical stress. The consequence of channel activity is an alteration of electrochemical gradients across those lipid membranes which harbor the proteins. Channels are mostly found to be involved in the electrochemical signal transduction in nerve fibers and in the brain or they are taking part of the signal transformation cascade in the retina. In general they are involved in a very specified mode of action which is driven by their ion selectivity and specific mode of activation.

On the other end of the ‘precision scale’ are the ‘pores’ such as antimicrobial peptides and toxins [43–45]. These proteins are released into and diffuse through an aqueous phase, attach and finally integrate into the lipid membrane [46,47]. Once assembled into homo oligomeric units they enable the flux of ions and small compounds across the lipid membrane in an ‘uncontrolled’ manner. No specific action is requested because simply the function of these proteins is to ‘kill’ the attacked cell via draining the cytosol of the cell.

Still the question remains, when exactly will a short protein classified as an ion channel [48] and when do we talk about a protein as ‘pore-like’. And more pressing, which technique should be used to decide on this issue. Neither techniques, permeability essays, bilayer recordings nor patch-clamp techniques with cells over expressing the respective proteins are free of pitfalls.

May be sequence features and structural models may help to classify the proteins to either side. Viroporins or channels are manufactured to be embedded within the membrane. The modes of action as for peptide antibiotics may find only limited applicability to describe the mode of action of the channel proteins. The way of how these proteins can assemble needs to be considered in more detail for suggesting a solution. For a single TMD it is hard to envision that in a homooligomeric assembly some of them suddenly may induce the formation of lipidic pores such as the peptide antibiotics whilst the others are not. It rather remains interesting to analyze whether the formation may lead to a ‘pore’ or ‘channel’ at all. With two TMDs within a protein one helix could eventually behave ‘detertgent’ like. 2B from Poliovirus may show such a dual-type of TMD system [49]. With 3 TMDs, such as in 3a from SARS-CoV, it may be worthwhile to compare the channel proteins with the larger host channels. The question remains whether we see a range of possible features where each of the features is limited to one particular protein. Or is it possible that a single protein covers multiple features and behaves channel/pore or detergent-like?

Based on a recent investigation it has been suggested that at least one of the proteins, may show a channel–pore dualism [40]. The proposal is based on conductivity experiments with a peptide representing the first 32 amino acids of Vpu from HIV-1, Vpu1–32, reconstituted into artificial lipid bilayers. The peptide, assumed to form bundles, shows conductance pattern in aqueous solutions of different alkali salts which reflect very weak selectivity. Selectivity in the pore is not driven by any strong electrostatic interaction of the permeating ion and the pore wall. It is also observed that the conductance is due to the simultaneous flow of cations and anions during an opening event. This will not allow for high selectivity and needs the respective pore diameter to be wide enough so that possibly also small molecules may permeate. Such an idea is supported by findings for 2B from Polio virus with 2 TMDs, where the pore size of the potential pore allows for size dependent substrate diffusion [35]. Structural data of the membrane morphology of 2B are not yet available. It is still an option for the 2B channel to adopt a ‘carpet’ like morphology [47] with one of the helices lying onto or strongly exposed to the membrane surface. A similar topology and uncertainty of membrane morphology like for 2B holds for p7 from HCV. In the case of the latter experimental data from cryo electron microscopic suggest that the two TMDs align antiparallel when in detergent environment [50]. Again, the question remains, whether this is the only morphology or can p7 also adopt various conformations under specific in vivo conditions. The concept of a weak channel with the consequence of a channel–pore dualism may not hold for all channels. With increasing TMDs per monomer, the likely-hood may vain. Either forth the name ‘channel’ is used for the proteins for simplicity.

2. Sequence alignment of Vpu with toxin and ion channels

The primary sequence of Vpu is aligned with ion channels and a toxin in anticipation that an environmental constraint spurs the development of a specific sequence and with it a similar structure within a protein. The focus is on the TMD of the proteins and therefore the ion channels chosen are those which adopt a helical TM motif similar to Vpu. The aligned TMDs are highlighted in the 3D structures of the respective channels and the toxin.

Sequence alignment of a representative number (10 isolates) of Vpu proteins (80 and 81 amino acids) taken from the Universal Protein Resource (UniProt) (www.uniprot.org) displays a large number of identical residues (10 residues) and conserved substitution (7 residues) within the hydrophobic TM region of Vpuα5–27 (Fig. 1A). HV1B8 and HV1SC isolates show insertions of threonine and serine,
respectively, at the N terminal side, whilst three isolates, HV1ND, HV1Z3 and HV190, replace Ile-24 by tyrosine at the C terminal side of Vpu6–27. Two isolates replace Ile-15 (HV1MN) Ala-7 as well as Val-8 (HV190) by glycine. A cladogram indicates that based on the amino acid sequence the different isolates adopt a very common ancestry (Fig. 1B). The numbering scheme mentioned refers to isolate AAB59750.1 (GenBank: AAB59750.1, www.ncbi.nlm.nih.gov) [51] which is taken as the sequence for alignment with the channels and toxin. Based on the almost perfect alignment the original isolate AAB59750.1 is chosen as an adequate representative of Vpu for sequence alignment with other channel and toxin proteins.

The following proteins have been used for sequence alignment with Vpu isolate AAB59750.1: cytolysin A (ClyA, toxin) [52], acid-sensitive potassium channel protein (TASK, UniProtKB/Swiss-Prot O14649, www.uniprot.org) [53], K⁺ channel from Streptomyces lividans (KcsA, PIR S60172) [54], nicotinic acetylcholine receptor (nAChR) [55], α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-sensitive, homotetrameric, rat glutamate A2 receptor (GluA2) [56], prokaryotic pentameric ligand gated ion channel (pLGIC) [57], pentameric formate channel (pFC) [58], large-conductance mechanosensitive channel (MsCl) [59], 3a from SARS-CoV (GenBank: ABA02268.1, www.ncbi.nlm.nih.gov) [60] and bone marrow stromal antigen 2 (BST-2, UniProtKB/Swiss-Prot Q10589, www.uniprot.org) [61]. Proteins have been pair wise aligned using ClustalW2 2.0.12 (www.ebi.ac.uk).

Sequence alignment of Vpu (Vpu1–81) with ClyA [52] shows no overlap of the TMD of Vpu with any parts of the toxin which is proposed to point into the lipid membrane (data not shown). When restricting the alignment to the first 100 amino acids of ClyA the hydrophobic part of the TMD of Vpu (Vpu6–27) has a considerable overlap with αA (residues 11–34) which is within the membrane in the assembled protein (Fig. 2A). The sequence αD/αE (residues 164 to 206) which is also suggested to interact with the membrane only slightly aligns with the C terminal side of Vpu (VpuW22–K31) (data not shown). The alignment leads to a break in the ClyA chain.

Comparison of the sequence of Vpu with human TASK-1 generates no alignment with the TMDs suggested by Hsu et al. [53] (data not shown). Aligning full length Vpu with the first 100 amino acids of TASK-1 reveals a match of the first 32 amino acids of both sequences as suggested by Hsu et al identifying 6 identical residues (Fig. 2B). The proposed TMD of TASK-1 (Leu-9–Glu-30) overlaps with the TMD of Vpu, residues Ala-9 to Arg-30. In the case of KcsA [54] residues Ile-19 to Ser-23 of Vpu and Ala-12 to Arg-27 of KcsA, as well as Ile-24 to Glu-28 of Vpu and Ile-38 to Ala-42 of KcsA align with one and two identical residues, respectively (Fig. 2C). This match is independent of the sequence used for either Vpu or KcsA. The structural motif indicates
that the overlap of the C terminal side of Vpu is with the outer helix of KcsA.

Alignment of Vpu with the primary sequence of either of the three ligand gated ion channels nAChR, GluA2 and pLGIC reveals no overlap with any of the individual TMDs of these channels (data not shown). Restricting the Vpu sequence to the first 30 amino acids, then an overlap with the TMDs of the ligand gated channels is observed. For nAChR sequence A/D (Fig. 2D) the N terminal side of Vpu3–12 matches parts of M1 (Pro-211–Ile-220), whilst the C terminal side, Vpu13–28, overlaps with M2 (Ile-247–Ile-264) (Fig. 2D, Sequence A/D, I). Omitting Vpu1–5 and Vpu28–30 leads to an extended overlap of the C terminal side of Vpu6–27 with M2 and a rather weak overlap of the N terminal side of Vpu6–27 with M1 (Fig. 2D, Sequence A/D, II).

The remaining sequences have in common an overlap of the TMD of

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**Fig. 2.** Similarity of Vpu [51] and various parts of it with respective toxin and non viral channel proteins. For detailed naming of the proteins see table caption of Table 1. Abbreviations and colors according to ClustalW2: ‘*’ = identical residues; ‘:’ = conserved substitution; ‘.’ = semi-conserved substitution; hydrophobic, aromatic (not Y) and small residues (AVFPMILW) are shown in red. The red bars indicate helical TMD of the proteins. The helices of the toxin and the non viral channels are named according to their respective notation in the literature.
Vpu_6-27 with M3 (Fig. 2D). For sequence B the N terminal side of M3 overlaps with the C terminal side of the TMD of Vpu_6-27 (Fig. 2D, Sequence B, I and II). Residue identity of Vpu_6-27-W22 with tryptophans of sequence C (Trp-331) and E (Trp-333) leads to disruption of Vpu_6-27 and a consequent overlap of Vpu_6-27 with M4 (Fig. 2D, Sequence C and E (II)). Favoring minimum disruption, overlapping of Vpu_6-27 with the sequences of nAChR sheds light of a potential overlap with M2 and M3.

The sequence of GluA2 allows an overlap rather with the Pre-M1 and N terminal side of the M1 domain of the receptor with the C terminal side of the TMD of Vpu_18-31 (Fig. 2E, I). Removing the ends in the Vpu sequence as mentioned for nAChR-Vpu alignment, shifts the overlap rather towards the N terminal side of the receptor which barely includes still the Pre-M1 domain (Fig. 2E, II).

Alignment of the Vpu_1-30 sequences with pLGIC results in a split of the Vpu sequence into three parts aligning with parts of α1 (Vpu1-10), α2 (Vpu11-23) and α3 (Vpu24-30) (Fig. 2F, I). The shorter Vpu_6-27 segment aligns solely with the α1 domain (Fig. 2F, II). For all three receptors the overlap of the longer sequence is driven by the Glu-Tyr-Arg (EYR-motif) of Vpu, whilst in the case of Vpu_6-27 Trp-22 is the driving amino acid for a consensus alignment. The overlap is with the domain which is at the outside of each of the subunits.

Unbiased alignment of Vpu with pFC results in an overlap of residues pFC-Ile-195 to pFC-Ser-204, which are allocated to the 5a domain which is at the outside of each of the subunits.

Fig. 2 (continued).
segment of the channel, with Vpu1–27 (Fig. 2G, I). The domain 5b of pFC overlaps with Vpu1–19. Vpu6–27 aligns this segment with pFC domain 4 (Fig. 2G, II). This is driven by overlap of Vpu-Trp-22 with pFC-Trp-231. Mutating Vpu-Trp-22 into alanine aligns the Vpu sequence Vpu6–27 with the pFC 2a domain (Fig. 2G, III). The result is that solely the TMD of Vpu has high sequence identity with an outside facing domain of pFC.

Unbiased sequence alignment of Vpu with TMD2 of MscL shows sequence identity with the TMD of MscL (Fig. 2H, I). A truncated sequence of MscL, MscL1–100, aligns the N terminal side of M-TMD1 with the C terminus of the Vpu-TMD (Fig. 2H, II). Aligning only the first 30 aa of Vpu with MscL1–174 delivers a match of both C termini of the TMD of Vpu with TMD2 (outer helix) of MscL (Fig. 2H, III).

Sequence alignment of Vpu with 3a reveals overlap with the proposed TMD3 of 3a and the cytoplasmic part of 3a (Fig. 2I, I). When aligned with the 3a1–150 the C terminal part of Vpu-TMD aligns with the N terminal part of 3a–TM1 (Fig. 2I, II). Taking Vpu1–150, overlap with the entire TMD1 of 3a is achieved (Fig. 2I, III). For Vpu6–27 the N terminal side (Vpu6–15) matches with the C terminal side of 3a–Leu-41 to 3a–Val-50 (Fig. 2I, IV). The position of TMD1 of 3a within the putative bundle is still under investigation and proposed to be at the outer side of the bundle [62].

Alignment of Vpu with TMD of BST-2 is almost a perfect match (Fig. 2).

2.1. Maximum overlap of the TMDs of Vpu with those of the toxin and ion channels

Alignment of Vpu6–27 with the selected proteins in this study reveals a maximum overlap with the TMD of pLGIC (Table 1). The TMDs of Vpu6–27 and α1 of pLGIC match over the entire 22 amino acids of α1 with only a single gap of only a single amino acid. TMD1 of TASK overlaps with 19 amino acids followed by BST-2 and MscL both showing 17 amino acids overlap of their TMD and TMD2, respectively. The nAChR and 3a still align 17 and 16 amino acids, respectively, with Vpu6–27. For nAChR the match is most pronounced for M2 (sequence A/D) followed by M3 with a match of 16 amino acids (sequences C and E). Sequence B allows 10 amino acids of Vpu6–27 to match with its M3 segment. With ClyA and pFC 15 and 14 residues, respectively, overlap, followed by GluA2 (10 amino acids) and KcsA (9 amino acids). Whilst for all channels mentioned just single gaps with up to 4 amino acids are observed, the TMD of KcsA is interrupted by a single gap of 10 amino acids of the channel. Ranking the perfect matches a sequence of MscL (7 matches), nAChR-C and TASK (5 matches each), pFC (4 matches), 3a, KcsA, pLGIC and nAChR-E (3 matches each). Taking maximum overlap and number of identical residues as a margin, pLGIC, TASK and MscL adopt the highest rank (‘top three’). All channels in common are an alignment driven by matches with tryptophans, phenylalanines and tyrosines of the respective channels with W-22 of Vpu6–27.

Highlighting the respective TMDs of each of the toxin and channels with which Vpu6–27 overlaps reveals that these TMDs are not always pore lining (Fig. 3A–C, orange helices). From the ‘top three’, pLGIC and MscL have matching TMDs rather at the outside of the proteins (Fig. 3B, C). For pFC the match is with the P segment which if fully embedded in the structure (Fig. 3C). A match with the pore lining domains is only found for two of the segments of the nAChR (Fig. 3B). Also for ClyA the matching segment α1 is a pore facing part of the giant pore (Fig. 3A). In most of the cases the TMD of Vpu matches with outside or embedded TMDs of the channels. For nAChR a duality is detected. It is concluded that the motif of the TMD of Vpu most likely harbors the capability of diffusing as a non-channel with some expertise to form a conducting channel. Channel formation need possibly specific conditions in vivo, such as lipid composition and dynamics or possibly also co-factors such as host membrane proteins or rafts to adopt a fully functional pore. For 3a the pore lining TMD is still to be elucidated, however the proposal of a match with TMD1 does not match with a proposed model in which TMD3 is pore lining [62] (Fig. 3C).

3. Structural modeling with in silico methods

The most advanced structural data are available for M2 from influenza A [63] and Vpu from HIV-1 [64] based on NMR studies. Most recently X-ray [65] and solution NMR structures [66] have been reported on M2 which deliver bundle structures even in the presence of drug candidates. Also for M2 of influenza B, BM2, a structure of the assembled TMDs including the cytoplasmic domain resolved with solution NMR spectroscopy is at hand [67]. The channel protein p7 is so far enlightened by cryo electron microscopy identifying its potential number of assembled units per channel to be most likely hexameric [23,50], albeit heptameric assemblies have also been reported [68].

On the computational level there are many options to achieve potential bundle models. One of the options is that the protocol for assembly can follow in vivo pathways as best as possible. This is that the protein monomers are produced in the endoplasmic reticulum (ER), diffuse within the ER and either interact – assemble – with other proteins, such as host proteins, or assemble with each other into homooligomers. In the latter case there can be pathways in which a putative bundle or monomeric unit is assembled by simultaneously approach of all units (concerted pathway). Alternatively, and most likely, the monomers assemble in a sequential manner (sequential pathway).

| Channel | aa of Vpu which overlap | Conserved substitution | Semi-conserved substitution | Identical residues | Aromatic aa |
|---------|-------------------------|-----------------------|-----------------------------|-------------------|------------|
| ClyA    | 15                      | 2                     | 2                           | 4                 | 7          | -          | -          | -          | W          |
| TASK    | 19                      | 5                     | 6                           | 1                 | 7          | -          | -          | -          | -          |
| KcsA    | 9                       | 3                     | 3                           | -                 | 2          | 1          | 10         | -          | W          |
| nAChR   |                         |                       |                             |                   |            |            |            |            |            |
| BST-2   |                         |                       |                             |                   |            |            |            |            |            |

Table 1

| Channel       | Similarity of Vpu6–27 [54] with respective toxin and non viral channel proteins: ClyA [52], TASK [53], KcsA [54], nAChR [55], GluA2 [56], pLGIC [57], pFC [58], MscL [59], 3a from SARS-CoV [60] and BST-2 [61]; Entry code in the protein data bank (www.rcsb.org) is given in brackets; ‘aa’ = amino acid; ‘*’ = identical residues; ‘-’ = conserved substitution; ‘?’ = semi-conserved substitution; ‘*’ = no specification suggested; ‘TMD’ = transmembrane domain. The helices of the toxin and the non viral channels are named according to their respective sequence number in the literature. W. B. Fischer, H.-J. Hsu / Biochimica et Biophysica Acta 1808 (2011) 561–571 |
|---------------|--------------------------------------------------------------|
| ClyA          | [2WCD] αA                                                   |
| TASK          | [M1/M2]                                                     |
| KcsA          | [1BL8] outer helix                                          |
| nAChR         | [2BG9]                                                      |
| GluA2         | [3KLY] Pre-M1/M1                                            |
| pLGIC         | [2VL0] α1                                                   |
| pFC           | [3KLY]                                                     |
| MscL          | [2OAR]                                                      |
| 3a            | [TM1]                                                       |
| BST-2         | [TM2]                                                       |
Most of the computational techniques adopted follow a kind of concerted pathways [69–74]. All potential TMDs are aligned and in a more or less fine grained step-width in respect of distances and angles. Conformational space is screened resulting in a moderate number of potential models. Ranking according to calculated potential energies allows the most appropriate model to be chosen also in accordance with all necessary constrains related with the general idea of a channel. In a more recent study the tetrameric M2 channel has been obtained.

Fig. 3. The toxin ClyA and the channel protein KcsA are shown in a side view (left) and a view from the respective top (middle) and bottom (right) of the protein (A). For the channel proteins only the TMDs are shown. The TMDs of nAChR, GluA2 and pLGIC are shown in the same way (B), so are the TMDs of the formate channel, MscL and 3a (C). The proteins are shown in green with the helical backbones sketched. Overlapping domains with Vpu6–27 are highlighted in orange.
from a replica exchange approach \cite{75}. Also here an enormous amount of conformational space is screened which depend nevertheless on the initial starting configuration.

In a more recently assembly method which is best described as a positioning approach combined with short energy minimization an extremely large number of structural models is achieved \cite{62}. In this method positioning is done for the backbone structure of a specific helix. The backbone structure is derived from a classical MD simulation of the monomer or monomeric unit embedded in a fully hydrated lipid bilayer. All the structures from the MD simulation are due to a principle component analysis (PCA). The frames belonging to the first eigenvector of the covariance matrix are averaged and used in the assembly method. Positioning is based on a rotational matrix for the Cα atoms in which rotational symmetry, tilt and rotation is altered with an angle and step widths chosen by the operator. In the assembly protocol for each positioning the side chains are consequently ‘attached’ to the individual Cα atoms on the bases of the most probably configuration taken from the software data base (MOE, www.chemcomp.com). At this stage short energy minimization steps follow and the models are ranked according to their potential energy. Structural integrity \cite{76} or ion selectivity \cite{77} of selected models can be further assessed stressing MD simulation techniques.

In the above mentioned assembly protocols all helices in a larger assembly, e.g. four or five helices, are moved, tilted or rotated simultaneously and in the same direction (concerted pathway). Sequential pathways have been so far rarely applied. It can be argued that it is most unlikely that all TMDs assemble at once. Most likely it seems that possibly two TMDs approach each other followed by either monomeric or dimeric TMDs and also oligomeric units. Along this line investigations have been conducted in which the alignment of the monomeric units of the three TMDs of 3a follows the so call sequential pathway (Hsu and Fischer; to be published).

For most of the models a helical motif of the TMD is assumed. In another approach using coarse-grained techniques the insertion and consequent formation of the secondary structural element is left to ‘in silico’ calculations \cite{78}. The formation is dependent on the quality of the force field based forces present in the environment of a lipid bilayer. Starting from a random structure outside the membrane Vpu from HIV-1 is seen to enter the membrane and adopt indeed a helical formation \cite{78}. Simulations on other proteins such as glycoporphin have proven that coarse-grained methods are reliable when assembling two TMDs from random positions. \cite{79}.

4. Drugs

Membrane proteins exhibit a variety of potential target sites. Most of these proteins express a considerable extramembrane domain which can be investigated separately from the TMDs. Usually crystallography and NMR spectroscopy can be used to obtain high resolution structural information followed by classical docking approaches for screening of the extramembrane parts of the proteins. Also the TMDs of these proteins may harbor potential target sites as has been shown recently with experiments for M2 of influenza A on a structural level \cite{65,66}. The antiviral drug amantadine has been detected inside the tetrameric bundle of M2 \cite{65} which is in accordance with other experimental \cite{80,82} and computational evidence \cite{83}. In a most recent solid state NMR investigation amantadine is found at both an inside and outside pose \cite{84}. The outside location of amantadine is detected to be on the ‘surface of the protein’ which is in contrast to the reported outside binding site of rimantadine which is identified to be at the protein–lipid interface \cite{66}.

A detailed evaluation of the currently ongoing controversy over the drug binding site in M2 is reviewed elsewhere \cite{85}. The conclusion from the current discussion in the field is that for future modeling of drug–protein interaction the lipid environment has to be more thoroughly taken into account in drug development.

Amantadines have been reported to become inefficient due to mutations within the TMD of M2 \cite{86,87}. Computational investigations reveal the reason for drug resistance is steric impair due to mutation at particular binding sites within the pore (see Khurana et al., this issue). Spiro-piperidines have been suggested as novel small molecule drugs to overcome viral resistance and reported to bind within the TMD of the M2 bundle \cite{88}.

Similarly, for Vpu from HIV-1 binding sites of potential drug candidates such as derivatives from amiloride are also reported to have the potential of inside and outside binding sites when docked to a pentameric bundle of the TMD of Vpu \cite{89,90}. Outside binding sites have been found as lowest poses for some of the compounds investigated. Blocking of hexamethylene amiloride has also been reported experimentally with a peptide corresponding to the TMD of Vpu \cite{91}. For p7 a series of drugs have been found to affect channel activity when p7 is reconstituted into artificial lipid membranes. The drugs identified are iminosugars \cite{22,92}, amantadine \cite{23,92}, hexamethylene amiloride \cite{93} and most recently substituted naphthyl guanidines \cite{94}. A specific binding site is so far proposed for amantadine to be inside a potential computationally derived hexameric p7 model \cite{73}. A direct link between the modulator effects on Vpu and p7 found in vitro and in vivo affects has yet to be established.

With the most recent findings that Vpu interacts with host factors such as BST-2 \cite{95,96} or eventually with TASK \cite{53} via a potential interaction of its TMD with those of the host proteins sparks the idea to target the viral proteins from ‘within’ \cite{13,97}. Here ‘within’ means the lipid membrane with the drugs preventing the oligomerization of the target protein either with itself or with the host. These ‘anti-oligomerization’ drugs (AODs) may either be peptides (anti-oligomerization peptide drugs (AOPDs), mimicking the potential interaction-partner in its sequence and conformation, or again small molecule drugs derived from conventional sources such as drug design, derivatives of existing drugs or herbs. Since screening for drug candidates needs to take the environment of the lipid membrane more thoroughly into account, this consequently imposes novel aspects on the docking approach. Peptide drugs have so far been reported to be a successful approach when interfering with the mode of action of fusion proteins from paramyxoviruses \cite{98}, HBV \cite{99} or gp41 from HIV-1 \cite{100} and one of HIV’s entry receptor protein \cite{101}. In all cases the peptide drug mimics parts of the viral fusion protein. Also membrane active proteins such as Nef from HIV are suggested to be the target of antiviral peptide drugs \cite{102}.

5. Conclusions

Some of the viral channel proteins are multi-tasking such as Vpu or Vpr, and some are known only in their role as a channel protein. In the latter case the protein consists of multi TMDs or as in the case of a single TMD (M2 from influenza A) is covalently coupled to a neighboring protein of the same kind (dimer of a dimer). Thus, increasing number of TMDs goes alongside with specificity. The single TMD protein Vpu shows motifs similar to pore lining helices but most likely also motifs of outer helices of large ion channels, which surround the pore lining helices. This supports a dual mode of action. Despite the tremendous success in viral channel research, questions remain, about the condition which is needed for the protein to finally start conducting ions after assembly. Is the ‘proper’ assembly already done during the mode of assembly or is there a reshuffling of the helices when assembled? Is eventually the reshuffling modulated by some other factors? Still an unknown field is the contribution of the extramembrane parts of the proteins. So far most of the results are based on separate investigations on either the TMDs or the cytoplasmic parts.

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References

[1] R. Adabae, A. Barco, L. Carrasco, Membrane permeabilization by poliovirus proteins 2B and 2BC, J. Biol. Chem. 271 (1996) 23134–23137.

[2] L. Carrasco, The inhibition of cell functions after viral infection, FEBS Lett. 76 (1977) 11–15.

[3] C.N. Na, Na and K changes in animal virus-infected HeLa cells, J. Gen. Virol. 65 (1984) 1135–1138.

[4] L. Carrasco, A.E. Smith, Sodium ions and the shut-off of host cell protein synthesis by picornaviruses, Nature 264 (1976) 807–809.

[5] A. Sakai, M. St. Claire, B. Sun, Severe acute respiratory syndrome-associated coronavirus 3a protein forms an ion channel that is blocked by the antiviral drug, amantadine, FEBS Lett. 535 (2003) 386–389.

[6] J.A. Mould, R.G. Paterson, M. Takeda, Y. Ohigashi, P. Venkataraman, R.A. Lamb, L.H. Lin, C. Schroeder, Deubel, B. Sun, Severe acute respiratory syndrome-associated coronavirus 3a protein forms an ion channel that is blocked by the antiviral drug, amantadine, FEBS Lett. 535 (2003) 386–389.

[7] J. Aittoniemi, J. Chang, P. Wentworth Jr., R. Dwek, P.C. Biggin, C. Chew, C. Huang, L. Li, Z. Wang, Y. Lu, Q. Bao, S. Chen, N. Wu, S. Cheng, J. Weng, Y. Zhang, J. Yan, L. Ding, H.W. Huang, Barrel-stave model or toroidal model? A case study on melittin pores, Biophys. J. 81 (2001) 1475–1485.

[8] X. Sui, L. Ding, H.W. Huang, Barrel-stave model or toroidal model? A case study on melittin pores, Biophys. J. 81 (2001) 1475–1485.

[9] K. Hsu, J. Seharaseyon, P. Dong, S. Bour, E. Marbán, Mutual functional destruction of the Golgi complex as the target organelle, J. Biol. Chem. 278 (2003) 1012–1017.

[10] F.J. Deu, M.P. Sansom, Viral ion channels: structure and function, Biochim. Biophys. Acta 1561 (2002) 27–55.

[11] J.F. Roeth, K.L. Collins, Human immunodeficiency virus type 1 nef: adapting to intracellular trafficking pathways, Microbiol. Mol. Biol. Rev. 70 (2006) 548–563.

[12] A. Fittipaldi, M. Giacca, Transcellular protein transduction using the Tat protein unitary current to the M2 ion channel protein of in... the antiviral drug, amantadine, FEBS Lett. 535 (2003) 386–389.

[13] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[14] A. Sakai, M. St. Claire, B. Sun, Severe acute respiratory syndrome-associated coronavirus 3a protein forms an ion channel that is blocked by the antiviral drug, amantadine, FEBS Lett. 535 (2003) 386–389.

[15] W.B. Fischer, M.S.P. Sansom, Viral ion channels: structure, function and drug design, in: A. Sakai, M. St. Claire, K. Faulk, S. Govindarajan, S.U. Emerson, R.H. Purcell, J. Bukh, Towards a mechanism of function of the viral ion channel Vpu from HIV-1, Proteins 70 (2008) 1488–1497.

[16] R. Dwek, P.C. Biggin, C. Chew, C. Huang, L. Li, Z. Wang, Y. Lu, Q. Bao, S. Chen, N. Wu, S. Cheng, J. Weng, Y. Zhang, J. Yan, L. Ding, H.W. Huang, Barrel-stave model or toroidal model? A case study on melittin pores, Biophys. J. 81 (2001) 1475–1485.

[17] Y. Shi, Z. Oren, From ‘carpet’ mechanism to de-novo designed diastereomeric cell-selective antimicrobial peptides, Peptides 22 (2001) 1629–1641.

[18] R.A. Lamb, L.H. Pinto, Do Vpu and Vpr of human immunodeficiency virus type 1 and NB of influenza A virus have ion channel activities in the viral life cycles? Virology 229 (1997) 1–11.

[19] G. Chang, R. Spencer, A.T. Lee, M.T. Barclay, D.C. Rees, Structure of the MscL cation-selective ion channel, Nature 452 (2008) 375–379.

[20] G.D. Ewart, T. Sutherland, P.W. Gage, C.B. Cox, The Vpu protein of human immunodeficiency virus type 1 forms cation-selective ion channels, J. Virol. 76 (1996) 7108–7115.

[21] A. Sakai, M. St. Claire, B. Sun, Severe acute respiratory syndrome-associated coronavirus 3a protein forms an ion channel that... the antiviral drug, amantadine, FEBS Lett. 535 (2003) 386–389.

[22] K. Hsu, J. Seharaseyon, P. Dong, S. Bour, E. Marbán, Mutual functional destruction of the Golgi complex as the target organelle, J. Biol. Chem. 278 (2003) 1012–1017.

[23] F.J. Deu, M.P. Sansom, Viral ion channels: structure and function, Biochim. Biophys. Acta 1561 (2002) 27–55.

[24] G.A. Woolley, B.A. Wallace, et al., Model ion channels: gramicidin and alamethicin, J. Membr. Biol. 129 (1992) 109–136.

[25] Z. Oren, Y. Shai, Mode of action of linear amphipathic α-helical antimicrobial peptides, Biopolymers 47 (1998) 451–463.

[26] K.A. Broden, Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat. Rev. Microbiol. 3 (2005) 238–250.

[27] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[28] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[29] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[30] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[31] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[32] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[33] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[34] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[35] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[36] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[37] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[38] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[39] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.

[40] L. Carrasco, Membrane leakiness after viral infection and a new approach to the development of antiviral agents, Nature 372 (1984) 674–660.
A. Kukol, P.D. Adams, L.M. Rice, A.T. Brunger, I.T. Arkin, Vpu transmembrane peptide structure obtained by site-specific tetrahydroxyperoxilatedmethyl radical and cluster calculations, Nature 451 (2008) 590–595.

J.R. Schnell, J.J. Chou, Structure of the M2 proton channel of influenza A virus, Nature 451 (2008) 591–595.

J. Wang, R.M. Pielak, M.A. McClintock, J.J. Chou, Solution structure and functional analysis of the influenza B proton channel, Nat Struct. Biol. 16 (2009) 1267–1271.

D. Clarke, S. Griffin, L. Beales, C.S. Gelais, S. Burgess, M. Harris, D. Rowlands, Evidence for the formation of a heptameric ion channel complex by the hepatitis C virus p7 protein in vitro, J. Biol. Chem. 281 (2006) 37057–37068.

A. Kukol, I.T. Arkin, Vpu transmembrane peptide structure obtained by site-specific fourier transform infrared dichroism and global molecular dynamics searching, Biochemistry 37 (1999) 1594–1601.

A. Kukol, P.D. Adams, L.M. Rice, A.T. Brunger, I.T. Arkin, 951–962, Experimentally based orientational refinement of membrane protein models: a structure for the influenza A M2 H+ channel, J. Mol. Biol. 286 (1999) 951–962.

L.R. Forrest, A. Kukol, I.T. Arkin, D.P. Tielemans, M.S. Sansom, Exploring models of the influenza A M2 channel: MD simulations in a phospholipid bilayer, Biophys. J. 78 (2000) 55–69.

F. Cordes, A. Kukol, L.R. Forrest, I.T. Arkin, M.S.P. Sansom, W.B. Fischer, The structure of the HIV-1 Vpu ion channel: modelling and simulation studies, Biochim. Biophys. Acta 1512 (2001) 291–298.

G. Patargias, N. Zitzmann, K. Schmidt-Rohr, W.B. Fischer, Modeling the hepatitis C virus channel p7, J. Med. Chem. 44 (2001) 3120–3130.

S. Faham, D. Yang, E. Bare, S. Yohannan, J.P. Whitelegge, J.U. Bowie, Side-chain flexibility artiﬁcial peptide, Biol. Chem. 388 (2007) 611–615.

M.S.P. Sansom, I.D. Kerr, Inhibition of amantadine binding site of influenza A virus from magic-angle-spinning solid-state NMR: the role of Ser31 in amantadine binding, J. Mol. Biol. 385 (2009) 1127–1141.

M.S. Cady, K. Schmidt-Rohr, J. Wang, C.S. Soto, W.F. DeGrado, M. Hong, Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers, Nature 463 (2010) 689–692.

R.M. Pielak, J.J. Chou, Flux channel drug resistance: a tale of two sides, Protein Cell 1 (2010) 246–258.

C. Wang, K. Takeuchi, L.H. Pinto, R.A. Lamb, Ion channel activity of influenza A virus M2 protein: characterization of the amantadine block, J. Virol. 67 (1993) 5585–5594.

X. Jing, C. Ma, Y. Ohigashi, F.A. Oliveira, T.S. Jardetzky, L.H. Pinto, R.A. Lamb, Functional studies indicate amantadine binds to the pore of the influenza A virus M2–proton-selective ion channel, Proc. Natl Acad. Sci. USA 105 (2008) 10967–10972.

J. Wang, S.D. Cady, V. Balanikin, L.H. Pinto, W.F. DeGrado, M. Hong, Discovery of spiro-piperidino inhibitors and their modulation of the dynamics of the M2 proton channel from influenza A virus, J. Am. Chem. Soc. 131 (2009) 8066–8076.

C.G. Kim, V. Lemaire, A. Watts, W.B. Fischer, Drug–protein interaction with Vpu from HIV-1: proposing binding sites for amiloride and one of its derivatives, Anal. Bioanal. Chem. 386 (2006) 2213–2217.

G. Patargias, G. Ewart, C. Luscombe, W.B. Fischer, Ligand–protein docking studies of potential HIV-1 drug compounds using the algorithm FlexX, Anal. Bioanal. Chem., accepted (2010).

W. Römer, Y.H. Lam, D. Fischer, A. Watts, W.B. Fischer, P. Göring, R.B. Wehrspohn, R. Gösele, C. Steinem, Channel activity of a viral transmembrane peptide in micro-BLMs: Vpu1235 from HIV-1, J. Am. Chem. Soc. 126 (2004) 16267–16274.

E. Steinmann, T. Whitfield, S. Kallis, R. Dwek, N. Zitzmann, T. Pietschmann, R. Bartenschlager, Antiviral effects of amantadine and iminosugar derivatives against hepatitis C virus, Hepatology 46 (2007) 330–338.

A. Premkumar, L. Wilson, G.D. Ewart, P.W. Gage, Cation–selective ion channels formed by p7 of hepatitis C virus are blocked by hexaminelathyrene amiloride, FEBS Lett. 537 (2004) 99–103.

C.A. Luscombe, Z. Huang, M.G. Murray, M. Miller, J. Wilkinson, G.D. Ewart, A novel Hepatitis C virus p7 ion channel inhibitor, BIT225, inhibits bovine viral diarrhea virus in vitro and shows synergy with recombinant interferon-α-2b and nucleoside analogues, Antivir. Res. 86 (2010) 144–153.

S.J.D. Neil, T. Zang, P.D. Bieniasz, Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu, Nature 451 (2008) 425–431.

N. van Damme, D. Goff, C. Katsura, R.L. Jorgensen, R. Mitchell, M.C. Johnson, E.B. Haberkorn, L. Fischer, J.-M. Pollok, B. Erbes, S. Seitz, S. Urban, Prevention of hepatitis B virus infection in vivo by entry inhibitors derived from the large chemokine receptors, Curr. HIV Res. 1 (2003) 51–57.

M. Montal, Vpu matchmakers as a therapeutic strategy for HIV infection, PLoS Pathog. 5 (2009) e1000246.

M. Porotto, C. Carta, Y. Deng, G.E. Kellogg, M. Whitt, M. Lu, B.A. Mungall, A. Moscona, Molecular determinants of antiviral potency of paramyxovirus entry inhibitors, J. Virol. 81 (2007) 10567–10574.

J. Bond, M.S.P. Sansom, Insertion and assembly of membrane proteins via simulation, J. Am. Chem. Soc. 128 (2006) 2697–2704.

C.S. Gandhi, K. Shuck, J.D. Lear, G.R. Dieckmann, W.F. DeGrado, R.A. Lamb, L.H. Pinto, Cu(II) inhibition of the proton translocation machinery of the influenza A virus M2 protein, J. Biol. Chem. 274 (1999) 5474–5482.

M. Yi, T.A. Cross, H.-X. Zhou, A secondary gate as a mechanism for inhibition of the M2 proton channel by amantadine, J. Phys. Chem. B 112 (2008) 7977–7979.

S.D. Cady, T.V. Mishana, M. Hong, Structure of amantadine-bound M2 transmembrane peptide of influenza A in lipid bilayers from magic-angle-spinning solid-state NMR: the role of Ser31 in amantadine binding, J. Mol. Biol. 385 (2009) 1127–1141.

M.S.P. Sansom, I.D. Kerr, Influenza virus M2 protein: a molecular modelling study on the ion channel, Protein Eng. (1993).

W.B. Fischer, H.-J. Hsu / Biochimica et Biophysica Acta 1808 (2011) 561–571