pH Dependent Conformational Change of Full-Length Influenza A M2 (M2FL) Protein Observed from Unambiguous Distance Measurement from Solid-state NMR Experiments

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Introduction

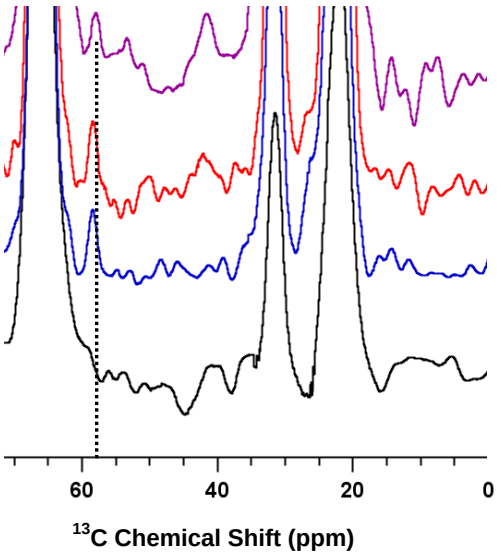
Influenza A M2 protein is an essential protein for this virus and a drug target for the treatment of this infectious disease. NMR has been widely used as a tool to study the structure and function of this homo-tetrameric proton channel. By identifying interhelical distances in M2FL, the tetrameric conformation can be characterized crucial for the development of therapeutic agents like channel blockers. Possible pH dependency for M2FL conformation has been previously suggested and in this study, we utilize unambiguous distance measurements from solid-state NMR experiments to study conformational variations and to observe the effect of a known channel blocker (amantadine) on this structure.

Experimental

Isotopically labeled M2FL is expressed in in E. coli followed by Ni^+ affinity purification. Then tetrameric samples were prepared with a mix of isotopically labeled M2FL protein monomers in detergents followed by detergent removal and reconstitution of M2FL into lipid bilayers. NHMFL 600 MHz wide bore NMR and an in house built 3.2 mm 1H-13C-15N CPMAS probe were used to carry out DARR (Dipolar Assisted Rotational Resonance) solid-state NMR experiments on these M2FL samples.

Results and Discussion

Results obtained to date clearly indicates backbone conformational changes from pH 7.5 to pH 5.0 (which resembles the closed and open state of the channel). Conformation of the open channel does not appear to be influenced by amantadine regardless of whether this drug is bound to M2FL in its open or closed state. How this channel blocker affects the backbone conformation of the closed channel and pH effects on the S31N mutant are being studied.



- Purple: Amantidine is added to the open channel (pH 5.0) of Valine and Leucine labeled mixture
- Red: Amantidine is added to the closed channel (pH 7.5) of Valine and Leucine labeled mixture. Spectrum is collected while channel is open. i.e. pH 5.0
- Blue: No amantadine is added to Valine and Leucine labeled mixture. Spectrum is collected while channel is open. i.e. pH 5.0
- Black: No amantadine is added to Valine and Leucine labeled mixture. Spectrum is collected

Fig.1 slices of 2-dimentional DARR spectra at the Cα valine frequency through valine α-carbon; dotted line shows chemical shift of Leucine α-carbon

Conclusions

Here, it is demonstrated that interhelical contacts in M2FL characterized by a mixed isotopic labeled strategy can be used to study conformational changes in M2FL.

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