A Secondary Gate as a Mechanism for Inhibition of the M2 Proton Channel by Amandatine

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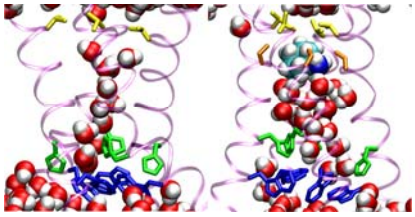
Introduction

The M2 protein is a proton-selective ion channel activated by low pH, and its channel activity is essential for the life cycle of the Influenza A virus. The antiviral drug amantadine inhibits the replication of the Influenza virus A by binding to the transmembrane domain (TMD) of the M2 proton channel. Recently, however, the high ratio (over 90 %) of amantadine resistant mutants among Influenza A viruses was reported. Understanding the inhibition mechanism of amantadine would be helpful for better drug design.

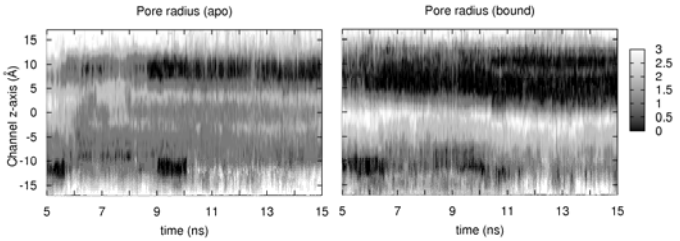
Molecular Dynamics Simulations

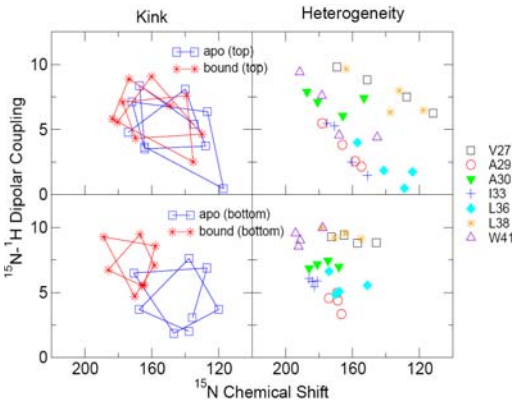
Both apo and amantadine bound M2-TMD using apo and bound structures solved by solid-state NMR [1] were simulated in the fully hydrated DMPC bilayer. The simulation started with energy minimization to remove bad contacts. The system was then heated up to 310 K for 50 ps under constant volume condition, and thereafter the simulation was continued at constant temperature and pressure up to 15 ns.

Results and Discussion



The simulation of the apo form revealed that Val27 can serve as a secondary gate near the channel entrance, which breaks the water wire down the channel pore. This gate arises from physical occlusion and the elimination of hydrogen-bonding partners for water molecules. In the presence of amandatine, the secondary gate formed by Val27 is reinforced by the nonpolar portion of amandatine and the water wire is now much more likely to break. Our study suggests a novel role of Val27 in the inhibition of the M2 proton channel by amandatine and an explanation for amandatine-resistant mutations on this residue. Despite the homogeneity of initial structure, apo M2-TMD showed the structural heterogeneity during the MD simulation which is consistent with the solid-state NMR observation (PISEMA). The heterogeneity of apo form can be interpreted as exchange of open and closed conformations of each helix. The protonation of His37 upon pH decrease and amantadine binding may simply perturb the pre-existing equilibrium between these two conformations. This is supported by the existence of spontaneous opening (continuous water wire with 0.03 % of the simulation time) and kinks (putative bound conformation) in the apo M2-TMD simulation.





Conclusions

Here we present a novel mechanism for M2 proton channel conductance mechanism and amantadine inhibition mechanism using MD simulations, which are consistent with the solid-state NMR experimental observations.

References

[1] Hu, J., *et al*., Biophysical Journal, **92**, 4335-4343 (2007).