Full Length M2 Protein Structural Characterization

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Introduction

H37 is the key residue functioning as the proton transporter in the Influenza A virus M2 protein [1]. The side chain conformation transition during pH titration and the pKas of H37 tetrad in M2 full-length (M2FL) protein have not been characterized previously. Our solid-state MAS NMR results indicate H37 tetrad in M2FL has a complex conformational transition process from neutral to acidic pHs while taking on charges. Meanwhile, the pKas turn out to be different from those obtained in the M2TM truncated version at room temperature. [2,3]

Experiments

Solid-state MAS NMR spectra were acquired on the Bruker Avance 600.1 MHz NMR spectrometer using an NHMFL Low-E triple-resonance biosolids MAS probe. The reversely ^{13}C, ^{15}N H37-labeled M2 mutant (H57,93Y) was reconstituted in DOPC/DOPE liposomes. 2d DARR with 50ms mixing time, 1d ^{15}N and 2d NC correlation experiments were recorded at 243K on samples with different pHs. The histidine ring ^{13}C and ^{15}N chemical-shift changes during the pH titration are monitored. With the help of 2d spectra, assignments are done for the charged and neutral histidine cross peaks. In every 1d ^{15}N experiments, a series of CP contact times were used and the spectra with the best signal to noise for both protonated and nonprotonated nitrogen signals were used for a clear view of peak position and shape changes during titration (Fig.1).

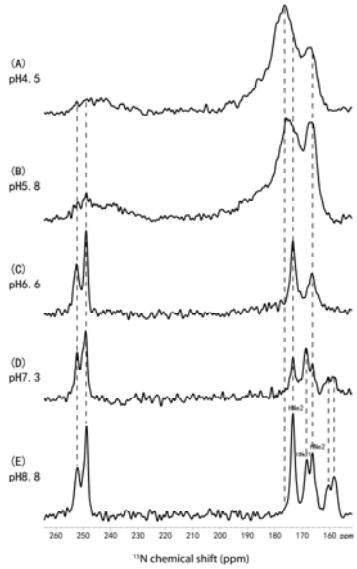

Fig.1 1d ^{15}N spectra of M2FL H37 aromatic nitrogens at different pHs at 243K .

Results and Discussion

Fig.1 shows 1d ^{15}N spectra of protonated and nonprotonated ring nitrogen signals of H37 at different pHs. There is no chemical shift change of any of the peaks between pH8.8 and pH7.3, thus no titration happened during this range. The ~160ppm peaks disappear below pH7.3. From pH6.6 to pH5.8, all the peaks become broader and a down-field shift of protonated ^{15}N signal is observed. At pH4.5, we still observe some nonprotonated nitrogen signal, which means +4 charged state of the H37 tetrad is not reached. While Fig.2 shows the H37 aromatic carbon signals at different pHs, we saw obvious changes happening during the titration. Together with the ^{15}N spectra, the results suggest titrations between pH6 and 7.

Conclusions

In the M2FL below the lipid phase transition, the H37 tetrad +1,+2 and +3 charged states seem to be formed in a narrow low pH range (around 6.0-7.0). It maintains at +3 charged state between pH6.0-4.5, when the channel is active for proton transfer. The H37 tetrad conformation transition process in M2FL is evidentially more complex than that in any of the truncated M2 versions [3,4]. We look forward to find some experiment evidence of how the charge is stabilized in the +2 state.

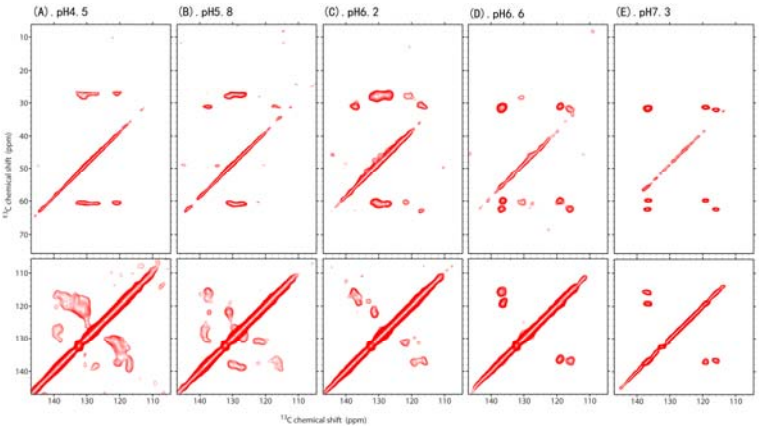

Fig.2 Aromatic regions of 50ms DARR of reversely ^{13}C, ^{15}N H37-labeled M2FL mutant (H57,93Y) at different pHs at 243K.

Acknowledgements

This work was supported by NIH AI074805, AI023007 and, the NHMFL via the State of Florida and NSF DMR 1157490.

References

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