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Effects of C479W Mutation on the Structure and Function of Band 3 Protein

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Abstract: Band 3, or anion exchanger 1 (AE1), is one of the indispensable transmembrane proteins involved in effective respiratory processes in the human body, mainly responsible for the exchange of bicarbonate and chloride ions on the plasma membrane of red blood cells. However, the effect of related gene mutations on Band 3 ion transport is not fully understood. In this work, we used all-atom molecular dynamics (MD) simulations to systematically investigate the effects of the mutation from cysteine (C) to tryptophan (W) at site 479 in Band 3 on the structure of Band 3 and its interaction with anions. We studied the kinetics of wild-type (WT) and mutant Band 3 interactions with bicarbonate and chloride ions on the microsecond scale. The results showed that the mutation at site 479 affected the stability of Band 3 structure and the distribution of anions, and changed the affinity of anions to different parts of Band 3. These findings provide some insights into the structural and functional effects of gene mutations in Band 3 and contribute to understanding Band 3 anion exchange.

Keywords: Band 3; Bicarbonate ions; Molecular dynamic simulations.

1. Introduction

Band 3, or anion exchanger 1 (AE1), is one of the primary glycoproteins located on the red blood cell membrane. Its main function is to facilitate the rapid exchange of bicarbonate and chloride ions across the membrane. [1]

Band 3 consists of 911 amino acids and is divided into two domains: the cytoplasmic domain (cdAE1, residues 1-360) at the N-terminus and the membrane domain (mdAE1, residues 361-911) at the C-terminus. [2] The two structure domains are responsible for interacting with the cytoskeleton and ion transport, respectively. [3] The mdAE1 typically exists as a dimer in the human body, with each subunit independently involved in ion transport. Each monomer contains 14 transmembrane (TM) helices arranged in two repeated topologies with similar folds. Based on their functional features, mdAE1 is divided into a core domain (TM1-4, 8-11) and a gate domain (TM5-7, 12-14). [4] The movement of these two domains provides an exchange pathway for bicarbonate and chloride anions to access the central binding sites. [5]

Studies have shown that mutations at specific sites on Band 3 are associated with a number of physiological diseases. [6] For example, hereditary stomatocytosis (HS) associated with anemia and distal renal tubular (dRTA) acidosis are associated with metabolic acidosis. [6, 7] Notably, C479W mutant on TM3 adversely affects Band 3 folding, potentially disrupting the packaging and impinging on Band 3 normal function.

Therefore, studying mutations in Band 3 is crucial for understanding their biological significance and for the prevention and treatment of related diseases. In this study, we particularly focus on the mutation of C479W, exploring the specific effects of this mutation on Band 3 protein folding and ion transport and providing some insights into related fields.

2. Models and Methods

2.1. System setup

In this study, we employed Band 3 monomer to investigate the impact of mutations on the structure and function of Band 3. We only considered the membrane domain of Band 3 with PDB: 4YZF. [8] We considered the wild-type and C479W mutant of Band 3. Both the C479W mutant and wild-type of Band 3 were embedded in lipid bilayers composed of dipalmitoyl-phosphatidylcholine (DPPC).

All models were represented as all-atom systems and simulated using the CHARMM36 force field, with CHARMM36m for proteins, CHARMM36 for lipids, and TIP3P for water. [9] The web-based CHARMM-GUI Membrane Builder was used to build the ternary lipid bilayers with Band 3. [10] The lipid bilayer was solvated with approximately 22500 water molecules. To mimic physiological conditions, 0.075 M NaCl and 0.075 M $NaHCO_3$ were added to the system, along with extra sodium ions to neutralize the negative charge carried by Band 3. The size of the simulation box is about $10\times10\times11$ nm^3.

2.2. Simulation details

Simulations were conducted using the GROMACS 2021.4-Plumed-2.7.3 software package with an integration time step of 2 fs. [11,12] Constant particle number, pressure, and temperature (NPT) ensemble simulations were employed. The system temperature was maintained at 298 K using the Nother-Hoover thermostat with a relaxation time of 1 ps. [13] The semi-isotropic Parrinello-Rahman barostat, with relaxation times of 5 ps, was used to maintain the pressure at 1.0 bar separately in the membrane plane (xy) and the normal direction (z) [14]. Long-range electrostatic interactions were calculated using the particle mesh Ewald (PME) method. [15] Bond lengths were constrained using the LINCS algorithm. [16] Periodic boundary conditions were applied in all three directions. Each system was simulated for a duration of 250 ns.

2.3. Data Analysis

The MD trajectory analysis was primarily carried out using tools from the GROMACS 2021.4-Plumed-2.7.3 software package. Visualization of the simulation results was achieved using Visual Molecular Dynamics (VMD) software. [17] The number of contacts between bicarbonate ions and Band 3

residues was calculated using the *gmx mindist* tool in GROMACS, with a cutoff distance of 0.4 nm. The root mean square deviation (RMSD) of Band 3 was computed using the *gmx rms* tool to estimate its conformational stability. The distribution of bicarbonate and chloride ions was calculated using the *gmx density* tool to characterize the interaction between anions and Band 3.

3. Results and Discussion

3.1. The effect of C479W mutation on the structure of Band 3

The site 479 is located at TM3, facing TM4 and TM8. Studies have shown that C479W mutation may affect protein folding. [18] As shown in Figure 1, we selected the snapshots of the wild-type and C479W mutant at the beginning and end of the simulation. We can see that the folding degree of the mutant Band 3 is smaller than that of the wild type, indicating that the C479W mutation affects protein folding.

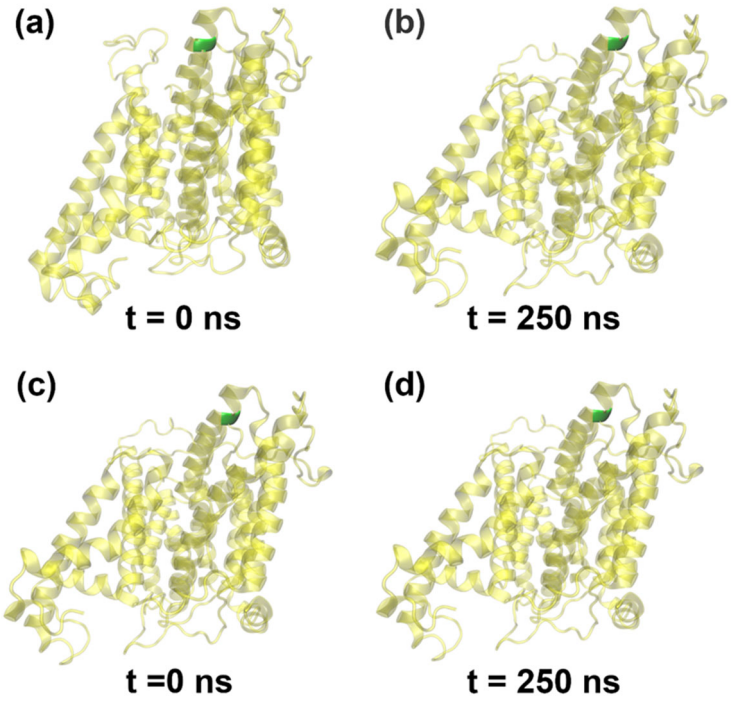

Figure 1. (a, b) Snapshots of the beginning and end states of the wild-type Band 3 in the simulation. (c, d) Snapshots of the beginning and end states of C479W mutant Band 3 in the simulation. Residue 479 is specifically labeled in green.

To further quantify the effect of this site mutation on the protein structure, we calculated the root mean square deviation (RMSD) of Band 3 during the simulation, as shown in the Figure 2. The RMSD of C479W mutant changed little, which may have played a certain role in inhibiting the further folding of Band 3.

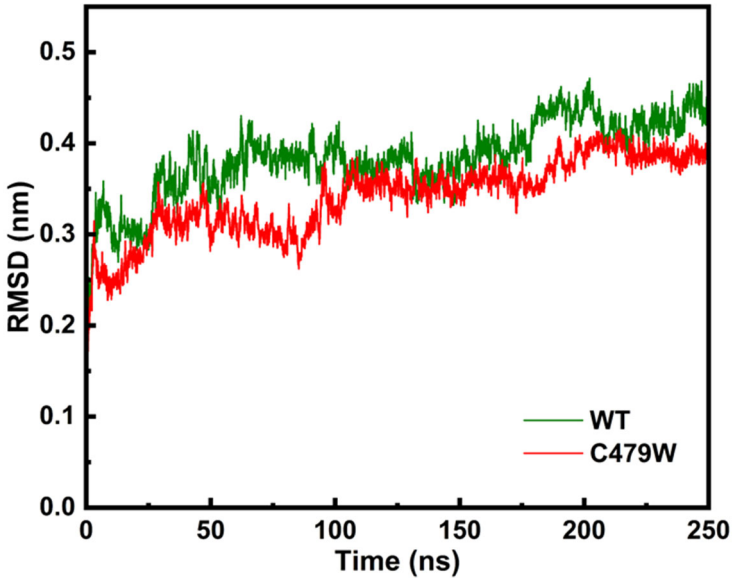

Figure 2. Root-mean-square deviation (RMSD) of wild type and C479W mutant as a function of simulation time.

3.2. The effect of C479W mutation on the function of Band 3

It is well known that the realization of transmembrane protein function is closely related to protein folding and conformation. In the last part of the work, we found that C479W mutation affect the conformation of the protein. In order to further explore the effect of mutation on Band 3 function, we calculated the number of bicarbonate ions and chloride ions along z direction for wild type and C479W mutant.

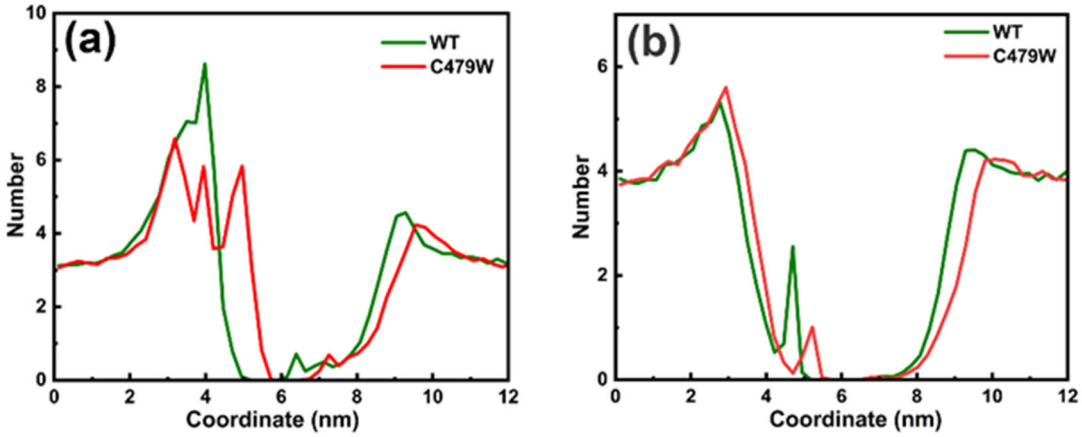

Figure 3. The number of anions along z direction. (a) The number of bicarbonate ions for wild type and C479W mutant along the z direction. (b) The number of chloride ions for wild type and C479W mutant along the z direction.

As shown in Figure 3, we found that the number of bicarbonate ions at 2-4 nm in wild type Band 3 is lower than that in C479W mutant. At 5.8 nm the number of mutant ions increased. It is clear that C479W mutant, a portion of the ions located outside the membrane plane are transferred to a position close to the membrane surface. There was little difference in the distribution of chloride between the wild type and C479W mutant.

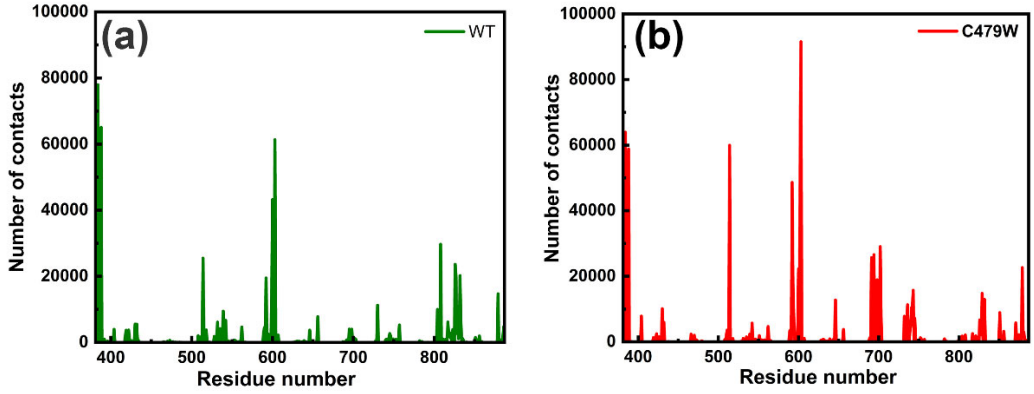

Figure 4. The number of contacts for (a) wild type and (b) C479W mutant.

We also calculated the number of contacts between bicarbonate ions and Band 3 for wild type and C479W mutants. As shown in Figure 4, for the wild type, the bicarbonate ions mainly contacts with residues near residue 381 and 600; for C479W mutant, the bicarbonate ions contacts with residues 514 and 603 more frequently. Residue 514 is located on the amphiphilic helix and 603 is located at the end of TM7. Both of these residues are located near the cytoplasm. In other words, the folding of C479W mutant may disturb the structure of the cytoplasmic end and change the distribution of ions on the cytoplasmic side.

4. Conclusion

In this paper, using all-atom molecular dynamics simulations, we study the effects of C479W mutation on the structure and function of Band 3. The results show that the structural stability of Band 3 is affected by the C479W mutant. In addition, C479W mutations change the distribution of anions on Band 3, which in turn affects the contacts between the anion and Band 3. These findings provide insights into the effects of Band 3 related gene mutations on Band 3 structure and function and may be helpful to understand the specific details of Band 3 conformational transformation.

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