DIRECT MEASUREMENT OF PROTON TRANSPORT THROUGH INFLUENZA VIRUS A M2 PROTEIN RECONSTITUTED VESICLES

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Introduction

The influenza virus M2 protein, target of the antiviral drugs, amantadine and rimantadine, forms an acid-activated tetrameric proton-conducting ion channel which functions during uncoating and maturation by modifying the pH in virions as well as in trans-Golgi vesicles. 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 hydrogen. This study directly measured the proton conductivity in vesicles containing reconstituted M2.

Experimental

The Udorn variety of M2 protein from influenza A was cloned into pET23d, overexpressed in *E coli*, purified using Ni affinity and DEAE ionic exchange chromatography. The protein was reconstituted into DMPC and DMPG lipids (4:1 molar ratio) at a 1:5 protein:lipid (w:w). The size of the resulting liposomes was measured by dynamic light scattering (90Plus Particle Size Analyzer, Brookhaven Instruments Corporation, Holtsville, NY). For experiments at pH 7 and pH 5 different intravesicular and extravesicular buffers were used. Proton flux across the vesicle membrane was measured by the method described by Cao, et al. (2001). The translocation buffer for experiments at pH 7 was composed of 190 mM Na_2SO_4, 0.1 mM KH_2PO_4 and 0.1 mM K_2HPO_4 and, for experiments at pH 5.4, this buffer was composed of 190 mM Na_2SO_4, 0.1 mM K_3Citrate and 0.1 mM KH_2Citrate.

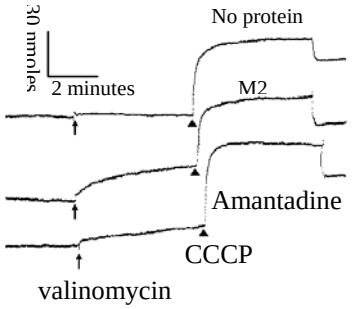

Fig. 1. Proton Flux Assay: Representative tracings of proton flux into vesicles. Addition of valinomycin and CCCP is marked by the arrowhead.

Results and Discussion

Figure 1 shows a typical result of pH change due to proton translocation by M2 reconstituted into vesicles at pH 7. The fast rise in pH immediately after valinomycin addition in the vesicles containing M2 is due to proton movement in response to valinomycin induced K^+ efflux. This fast signal is not observed in control vesicles. The 10 – 20 second slope after initial addition of valinomycin is proportional to H^+ influx. This figure also shows the pH rise after CCCP addition with both M2 and control vesicles. This is due to the exchange of K^+ for H^+ in all vesicles that do not contain M2. The total signal is a combination of the valinomycin and CCCP signal which is proportional to the total volume entrapped inside the vesicles and depends on the buffer strength inside and outside the vesicles. Figure 1 also shows the effect of external amantadine on M2-induced proton flux. Consistent with amantadine block, the valinomycin signal is reduced indicating the reduction of M2-induced proton flux.

Conclusions

In summary, the proton flux assay has the potential to provide very sensitive and accurate measurements of M2 channel activity. The present work reports our first attempts to measure proton flux through the M2 protein reconstituted into lipid vesicles by monitoring external pH after addition of valinomycin to vesicles with 100-fold diluted external $[K^+]$. Proton uptake was not significantly altered by acidification. Under neutral conditions, proton conductance was inhibited by external addition of 1 mM amantadine. Such experiments validate our NMR studies on these preparations.

References

Cao, N.J.Y., W.S.A.Brusilow, J.J.Tomashek, and D.J.Woodbury. 2001. *Cell Biochemistry and Biophysics* 34:305-320.

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