Solid-State NMR Characterization of s31n m2 Transmembrane Domain Bound to Novel Adamantanes with Persistent *in vitro* Efficacy

A.K. Wright (FSU, Molecular Biophysics, NHMFL); I. Hung (NHMFL); C. Tzitzoglaki, H. Ioannidis (National and Kapodistrian U. of Athens, Pharmacology and Medicinal Chemistry); D.D. Busath (Brigham Young U., Physiology and Developmental Biology); A. Kolocouris (National and Kapodistrian U. of Athens, Pharmacology and Medicinal Chemistry); T.A. Cross (FSU, Chemistry and Biochemistry, NHMFL)

Introduction

As a result of amino acid mutations, in the residues lining the pore of the M2 proton channel FDA-approved antiviral agents, amantadine and rimantadine[1] are no longer suitable therapeutics for the infections. More than 95% of the circulating strains isolated from human clinical cases bear the S31N mutation[2,3]. Efforts in rational drug design targeting S31N 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.

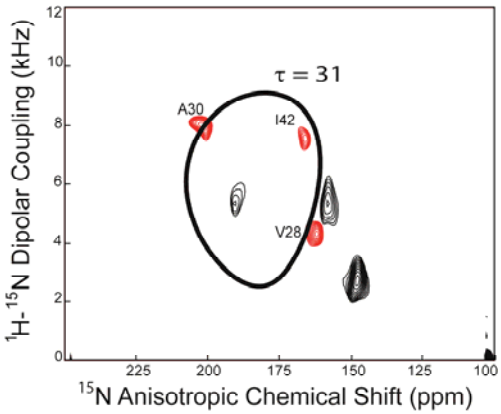

Fig. 1: Superimposed SAMPI spectra of the S31N M2 TMD (residues 22-46), with (red) and without (black) compound **14.**

Experimental

^{15}N-$V_{28}A_{30}I_{42}$ S31N M2 transmembrane domain (TMD) was synthesized using Fmoc (9-fluorenylmethoxycarbonyl) chemistry and incorporated into dimyristoylphosphatidylcholine (DMPC) liposomes. Samples were packed into a 3.2 mm rotor or uniformly oriented on glass slides, following compound addition. NMR experiments were performed at the NHMFL NMR facility. 21.1 Tesla ultra wide bore, 16.9 Tesla and 14.1 wide bore spectrometers, equipped with "Low E" static and magic angle spinning probe capabilities, were used for all the data obtained in this study.

Results and Discussion

Oriented sample solid state NMR experiments of S31N M2 TMD in Fig.1, indicate that the compound is bound in the channel pore, inducing a change in the helical tilt relative to the bilayer normal. The kink in the monomer between two helical fragments, observed in the wild type M2 TMD bound with amantadine, is absent for the S31N M2 TMD bound to all inhibitor molecules tested to date. Furthermore, chemical shifts observed in 2D NCA spectra in Fig.2 confirm inhibitor binding in the channel and rule out topological changes (Fig1) as a function of non-specific interactions.

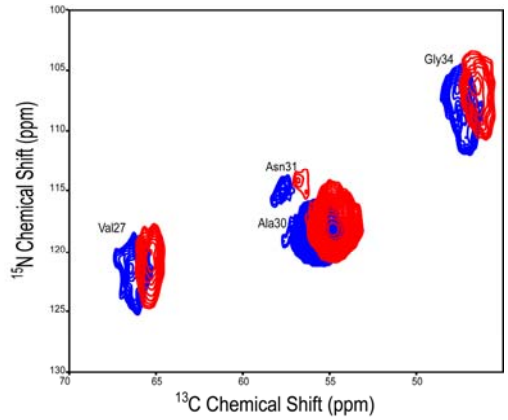

Fig. 2 Superimposed 2D NCA spectra of ^{13}C,^{15}N-V_{27},A_{30},G_{34} S_{31}N M_2 TMD with **14** (red) and without (blue).

Conclusions

Binding data for all inhibitors tested to date suggests a different mechanism of inhibition than for the wild type channel. Further experiments are in progress to elucidate the specificity of the interaction.

Acknowledgements

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References

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[3] Y. Lan, *et al.*, Antiviral therapy**, *15*(6)**, 853-9 (2010).