Elucidating the Mechanism of M2 Proton Channel Inhibition Using Solid-State NMR to Aid in Design of Compounds with Broad Specificity

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Introduction

Despite numerous drug design efforts, there are only two classes of therapeutics targeting Influenza A virus. Clinical and agricultural use of these pharmaceuticals has generated multiple drug resistant viral strains [1]. Efforts in drug design, targeting the M2 channel, have been impeded by the limited number of experimental techniques with capabilities for structural characterization of the protein-ligand interaction in native-like membrane mimetic environments. Elucidating the mechanism of inhibition in the **full length** channel using Solid State Nuclear Magnetic Resonance (ssNMR) ensures that native interactions between the channel and lipid bilayer are retained and channel pore is not artificially constricted or kinked, as it might be in a detergent micelle environment. Using ssNMR and deuterated rimantadine enantiomers as well as novel inhibitor of the wild type channel, binding specificity and stabilizing interactions are observed in the wild type channel. While structural characterization of the S31N mutant conductance domain yields structural information to aid in drug design of novel small molecules with broad spectrum specificity, targeting both mutant and wild type variants.

Experimental

3D NCA correlation experiments were performed on ^{13}C,^{15}N-site specifically labeled full length M2. Isotropic chemical shift changes were observed to confirm that drug binding took place. Differences in the bound peak volume indicated binding specificity of each enantiomer. 2D ^{13}C-^{13}C correlation experiments were utilized to characterize previously unseen structural perturbations due to binding of a novel inhibitor. NMR experiments were performed at the NHMFL NMR facility in Tallahassee, FL. 21.1 T ultra wide bore, 16.9 T and 14.1 T wide bore spectrometers, equipped with “Low E” static and magic angle spinning probe capabilities, were used for the data obtained in this study.

Results and Discussion

Inhibitor molecule binding in the shorter construct of M2 has been previously characterized with a 3D NCA correlation experiment [2]. Similarly in the full length protein, significant chemical shift changes in the ^{15}N dimension can be seen in Figure 1 from the apo S31-Ca to the drug bound S31-Ca’. Two distinct sets of peaks with different peak volumes are indicative of different fractions of the bound and unbound population. Identifying a single enantiomer with higher specificity is the first step in designing an improved inhibitor targeting multiple viral strains.

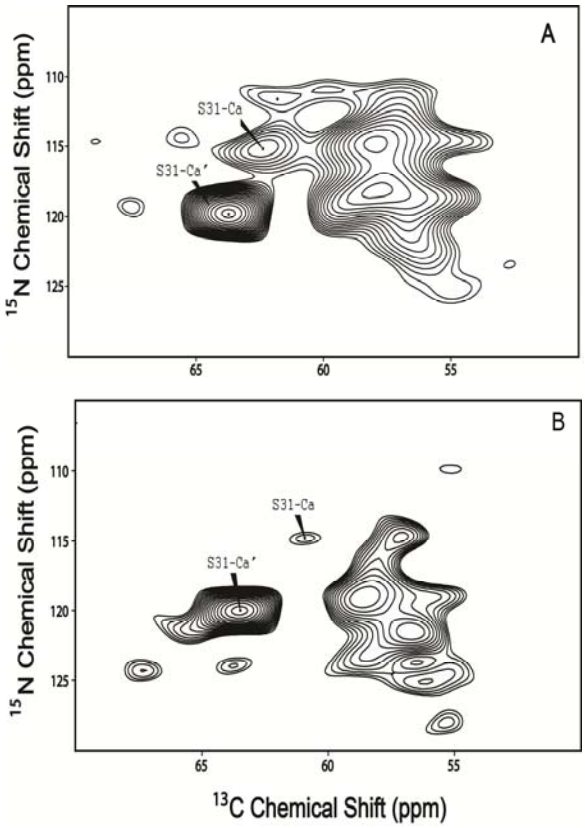

Fig.1 2D NCA correlation spectra for ^{13}C, ^{15}N-Ser,Gly M2 FL wild type bound to rimantadine enantiomers. Bound signal is indicated with a prime.

Acknowledgements

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References

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