Dynamics of Ala_{29} on the Transmembrane Segment of the Influenza A M2 Proton Channel

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Introduction

M2 proton channel is essential for the influenza A virus life cycle [1]. Based on the known backbone structure of the M2 conductance domain [2] the dynamics of the Ala_{29} backbone and side chain were characterized.

Experimental

M2 transmembrane (22-46) (M2TM) was labeled at Ala_{29} (L-Ala-d4). ^{2}H static NMR spectra of M2TM reconstituted in DOPC:DOPE bilayers at pH 7.5 were acquired at -40°, -20°, 15°C using various echo times. Experiments were carried out at the NHMFL using the 19.6 T 31 mm and the 21T 105 mm bore spectrometers equipped with "Low E" probe technology built inhouse. Simulations were performed using Express [3].

Results and Discussion

M2TM(d4Ala_{29}) spectra at -40° and -20°C, where rotational motion of the channel is arrested, indicates that the Cα-D bond is undergoing libration motion with an amplitude of ±12.5°. This motion is a reflection of the libration of the peptide plane. Simulated spectra of tetrahedral methyl deuterons (CD_3) with peptide plane libration of ±12.5° and rotation of the CD_3 with a correlation time (τ_{methyl}) of ns, agrees with the experimental spectra (Figure 1A). At 15°C the spectrum collapses (Figure 1B) indicating that the channel is undergoing rotation motion about its main longitudinal axis. The insert in Figure 1B shows a broad area of the spectrum attributed to the Cα-D which, at 15°C, is affected by the rotation of the channel around its main axis. This broad region can be fitted to a simulated spectrum correspondent to libration of ±15° amplitude and $\tau_{channel}$ of μs. The ±15° amplitude is in agreement with previous studies of M2TM in DMPC bilayers [4]. Simulations of line shapes representing the motion of CD_3, with same parameters used for Cα-D, fits well with the experimental spectrum using an echo time of 10 μs. This simple model fails to explain line shapes at larger echo times. Dependence of peak area with echo time (data not shown) is well represented by a double exponential with two T_2 values, 200 and 12.5 μs. Figure 2 illustrates modes of motion described.

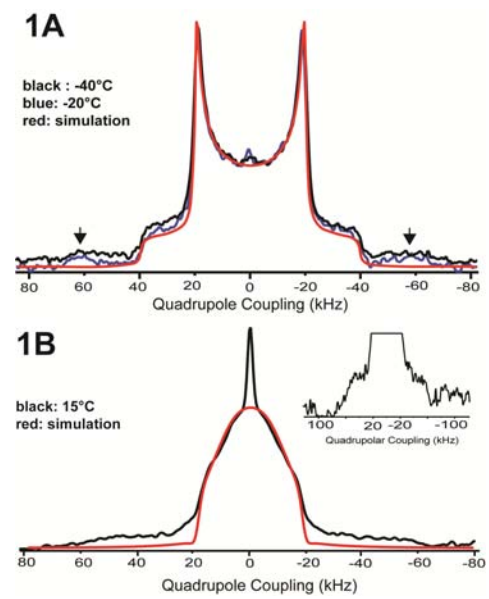

Figure 1. A) Spectra of M2TM(d4Ala_{29}) at -20°C (black) and -40°C (blue) and simulated spectra (red). B) Spectrum of M2TM(d4Ala29) at 15°C and 10μs echo time (black) and simulated spectra (red). Insert on the top right corner expands region of broad intensity attributed to

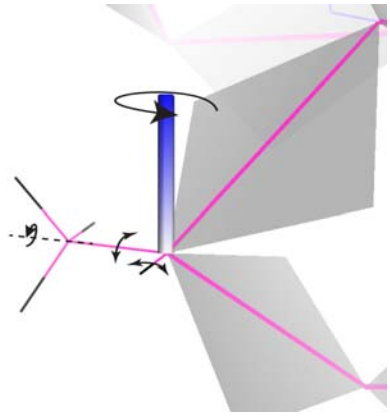

Figure 2. Ala_{29} at M2TM. Grey area: peptide plane. Pink: carbon. Black: hydrogen. Blue: channel longitudinal axis positioned at Cα. Double head arrows: motion on the surface of cone of defined angular amplitude. Single head arrows: motion about an axis.

Conclusions

At low temperatures ^{2}H NMR spectra of M2TM(d4Ala_{29}) is determined by libration of the peptide plane with ± 12.5° amplitude and τ_{methyl} on the ns time scale. Upon increase in temperature rotation of the channel yields an averaged spectrum. The spectrum at 10μs echo can be simulated by a $\tau_{channel}$ of μs. The dependence of M2TM(d4Ala_{29}) spectra with the echo time indicates that more than one type of motion is responsible for the observed line shape.

Acknowledgements

This work was supported by NIH/NIAID (5R01AI073891) grant and the NHMFL, a cooperative agreement between the State of Florida and the NSF (DMR1157490).

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