ELECTROPHYSIOLOGY STUDIES OF M2 PROTEIN FROM INFLUENZA VIRUS A

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Introduction

The M2 protein of influenza A virus forms a homotetramer proton channel in the lipid membrane and plays an important role in the life cycle of the virus. The channel conductance for protons can be blocked by amantidine and ramantidine. Electrophysiological measurements have been reported for ionic permeability due to M2 expressed in CV-1 cells, Xenopus oocytes, mouse erythroleukemia cells, and planar lipid bilayers. The currents were pH-activated, amantadine sensitive, and selective for protons. However, these features in lipid bilayers were less convincing than expected and it has brought into question standard protocols for the reconstitution of protein from typical expression systems. This has very broad implications for the study of membrane proteins.

Experimental

The Udorn variety of M2 protein from influenza was cloned into pET23d and pTBGS vector with a six-His tag at the C and N-terminus. The expressed protein was purified by Ni-affinity chromatography. The protein was reconstituted into DMPC:DMPG (4:1, all lipids from Avanti Polar lipids) liposomes in 1:10 (protein:lipid w/w) ratio using the detergent dialysis method. Membrane currents were measured using a Warner BC-525C Bilayer Clamp amplifier. To determine the selectivity among different cations, bionic experiments were performed using a Delrin cup (Warner Instruments) with asymmetrical solutions containing 250 mM of test ions (NaCl, KCl, CsCl, or methylammonium chloride, 150 mM, pH 5.3) in cis chamber and a mixture of 100 mM NH4Cl and 150 mM test ion in trans chamber. Currents were then recorded at +90 mV and -90 mV. Analysis of channel current and lifetime was performed using TAC and TACfit from Bruxton Corp. Digital filtering was applied with a cut-off of 30 Hz.

Results and Discussion

Single channel proton currents have been observed with the reconstituted protein. This was a major breakthrough after over 15 years of efforts by several labs, and put us in a position to analyze the other behaviors of the protein. We have studied the permeability of the channel to ammonium, chloride, sodium, potassium, cesium, and methylammonium, the sensitivity to the antiviral drug, amantadine, and the effect of pH on permeability, assayed with ammonium currents.

We have also measured the ammonium permeation properties of several structural variants of the protein, including one with a His-tag (added for purification purposes) remaining on the C-terminus, one with it remaining on the N-terminus, one with fluorinated Trp at the three Trp positions (including Trp41, which is expected to project into the channel selectivity filter), and one with the small Ala residue replacing His37, which is also expected to project into the selectivity filter.

We expect changes in the selectivity filter to strongly affect ion selectivity and permeability properties. In addition, the Trp41 residues from the four subunits have been shown to be important for acid activation. Some studies have shown that amantadine may block the channel (and prevent viral propagation) by inserting into the channel near the selectivity filter.

Our results seem to imply that the physiological channel has not been obtained in our reconstitution. On the one hand, the channel is selective for protons, but the native channel has been found to be approximately 1000-fold more selective. The channel is amantadine sensitive, but only at about 0.03 times as much as the naturally expressed channel. Finally, the variants we have tested have behaved essentially identically with the native protein in ammonium and hydrogen permeability and amantadine sensitivity, indicating that slight changes in the structure do not fully restore normal function.

Conclusions

Thus the reconstituted protein is behaving as if there is some change in the structure relative to that in cellular expression systems. It is as if the His residue were absent from the pore, because mutation doesn’t affect permeation. Likewise, the selectivity sequence, low amantadine affinity, and lack of apparent acid activation are consistent with a channel of narrow diameter, but not “essentially occluded” as predicted by current models for the transmembrane domain in membranes based on solid state NMR constraints.

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