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A Computational Approach to Study the Energy Transduction Mechanism in the Na$^+$/K$^+$-ATPase

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The Na$^+$/K$^+$-ATPase pumps ions across the membrane which is necessary for maintaining the membrane potential. The energy for this active ion transport is provided by binding and hydrolysis of ATP and has to be transferred from the cytoplasmic nucleotide binding site to the transmembrane domain of ion transport. This transport cycle can also be induced experimentally by applying voltage jumps across the membrane. We simulated the applied electric field by an ionic capacitor and studied the impact on the Na$^+$/K$^+$-ATPase by a combination of multiconformation continuum electrostatics (MCCE) and molecular dynamics (MD). Our calculations show a selective activation of the helices M5, M6 and M8 by the electric field. Those helices are likely to act as energy transduction elements.

1 Introduction

The Na$^+$/K$^+$-ATPase is an integral membrane ion pump belonging to the superfamily of P-type ATPases. All members of the subfamily (P-type ATPases II) share common structural similarities. These proteins consist of three cytoplasmic domains (**n**ucleotide binding-, **a**ctuator- and **p**hosphorylation domain) and a transmembrane domain (with the ion binding sites) which is composed of 10 α-helices (M1-M10). The cytoplasmic domain is connected to the transmembrane domain by the stalk region (S2-S5). Because of this structural and functional similarity, the mechanism of pumping ions across a membrane is supposed to be common for all P-type ATPases and is described by the Post-Albers scheme (with two main conformational states E1 and E2). In the reaction cycle of the Na$^+$/K$^+$-ATPase, three Na$^+$-ions are pumped out and two K$^+$-ions are pumped into the cell. Thus the Na$^+$/K$^+$-ATPase is electrogenic which is essential for maintaining the membrane potential. Cations have to be transported actively against a gradient. Therefore energy is needed which is provided at the nucleotide binding site by binding and hydrolysis of ATP. This energy has to be transferred to the transmembrane ion binding sites which are located approx. 50 Å apart. Experiments show that the transport cycle can not only be induced by ATP but also by an electric field that is applied to the membrane[1]. These voltage-clamp fluorometry experiments indicate an important role of the highly conserved transmembrane helix M5 and M6 for the energy transduction mechanism of the Na$^+$/K$^+$-ATPase. To study the energy transduction theoretically, a combination of electrostatic calculations (MCCE) and molecular dynamic simulations (MD) is used to evaluate the impact of a simulated electric field on the Na$^+$/K$^+$-ATPase.

2 Methods

Multiconformation continuum electrostatic (MCCE) calculations simulate simultaneously the residue ionization and side chain rotamers[2,3]. The electric field that is applied to the

transmembrane region of the Na^+/K^+-ATPase (pdb-id: 3B8E ; resolution: 3.5 Å ; E2 enzyme state)[4] was simulated by an "ionic capacitor". Therefore ions were placed above and below the protein and parameterized in MCCE. The atomic model of the Na^+/K^+-ATPase was embedded in a POPC (palmitoyl oleoyl phosphatidylcholine) membrane and the gap between the protein and the membrane was closed with a molecular dynamic (MD) simulation (NAMD)[5]. The exact position of the capacitor and the number of ions that were inserted (strength of the applied electric field) were varied to study the influence of the simulation setup. To include conformational dynamics and protein backbone flexibility, structural snapshots of the MD simulations have been chosen as input coordinates for the electrostatic calculations. The impact of the electric field on the helices M1-M10 and the stalk region S2-S5 was observed by the number of residues per helix that change their conformer. Conformer changes consider both, rotamer changes and changes of the residue ionization.

3   Results and Conclusions

Number of Residues per Helix										
Helix	M1	M2+S2	M3+S3	M4+S4	M5+S5	M6	M7	M8	M9	M10
	21	33	26	36	39	21	23	20	19	21
Number of Conformer Changes for Selected MD Snapshots										
time [ps]	M1	M2+S2	M3+S3	M4+S4	M5+S5	M6	M7	M8	M9	M10
0	2	6	5	8	14	6	3	6	2	3
125	2	10	5	8	11	6	2	8	1	4
250	1	5	3	8	13	6	4	7	1	5
500	1	7	4	12	12	6	2	6	3	5
750	1	6	2	8	11	7	2	7	1	5
1000	3	7	4	8	12	6	2	8	3	5
Averaged Residue Changes per Helix [%]										
	7.9	20.7	14.7	22.2	31.2	29.4	10.9	33.3	8.8	21.4

Table 1. Impact of an electric field on the transmembrane helices of the Na^+/K^+-ATPase including the stalk region.

Table 1 shows that the helices M2, M4, M5, M6 and M8 and M10 respond intensely with residues conformer changes to an applied electric field. The helices M5, M6 and M8 change approximately one-third of their conformers. In addition, the conformer changes of these helices are evenly distributed across the complete helix and not only at the helix ends close to the capacitor as it is the case for the helices M2, M4 and M10. The impact of the simulation setup was tested by varying the geometry of the ionic capacitor and the strength of the applied electric field. Independent of the setup, the number of conformers that change remained high and nearly the same for the helices M5, M6 and M8 as compared to the other transmembrane helices. The strength of the electric field has to be simulated much

higher than the fields that were applied in the experimental setup in order to obtain any effects on conformer occupancy. This can be explained by the limited backbone flexibility in our calculations. However, a qualitative analysis is feasible by this simulation approach. The different structural MD snapshots showed only a small variation in conformer changes as compared to the crystal structure coordinates. In particular, the changes on the helices M5, M6 and M8 remained high for the different structural snapshots. Thus these helices are selectively activated by the electric field supporting the experimental hypothesis that these helices are likely to act as energy transduction elements. The contribution of the helices M5 and M8 to the energy transduction mechanism could also be concluded from our previous electrostatic calculations with modeled structures of the Na^+/K^+-ATPase and with the Ca^{2+}-ATPase, another member of the P-type ATPase family[6]. Further support is provided by calculations on a mutant of the Na^+/K^+-ATPase. This mutant (N776D) shows no voltage dependence in experiments[7] what could be confirmed by our calculations. The number of conformer changes due to the applied electric field is also reduced for M5, M6 and M8. The number of conformer changes is reduced to two-third for the helix M6 and even to the half for the helices M5 and M8.

Acknowledgments

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