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Review

Drug sensitivity, drug-resistant mutations, and structures of three conductance domains of viral porins

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ABSTRACT

Recent controversies associated with the structure of the M2 protein from influenza A virus and the binding site of drug molecules amantadine and rimantadine motivated the comparison here of the drug binding to three viral porins including the M2 proteins from influenza A and B as well as the viral protein 'u' from HIV-1. While the M2 protein from influenza B does not normally bind amantadine, chimeras with the M2 protein from influenza A show blockage by amantadine. Similarly, Vpu does not normally bind rimantadine, but the single site mutation A18H converts a non-specific channel to a selective proton channel that is sensitive to rimantadine. The comparison of structures and amino acid sequences shows that the membrane protein sample environment can have a significant influence on the structural result. While a bilayer surface bound amphipathic helix has been characterized for AM2, such a helix may be possible for BM2 although it has evaded structural characterization in detergent micelles. A similar amphipathic helix seems less likely for Vpu. Even though the A18H Vpu mutant forms rimantadine sensitive proton channels, the binding of drug and its influence on the protein structure appears to be very different from that for the M2 proteins. Indeed, drug binding and drug resistance in these viral porins appears to result from a complex set of factors.

Contents

1. Introduction

Recently, there has been interest in the A18H mutant of Vpu, viral protein 'u' of HIV-1, that induces highly selective H^+ conductance not present in the wild type and that is blocked by rimantadine [1,2]. This mutation generates the proton-conductance signature sequence HxxxW

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found in two M2 proteins from influenza virions [3]. While the influenza AM2 conductance is amantadine and rimantadine sensitive, influenza BM2 conductance is not [4]. These three proteins have essentially no sequence homology except for the HxxxW motif. Here, we compare the sequences, and what is known about the structures of these three proteins for the domain responsible for the H^+ selective conductance. We will also discuss mechanisms for drug binding and drug resistance.

Of these three proteins, AM2 has been the subject of numerous conductance, structural, and computational studies. As with Vpu and BM2, there are no full-length structures, although for BM2 two overlapping constructs have been used to develop the first full-length structural model [5]. A variety of membrane mimetic environments have been used for the structural and functional studies. Most of the structural efforts have focused on the transmembrane (TM) domain, a single helix that as a tetramer for AM2 and BM2 conducts protons across the viral membranes, an essential function for the life cycle of the virus [3,6–9]. Based on detailed conductance studies by Pinto and coworkers [10], the minimal sequence that accounts for the conductance properties of AM2 near neutral pH includes not only the TM helix, but an additional sequence following the TM helix. This latter sequence is known to form a bilayer-bound amphipathic helix [11,12]. The drugs, amantadine and rimantadine, were effective against influenza A until recently when the influenza A strains became dominated by an S31N mutation displaying drug resistance. A variety of other naturally occurring mutants also display drug resistance. While there is no direct proton-conductance function associated with Vpu and HIV-1, the TM domain has been associated with non-specific ion conductance and the ability to enhance virion release from infected cells [13–15], such that a chimera of Vpu including the TM domain of AM2 appears to function normally in HIV-1 and displays proton selective currents [2,15].

The results discussed here reflect data obtained from a variety of technologies utilizing different membrane mimetic environments for membrane proteins. These environments have structural, dynamical, and functional implications that cannot be ignored if the goal is to describe the native state. Unfortunately, we are not yet able to characterize membrane proteins in their native membrane environment, but it is possible to characterize proteins in lipid bilayers, even liquid crystalline environments. Such environments are similar to native membranes in many respects, including a well-defined hydrophobic thickness, a complex interfacial region, and extreme dielectric and water concentration gradients. Yet it is important to acknowledge that synthetic bilayers and other model membrane environments may fall far short of complex native membranes.

2. Materials and methods

This paper is primarily a review paper; however, there are a few previously unpublished results that are included and hence this Materials and methods section.

2.1. Sample preparation

^{15}N-amantadine•HCl was synthesized according to the literature procedure [16]. ^{15}N-labeled acetonitrile (Isotec, Miamisburg, Ohio) was used to provide the ^{15}N source. The final product was verified by Mass and ^{1}H solution NMR spectroscopy. The M2 TM domain peptide, NH_2-Ser22-Ser-Asp-Pro-Leu-Val-Val-Ala-Ala30-Ser-Ile-Ile-Gly-Ile-Leu-His37-Leu-Ile-Leu40-Trp-Ile-Leu-Asp-Arg-Leu46-COOH, was chemically synthesized by solid-phase synthesis on an Applied Biosystems 430A Synthesizer using ^{15}N-labeled FMOC amino acids obtained from Isotec and Cambridge Isotope Labs (Cambridge, Mass). The peptide was purified and characterized as described previously [17].

Oriented samples of the ^{15}N-labeled peptide in hydrated DMPC bilayers were prepared by first co-dissolving M2 TM domain (10 mg) and DMPC (100 mg) in 5 ml TFE (trifluoroethanol). TFE was removed by rotary evaporation and dried under high vacuum. Fifteen milliliters of 20 mM CBP (citrate-borate-phosphate) buffer (~37 °C, pH 8.0) with 1 mM EDTA (ethylenediamine tetra-acetic acid) was added to the dried mixture and shaken at 37 °C. This lipid suspension was bath sonicated for 10 min intermittently. The sonicated suspension was loaded into a 3-kDa MW cutoff dialysis bag. The dialysis bag was placed in 1 L of 20 mM CBP buffer overnight to adjust the pH of the M2 TM domain/DMPC liposomes. For an M2 TM domain sample with 10 mM amantadine, 46.9 mg (250 μmol) amantadine hydrochloride (Fisher Scientific, GA) in 5 ml CBP buffer was added to an M2 TM domain loaded vesicle suspension (20 ml). The suspension was incubated at room temperature overnight and pelleted in 2.5 h by ultracentrifugation at 196,000×*g*. The pH value of the pellet was inferred from a measurement of the supernatant. The pellet was agitated at 37 °C for 1 h until fluid. For M2 TM domain studies with amantadine, proteoliposomes were prepared first without amantadine and then amantadine was added to a 1-ml suspension of liposomes at the desired protein:drug ratio and incubated overnight before sample preparations. This thick fluid was spread onto 50 glass slides (5.7 × 12.0 mm) (Marienfeld Glassware, Lauda-Königshofen, Germany) and dried in a humidity (70–75% relative humidity) chamber using an N_2 atmosphere. The partially dehydrated slides were stacked together, inserted into a glass tube, and rehydrated in a 96% relative humidity (saturated K_2SO_4) chamber at 40 °C for one week. Finally the glass tube was sealed with wax.

2.2. PISEMA spectroscopy

All PISEMA spectra were acquired at 600 MHz utilizing low-E probes at the NHMFL except for the full-length M2 PISEMA spectrum that was obtained at 400 MHz as described previously [11]. The PISEMA spectra of the M2 TM domain were obtained with the following parameters: 800 μs cross-polarization contact time, 4 ms acquisition time, 6 s recycle delay, and ^{1}H decoupling with the SPINAL scheme [18]. Spectra were typically acquired with 32 t_1 increments resulting in total acquisition times ranging from 6 h to 3 days. All experiments were conducted at either 30 or 40 °C, well above the gel to liquid crystalline phase transition for the bilayer systems employed. Spectra were processed using in-house scripts written for NMRPipe [19]. Processing scripts included the following: zero filling of t_2 to 1024 points, linear prediction of t_1 to 128 points; exponential multiplication window function for t_1 and t_2 domains with 164 Hz Lorentzian line broadening to enhance signal to noise; shifted sine bell curve for both t_1 and t_2 domains; Lorentz-to-Gauss window function to reduce linewidths; Fourier transformation of both t_1 and t_2 time domains; correction of the ^{15}N–^{1}H-dipolar coupling dimension by a scaling factor of 0.816 (sin54.7°). ^{15}N-chemical shifts were referenced to liquid ammonia at 0 ppm via a saturated solution of $^{15}NH_4NO_3$ at 26 ppm.

3. Results and discussion

3.1. Sequence analysis of viral ion channels

All three proteins are single pass membrane proteins with a TM domain flanked by hydrophilic domains (Fig. 1). The focus here will be on the TM helix and the subsequent amphipathic sequence. With the exception of a few key functional residues lining the channel pore, amino acid composition of the TM helices is largely hydrophobic. For such a composition, the possibilities for hydrogen bonds and electrostatic contacts at the helix–helix interface are rare. TM helical association is therefore largely determined by weak van der Waals interactions. In fact, stronger inter-helical interactions might considerably hinder the dynamics important for channel function. The amphipathic sequence has a net positive charge and its amphipathic composition suggests a helical structure.

The TM domains of AM2 and BM2 feature the proton channel signature sequence, HxxxW [3]. Otherwise, the TM sequences of AM2