The Histidine Tetrad of the M2 Proton Channel: Proton Selectivity and Conductance Explained

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Introduction

The Influenza A M2 protein forms an acid-activated proton-selective tetrameric channel, in which His-37 (H37) acts as both the pH sensor and selectivity filter. The side chain conformational transitions during pH titrations 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 as a function of pH while taking on charges. The first two protons are added in a narrow pH range, which suggests cooperativity.

Experimental

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 liposomes. 1D ^{15}N variable CP, ^{1}H T1ρ measurement, 2D ^{13}C-^{13}C PARIS, EXSY, ^{13}C-^{15}N TEDOR, ^{1}H-^{15}N HETCOR experiments were recorded on samples at various pHs. The H37 tetrad tautomeric and conformational compositions as a function of pH were studied. The titration was accomplished by calculating the amount of charged and neutral histidine at different pHs.

Results and Discussion

Fig.1 shows 2D ^{13}C-^{15}N TEDOR spectra of H37 side-chain signals at different pHs at 243K. At pH7.3, there are 4 neutral states. At pH6.6, when the charged H37 appears, a new neutral state arises to interact with the new charged state. At pH6.2, there are more charged residues. At pH4.5, most of the His residues are charged. The TEDOR spectra provide hints as to how the H37 tetrad changes conformation and charged states as a function of pH which informs us about the conductance mechanism.

Conclusions

The spectra of the M2FL His tetrad is much more complex than that of the M2 truncated versions and those in non-native like lipids [1,2] emphasizing the importance of performing these studies with M2FL in native-like lipid bilayers. With the data we now have we anticipate that a functional mechanism can be achieved.

Acknowledgements

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References

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[2] Hu, F., *et al.*, JACS, **134**, 3703 (2012).

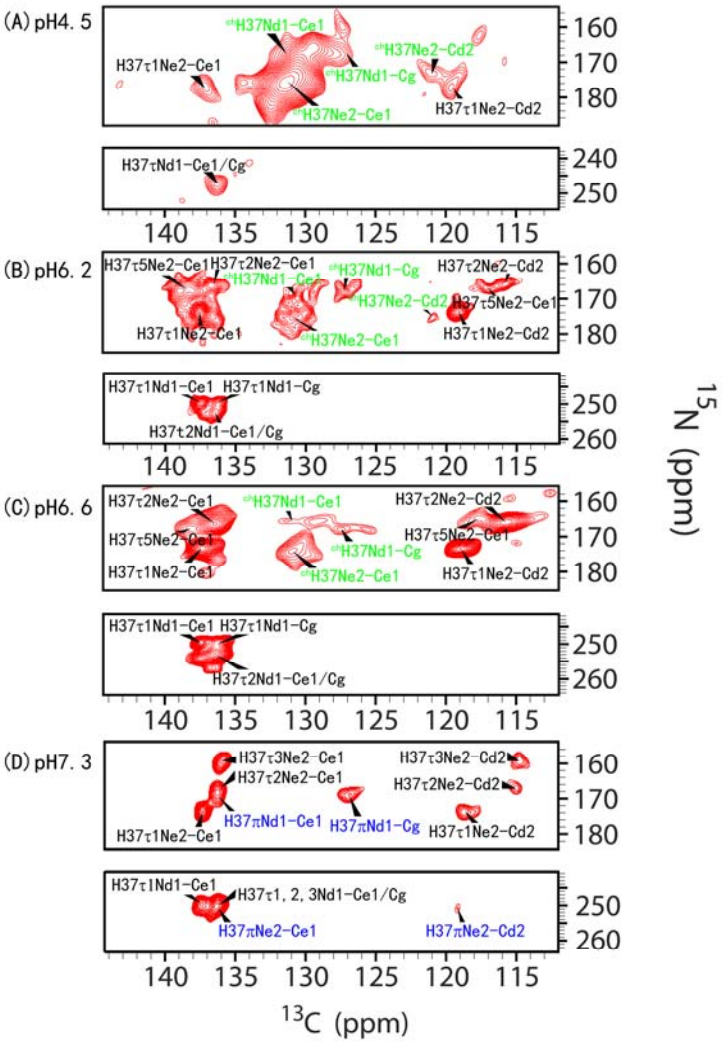

Fig. 1 2D ^{15}N-^{13}C zf-TEDOR spectra of different pHs. (A) pH4.5. (B) pH6.2. (C) pH6.6. (D) pH7.3. The neutral t H37 resonances are labeled in black; the neutral π H37 resonances are labeled in blue; the charged ^{ch}H37 resonances are labeled in green. The total TEDOR mixing time of 1-1.3 ms was used to get one-bond ^{15}N-^{13}C correlations.