Structure and Dynamics of Spin Label Side Chains In KvAP Voltage-Sensing Domain: An All-ATOM MD Simulation Study

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Abstract
Spin labeling technique in combination with continuous-wave electron paramagnetic resonance spectroscopy has become a useful approach to investigate the structure and dynamics of biomolecules, especially membrane protein. In this method, spin label side chains due to their motion highly depending on the local environment were employed as external molecules to explore the structure and dynamics of the membrane protein. The close relationships between the spin label side chain dynamical feature and protein structure have been investigated for T4 lysozyme. Besides, molecular dynamics (MD) simulations were widely applied as an effective approach to study the microscopic behavior of the proteins, completing experimental approaches. Here, we presented an atomistic MD simulation study of the nitroxide spin label (MTSSL) side chains in a potassium ion channel voltage-sensing domain (VSD) from Aeropyrum Pernix (KvAP-VSD).

Keywords: Membrane Protein, Spin Labeling, MD simulations, MTSSL, KvAP

Research Highlights
♦ This work is one of the pioneer computational investigations applying SDSL for all residues in a membrane protein and performing MD simulations.
♦ The dynamics of spin label side chains were well consistent with the experimental EPR data.
♦ Spin label side chain can reflect the conformational changes and flexibilities of membrane protein.
Graphical Abstract

Research Objectives

In this research, we aim to model structures of cysteine-mutated membrane protein with nitroxide spin label attaching at various positions and then investigate typical structural and dynamical features of spin label in membrane protein.

Methodology

- To model attached-nitroxide spin label membrane protein systems: CHARMM-GUI (1) web-based service was used and setting up systems by VMD software (2) (version 1.9.3). Spin label-attached KvAP-VSDs were embedded in a POPC lipid bilayer and solvated with TIP3P water molecules. The simulation box is approximately 84 × 81 × 90 Å³ in size. Na⁺ and Cl⁻ were used as counterions.

- To perform MD simulations, NAMD program (3) (version 2.11) with CHARMM36 force fields (4) were employed. Switch and cutoff distances were 10 and 12 Å. Simulation time was 4 ns for each system at 298 K and 1 atm. Time step was 1 fs. Trajectories were saved every 1000 fs.
To analyze the MD trajectories: Root-Mean-Square Fluctuation (RMSF), dihedral angles ($\chi_1$-$\chi_5$) and Cα-nitroxide distance ($r$) of spin label side chains were calculated. The obtained results were compared with experimental data from our collaborators.

**Results**

For each system, a spin label was covalently attaching to one amino acid of the X-ray structure of KvAP-VSD (5) by site-directed mutagenesis. Continuing this procedure for all amino acid positions (residues 20-151), there were a total of 132 obtained systems. It was found that the dynamics from RMSF calculations and mobilities data from EPR (6) had similar patterns as shown in graphical abstract. The loop sites were more flexible than the segment sites (S1-S4). Besides, the comparable tendencies can be seen for the dynamics of the backbones when attaching/without spin labels. As for the dihedral angles, they were ranged from -180° to +180° with a confidence interval of ±30°. The $\chi_1$ rotations were equal to ±60° and ±180°. $\chi_2$ angle values were located at ±75° and ±180°. The $\chi_3$ configurations distributed at 90° and -90°. $\chi_4$ ranged from ±60° to ±180°. For $\chi_5$, they spread from 0-120°. These distributions showed a good agreement with Tombolato F. and co-worker study (7). The variation of the nitroxide-Cα distances ($r$) were from 6.5 to 10 Å whereas DeSensi, S. C., et. al. found it ~7 Å when attaching to T4 lysozyme (8).

**Findings**

We constructed structures of the cysteine-mutated membrane protein with the nitroxide spin label attaching at all amino acid sites. The SDSL technique and MD simulations are such a valuable combination to study the structure and dynamical properties of spin label side chain which are thoroughly sensitive to reflect the membrane protein conformational fluctuation.

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**REFERENCES**


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