CHAPTER 5

CONCLUSION

This study has exploited the spin labeled EPR data to model the closed and intermediate conformation of *E*.coli mechanosensitive channel of large conductance by means of Pseudoatom-Driven Solvent Accessibility Refinement. The closed conformation was constructed from the x-ray crystal structure of MscL homolog from *Mycobaterium tuberculosis*. The refined homology model of cl-ecoMscL was obtained from the PaDSAR approach using restraints derived from ΠO_2 and ΠNiEDDA data of spin labeled cysteine mutants reconstituted in 18:1 dioleoyl-phosphatidylcholine (PC18). Then the EPR solvent accessibility restraints derived from the mutants reconstituted in 12:1 dilauroyl-phosphatidylcholine (PC12) were employed in the PaDSAR simulation to drive the closed conformation to the intermediate model.

Structure models derived from PaDSAR revealed small changes between closed to intermediate conformations. The model of closed state and intermediate state validated by ramanchandran plot and analyses of pore diameter. Inspecting the pore size in the intermediate conformation revealed about ~4Å expansion in the hydrophobic constriction region of the inner pore near the hydrophobic gate. The results suggest that the change of the pore radius is associated with the movement of hydrophobic residues Leu-19 and Val-23. The overall structure of the intermediate conformation is similar to that of the closed model, supporting the pre-expanded closed-state model in the conformation transition pathway of MscL.

The 100ns all-atom molecular dynamics simulations were performed in hydrated membrane bilayer to investigate structural and dynamics properties of its closed and intermediate conformations. The results show structure stability of MscL during the course of MD simulation. From the MD trajectory, RMSD and RMSF revealed the two transmembrane helices segments, TM1 and TM2, are the least mobile compared with the N- and C-terminal helices. The intermediate conformation is more flexible than the closed conformation. The relative mobility between the two transmembrane segments obtained from the simulations is consistent with the experimental mobility data obtained from SDLS/EPR technique.