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Preexisting domain motions underlie protonation-dependent structural transitions of the Ca^{2+} -ATPase

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Abstract

We have performed microsecond molecular dynamics (MD) simulations to determine the mechanism for protonation-dependent structural transitions of the sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA). Release of two H^+ from the transport sites activates SERCA by inducing a structural transition between low (E2) and high (E1) Ca^{2+} -affinity states (E2-to-E1 transition), but the structural mechanism by which transport site deprotonation facilitates this transition is unknown. We performed microsecond all-atom MD simulations to determine the effects of transport site protonation on the structural dynamics of the E2 state in solution. We found that the protonated E2 state has structural characteristics that are similar to those observed in crystal structures of E2. Upon deprotonation, a single Na^+ ion rapidly (<10 ns) binds to the transmembrane transport sites and induces a kink in M5, disrupts the M3–M5 interface, and increases the mobility of M3/A-M3 linker. Principal component analysis showed that counter-rotation of the cytosolic N-A domains about the membrane normal axis, which is the primary motion driving the E2-to-E1 transition, is present in both protonated and deprotonated E2 states; however, protonation-dependent structural changes in the transmembrane domain control the hierarchical organization and amplitude of this motion. We propose that preexisting rigid-body domain motions underlie structural transitions of SERCA, where the functionally important directionality is preserved while transport site protonation controls the dominance and amplitude of motion to shift the equilibrium between the E1 and E2 states. We conclude that ligand-induced modulation of preexisting domain motions is likely a common theme in structural transitions of the P-type ATPase superfamily.

Graphical abstract

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We propose that control of the hierarchical structure and amplitude of preexisting domain motions underlie protonation-dependent structural transitions of the calcium pump.

Introduction

The sarcoplasmic reticulum (SR) Ca^{2+} -ATPase (SERCA) is a member of the large family of evolutionarily related ion and lipid P-type pumps that are found in bacteria, archaea, and eukaryotes. SERCA clears cytosolic Ca^{2+} in most cells, thus playing a dominant role in Ca^{2+} homeostasis,¹ signaling,^{2–4} cell differentiation,⁵ and muscle contractility.⁶ SERCA uses the energy produced by hydrolysis of a single ATP molecule to pump two Ca^{2+} ions into the SR lumen and countertransport two H^+ from the lumen to the cytosol.^{7, 8}

At the beginning of each Ca^{2+} transport cycle, two H^+ from the transport sites of SERCA are released to the cytosol.^{9, 10} Proton release induces a large structural transition between low (E2) and high (E1) Ca^{2+} -affinity states.^{11, 12} This structural transition involves a large-scale rearrangement of the transmembrane (TM) helices as well as a reorganization of the cytosolic headpiece, formed by the nucleotide-binding (N), actuator (A) and phosphorylation (P) domains. Following this structural transition, SERCA binds two Ca^{2+} ions to the TM transport sites and one molecule of ATP in the N domain, resulting in a structural arrangement that is suitable for ATP hydrolysis and SERCA autophosphorylation.¹³ SERCA autophosphorylation then induces a 110° rotation of the A domain,¹⁴ which shifts the equilibrium toward the E2 state; this structural change facilitates Ca^{2+} release into the SR lumen and H^+ occlusion in the transport sites.^{15–19} Finally, SERCA is dephosphorylated,^{9, 10} populating the unphosphorylated E2 state required for the next Ca^{2+} transport cycle of the pump.^{11–13}

Transport site protonation/deprotonation plays a central role in the transport cycle of SERCA, underscored by its striking capability to stabilize the E2 structural intermediate^{16, 17} and modulate large-scale E2-to-E1 structural transitions of the pump.^{20–22} Several studies have characterized the complex structural and functional aspects of E2-to-E1 transition. Experimental characterization of protonation-dependent structural transitions of SERCA in atomic-level detail has proven difficult, so molecular simulations have been used as a complementary approach. For example, coarse-grained molecular dynamics (MD)

simulations were used to characterize the changes in entropy and enthalpy along the E2-to-E1 transition.²³ This study, motivated by an experimental study of insertion mutants in the A-M3 linker region,²⁴ revealed an enthalpy-entropy compensation for the E2-to-E1 transition.²³ Another study reported a computational method that uses a tree diagram that describes a conformational change between two structures, and provides information of the coupled rigid-body motions of SERCA along individual reaction steps. This study showed the presence of several functional regions that play a role in the E2-to-E1 transition, e.g., the M1–M3 helices and A domain.²⁵ More recently, a multiscale methodology was used to determine all-atom transition pathways for the E1-to-E2 transition of the pump.²⁶

While these studies have yielded important insights into the structural transitions between the functional E1 and E2 states, major questions remain regarding the mechanisms for protonation-dependent structural transitions of SERCA: How does protonation facilitate the large domain motions that shift the equilibrium between E1 and E2 states? What are the regions of SERCA responsible for transmitting the protonation-dependent signal from the transport sites to the cytosolic headpiece? How does transport site protonation act upon SERCA in a Ca^{2+} -independent manner? To address these questions, we have performed microsecond MD simulations of protonated and deprotonated SERCA in solvated lipid bilayer starting from crystal structures of the E2 state.

Methods

Preparation of protonated and deprotonated E2 state of SERCA

We used the crystal structures 2agv¹⁹ and 1iwo⁹ to simulate the structural dynamics of protonated and deprotonated E2 state, respectively. We used these crystal structures on the basis of previous studies showing that at intracellular pH (7.1–7.2),^{27, 28} the gatekeeper residue Glu309 is protonated in 2agv ($\text{p}K_a=8.7$), and deprotonated in 1iwo ($\text{p}K_a=6.4$).^{17, 29} Extensive $\text{p}K_a$ calculations and in vitro mutagenesis experiments have shown that stabilization of the E2 state requires protonation of residues Glu309, Glu771 and Glu908, whereas protonation-induced E2-to-E1 structural transitions require deprotonation of Glu309 and Glu771.^{17, 22, 29} Therefore, we modeled side chains of Glu309 and Glu771 as neutral and ionized to simulate the protonated and deprotonated E2 states, respectively. We used PROPKA^{30–33} to adjust the ionization state of all other titratable residues to a normal intracellular pH range (7.0–7.2).^{27, 28}

SERCA structures were inserted in a pre-equilibrated 12×12 nm bilayer of palmitoyl-2-oleoyl-sn-glycerolphosphatidylcholine. This SERCA-lipid system was solvated using TIP3P water molecules with a minimum margin of 1.5 nm between the protein and the edges of the periodic box in the z -axis. Finally, Na^+ , and Cl^- ions were added to neutralize the electric charge of the protein-lipid-water system, and to produce a NaCl concentration of approximately 50 mM. This salt concentration was chosen to match the experimental conditions previously used to study the effects of pH on SERCA *in vitro*.²² The final size of each protein-lipid-water-ions system was approximately 220,000 atoms. CHARMM36 force field topologies and parameters were used for the protein, lipid, water, and ions.^{34, 35}

Molecular dynamics simulations

We performed MD simulations of SERCA in the protonated and deprotonated E2 states using NAMD,³⁶ with periodic boundary conditions³⁷, particle mesh Ewald,^{38, 39} a non-bonded cutoff of 0.9 nm, and a 2-fs time step. The NPT ensemble was maintained with a Langevin thermostat (310K) and an anisotropic Langevin piston barostat (1 atm). Fully solvated systems were first subjected to energy minimization, followed by gradually warming up of the systems for 200 ps. This procedure was followed by 10 ns of equilibration with backbone atoms harmonically restrained using a force constant of 1000 kcal mol⁻¹ nm⁻². Each protonated and deprotonated E2 states were simulated without restraints for 3.6 μ s, yielding total simulation time of 7.2 μ s.

Cartesian principal component analysis

We performed Cartesian principal component analysis (cPCA) using CARMA⁴⁰ to characterize the protonation-dependent motions of SERCA. cPCA uses the actual dynamics of the protein to generate the appropriate collective coordinates that capture the important structural and dynamic features of the native states of the protein.⁴¹ We used cPCA to analyze the functional motions of SERCA in the MD trajectories of both protonated and unprotonated E2 states. We also performed PCA on an ensemble of combined E1 and E2 crystal structures to directly compare our MD trajectories with available experimental data. For the ensemble of E1 structures, we used the following structures from the PDB: 1su4,⁴² 1vfp,¹³ 1t5s,⁴³ 3ar2,⁴⁴ 3n8g,⁴⁵ 3w5a,⁴⁶ 3w5b,⁴⁶ and 4h1w.⁴⁷ The structure of SERCA bound to phospholamban (PDB: 4kyt) was originally designated as a E2 state,⁴⁸ but extensive MD simulations showed that this phospholamban-bound SERCA structure represents an E1 state of the pump protonated at residues Glu771 and Glu908.²⁹ Therefore, we also included this crystal structure in the ensemble of E1 structures. For the E2 ensemble, we used the following crystal structures: 1iwo,⁹ 2agv,¹⁹ 2ear,⁴⁹ 2eat,⁴⁹ 2c8k,⁵⁰ 2eau,⁴⁹ and 3w5c.⁴⁶ For all analyses, we removed the translations and rotations of the protein by aligning SERCA structures using the TM domain as a reference.

Analysis and visualization

We used the program VMD⁵¹ for analysis, visualization, and rendering of the structures. We used the program STRIDE⁵² to analyze the evolution of the secondary structure of the TM domain helices of SERCA. STRIDE recognizes secondary structural elements in proteins from their atomic coordinates. This program utilizes both hydrogen-bond energy and main chain dihedral angles to define the secondary structure pattern. It relies on database-derived recognition parameters with the crystallographers' secondary structure definitions as a standard-of-truth.

Results

Structural dynamics of protonated and deprotonated E2 SERCA

Figure 1A compares the structure of protonated and deprotonated E2 at the end of the 3.6- μ s MD trajectories with the crystal structure of SERCA in the E2 state. Analysis of the root mean square deviation (RMSD) revealed that the structure of the TM domain of protonated

E2 undergoes a 0.17-nm drift at the beginning of the simulation, but the RMSD settles a plateau at $t > 0.05 \mu\text{s}$ (Figure 1B, black line). This small change in RMSD indicates that transport site protonation stabilizes a structure of the TM domain that is similar to that determined by x-ray crystallography.¹⁹ The RMSD of the P domain settles a plateau at 0.17 nm (Figure 1B, blue line), indicating that the orientation of the P domain does not substantially change in the MD trajectory.). The RMSD of the A domain settles a plateau at ~ 0.3 nm after $t > 1 \mu\text{s}$, but retains some mobility (RMSD= 0.29–0.36 nm) for the rest of the trajectory (Figure 1B, red line. The position of the N domain also equilibrates at $t > 1 \mu\text{s}$ and retains some mobility (RMSD=0.35–0.46 nm) for the remainder of the MD trajectory (Figure 1B, green line). Although the N and A domains are fairly mobile in the protonated E2 state, these results indicate that transport site protonation stabilizes a structure that is similar to that determined by x-ray crystallography.

Deprotonation induces a substantial structural rearrangement of the pump (Figure 1A). Transport site deprotonation induces a 0.35-nm shift in the RMSD of the TM domain, twice as large that that calculated in the trajectory of protonated E2 state (RMSD=0.17 nm). This structural change occurs in the first 0.8 μs of the MD trajectory (Figure 1C, black line). The TM domain of deprotonated E2 state settles at a plateau of 0.33 nm for the remainder of the simulation. The initial orientation of the P domain is shifted by 0.44 nm during the first 1.1 μs , but settles to a plateau at 0.43 nm (Figure 1C, blue line). The N and A domains undergo a 0.5-nm and 0.63-nm change in the RMSD for the first 1.3 μs of simulation, respectively (Figure 1C, green and red lines). After $t = 1.3 \mu\text{s}$, the RMSD of both N and A domains increase to a RMSD=0.81 nm until $t = 2.5 \mu\text{s}$. The RMSD of the N and A domains fluctuates between 0.77 nm and 0.9 nm for the remainder of the MD trajectory (Figure 1C). Deprotonation of the E2 state occurs spontaneously at physiological pH,²⁰ so these findings indicate that the deprotonated E2 state populates a structure that is substantially different to that determined at non-physiological pH conditions.⁹

Structural arrangement of the transport sites and their interaction with metal ions in protonated and deprotonated E2 SERCA

The protonation-dependent structural changes captured by the MD simulations (Figure 1) are likely coupled to structural changes in the transport sites, so we analyzed the structural arrangement of these sites and their interaction with metal ions. Transport site protonation stabilizes a network of salt bridges Val304-Glu309 and Glu771-Asn796 (Figure 2A, left panel). These salt bridges are stable in the MD trajectory (Figure 2B, left panel). The structural arrangement of the transport site in the trajectory of protonated E2 is similar to that in crystal structures of E2 (RMSD<1 nm). These salt bridges populate a closed-pack structural arrangement and facilitate transport site burial in the interior of the TM domain (Figure 2A, left panel). Transport site protonation prevents metal ions from binding to the transport sites, in agreement with studies showing that transport site protonation is incompatible with metal ion binding.^{17, 22}

Deprotonation of the E2 state disrupts salt bridges Val304-Glu309 and Glu771-Asn796 (Figure 2A–B). In this structural arrangement of the transport sites the cytosolic pathway (M1, M3 and M4)⁴⁵ is open and the transport sites are exposed to the cytosol (Figure 2A,

right panel). We found that the open transport sites favor Na^+ binding to transport site I. Na^+ binding follows the M1 pathway, guided by residues Glu55, Glu58, Glu59 and Glu109 toward site II before reaching site I. We did not find any evidence of another cytosolic metal-ion entry pathway to the transport sites (i.e., via M8/M9⁵³). This ion pathway is similar to that found in the E1 state of the pump,^{54, 55} suggesting that the mechanism for metal ion recognition and binding via the cytosolic pathway is similar in both E2 and E1 states.

In the deprotonated E2 state, the transport site I is occupied by a single Na^+ ion for the entire 3.6 μs of simulation time. Na^+ binds to three different positions in the transport site I of deprotonated E2 SERCA (Figure 3A). At time intervals 0–0.2 μs and 0.4–0.6 μs (Fig. 3B), Na^+ primarily binds to the side chains of residues Glu771 and Asp800 (Figure 3A, position 1). The estimated interaction energy (E_{int}) of Na^+ in position 1 is $-290 \pm 24 \text{ kcal mol}^{-1}$. Na^+ interact with the side chain of residues Glu771, Glu908, Asp800 and Thr799 (Fig. 3A, position 2) between 0.9 μs and 1.2 μs (Figure 3B). Na^+ is bound to position 2 for 7% of the total simulation time (Figure 3B); in this position, Na^+ interacts with the to the transport site I ($E_{\text{int}} = -273 \pm 18 \text{ kcal mol}^{-1}$). Finally, Na^+ is occluded in position 3 for most of the simulation time (Figure 3A–B). In this position, the coordinating ligands for Na^+ are the side chains of residues Thr799, Asp800, Glu771 and Glu908. In position 3, Na^+ also binds a location that virtually overlaps with the site occupied by Ca^{2+} in the transport site I of the E1-2 Ca^{2+} state. The interaction energy of Na^+ in this site ($E_{\text{int}} = -275 \pm 17 \text{ kcal mol}^{-1}$) is substantially weaker compared to that of Ca^{2+} in the transport site I ($E_{\text{int}} = -830 \text{ kcal mol}^{-1}$).⁵⁶ These findings suggest that H^+ - Na^+ exchange and weak Na^+ binding to the transport site I is sufficient to produce competent transport sites upon deprotonation of the E2 state.

Effects of transport site protonation of the E2 state on the structure of the TM domain

We measured the changes in the RMSD of individual TM helices to determine the structural changes induced by transport site protonation. In the protonated state, the position of each TM helix is similar to that found in the crystal structure of the E2 state (e.g., RMSD 0.2 nm). In the deprotonated state, the position of helices M1, M2 and M7, and M9 does not change substantially (RMSD < 0.1 nm, Figure 4A), but the RMSD of helices M3–M6, M8 and M10 increase substantially upon transport site deprotonation (RMSD > 0.27 nm, Figure 4A).

We used STRIDE⁵² to analyze the changes in the secondary structure of helices M3–M6, M8 and M10. Helices M3, M4, M6, M8 and M10 retain a α -helical structure in both protonated and deprotonated E2 states. TM helix M5, which contains transport site residue Glu771, loses its α -helical structure at Ile765–Asn768 upon deprotonation (Figure 4B). Visual inspection of the structure of the deprotonated E2 state showed that this local loss in α -helical structure induces a kink in M5 (Figure 5A), so we measured the time-dependent changes in M5 kink in the trajectories of protonated and deprotonated E2 states. The kink angle was measured by fitting residues Ala746–Leu764 and Val769–Ala780 to two cylinders; the angle between the axes of the cylinders represents the kink angle of M5. In the protonated E2 state, the kink angle of M5 but settles a plateau at $\sim 5^\circ$ for most of the simulation time (Figure 5B). Upon deprotonation, the kink angle of M5 increases by 60°

during the first 0.5 μ s of simulation time (Figure 5B). At $t > 0.5 \mu$ s, the kink angle of M5 remains virtually unchanged at a value of 53° . This large structural change in M5 displaces helices M4, M6, M8 and M9, thus explaining the differences in RMSD observed in Figure 4.

We determined the structural mechanism by which deprotonation facilitates kink formation in M5. In the protonated E2 state, a O $\cdots$ H-N hydrogen bond between residues Ser767 and Glu771 stabilizes the α -helical structure of residues Ile765-Asn768 (Figure 6). Transport site protonation stabilizes this hydrogen bond (Figure 6), in agreement with secondary structure measurements showing that Ile765-Asn768 populate a α -helix in the protonated E2 state of the pump (Figure 4). Upon deprotonation, the hydrogen bond between Ser767-Glu771 becomes disrupted (Figure 6). We found that the first coordination shell of Na $^+$ in the transport site I includes the backbone oxygen of residue Ser767, so Na $^+$ directly competes with the backbone hydrogen bond between Ser767 and Glu771. The correspondence of time scales for Na $^+$ -induced disruption of the hydrogen bond Ser767-Glu771 and M5 kink formation (Figure 6) indicates that these two events are coupled.

Deprotonation-induced kink in M5 alters the structure of the M3-M5 interface (Figure 7A). We calculated the fraction of native contacts between TM helices M3 and M5 (Q_{M3-M5}) to measure the effect of transport site protonation on the stability of the M3-M5 interface. Q_{M3-M5} is defined by a list of native contact pairs (i, j). All pairs of heavy atoms i and j belonging to residues X_i and X_j are in contact if the distance between i and j is < 0.7 nm. Q is expressed as a number between 1 and 0, and calculated as the total number of native contacts for a given time frame divided by the total number of contacts in the crystal structure. In the trajectory of protonated E2, $Q_{M3-M5} = 0.8$ for $> 90\%$ of the simulation time (Figure 7B), indicating that the M3-M5 interface retains a significant amount of contacts resolved in the crystal structure of the E2 state. Upon deprotonation, Q_{M3-M5} decreases to a value of 0.2 during the first 0.1 μ s of simulation time, but at $t > 0.3 \mu$ s, Q_{M3-M5} settles a plateau at a value of 0.3 (Figure 7B). These findings indicate that deprotonation induces a decrease from 80% to 30% in the native contacts of the M3-M5 interface.

TM helix M3 is connected to the A domain by the A-M3 linker (Figure 8A), we measured the Ca mean square displacement (MSD) of the A-M3 linker and TM helix M3. We found that compared to the protonated E2 state, transport site deprotonation increases the MSD of the cytosolic segment of M3 and the A-M3 linker (Figure 8B). Assuming a linear relationship between the time-dependent RMSD of M3, M5 and the cytosolic, the correlation coefficient r of M3/AM-3 linker-cytosolic headpiece and M5-cytosolic headpiece at time interval $t = 0-1 \mu$ s are 0.75 and 0.78, respectively. However, at time interval $t = 1-3.6 \mu$ s, only the RMSD of M3/A-M3 linker maintains a strong linear relationship with the RMSD of the cytosolic headpiece ($r = 0.8$). These findings indicate that protonation-dependent signal transmission primarily propagates from the transport sites to the cytosolic headpiece through M5, M3 and the A-M3 linker.

Effects of protonation and deprotonation of the E2 state on the structural dynamics of the cytosolic headpiece

The MD data suggests that the structural changes in the TM induced by deprotonation propagate to the cytosolic headpiece. Therefore, we investigated the specific effects of E2

protonation and deprotonation on the structural dynamics of the cytosolic domains. We plotted the time-dependent interdomain distance evolution of the carboxyl-carboxyl pairs between centrally positioned residues of the N, P and A domains. Interdomain distances of N-A, N-P and A-P domains were calculated using Ca-Ca distances of the following amino acid pairs: Lys515-Thr171 (N-A domains); Lys515-Glu680 (N-P domains); and Thr171-Glu680 (A-P domains) (Figure 9).

Interdomain distances in the trajectory of the protonated E2 state are 0.1–0.34 nm shorter than those calculated in the crystal structures of E2 (Figure 9). Upon deprotonation, we detected substantial changes in the spatial separation of the cytosolic domains. During the first 2 μ s of simulation time, the distances of N-A domains and N-P domains fluctuate between 2.8 and 3.3 nm (Figure 9). At $t > 2 \mu$ s, the distances of N-A and N-P domains settle a plateau at 3.5 and 3.2 nm, respectively (Figure 9). The distance between the A-P domains increases by 0.4 nm during the first 2.6 μ s, but settles a plateau at 3.8 nm for the rest of the trajectory (Figure 9). These measurements indicate that transport site protonation state populates a compact structure of the cytosolic headpiece, and deprotonation destabilizes it to produce a more open structural arrangement of the headpiece.

The assumption that a closed-to-open structural change in the headpiece is linked to the E2-to-E1 transition is problematic because the headpiece closed and open structures exist in both E1 and E2 states.^{29, 55–60} Therefore, we used cPCA to characterize the essential space of the E2 state, and to determine whether the structural changes observed in our MD simulations are associated with the E2-to-E1 transition. We first projected the trajectories of protonated and deprotonated E2 states into the phase space; the projection of a trajectory on the eigenvectors of its covariance matrix is called principal component (PC). We then constructed probability histograms of each PC. Each histogram is then fitted to a one-Gaussian distribution to determine whether a PC belongs to essential phase space.⁴¹ Distributions with a coefficient of determination $r^2 < 0.9$ is considered to be non-Gaussian, and thus belong to the essential phase space.⁶¹ Distributions with r^2 values between 0.9 and 0.98 indicate that the PC retains some significant non-Gaussian features, therefore contribute to the essential phase space. Finally, PC distributions with $r^2 > 0.98$ are Gaussian fluctuations, thus not considered part of the essential phase space.⁶¹

The histograms of the six PCs calculated for the trajectory of the protonated E2 state are non-Gaussian ($r^2 < 0.9$, Figure 10A) or retain some non-Gaussian features with ($r^2 = 0.9–0.98$, Figure 10A). Beyond PC6, all consecutive histograms fit to a single Gaussian distribution (Figure 10A). In the deprotonated E2 state, the first five PCs belong to the essential space ($r^2 < 0.81$, Figure 10B). PC6, PC7 and PC8 retain some non-Gaussian features ($r^2 = 0.92–0.96$, Figure 10A), and all consecutive PCs belong to the non-essential space ($r^2 \geq 0.99$, Figure 10B). This data shows that in the course of 3.6 μ s MD simulations, the first 4 and 5 PCs represent the essential phase space of protonated and deprotonated E2 states, respectively. The low dimensionality of the essential phase space facilitates subsequent analysis of the headpiece structural dynamics. In this context, low dimensionality refers to the fact that important dynamics of a protein are confined within a handful of collective motions described by the essential phase space.⁴¹

We used cPCA on ensembles of E1 and E2 crystal structures to determine the primary structural motion for the E2-to-E1 transition. cPCA showed that the first PC of the experimental ensemble is non-Gaussian ($r^2 < 0.9$) and accounts for 64% of the total space. The motion captured by this PC is the counter-rotation of the N and A domains about the membrane normal axis (Figure 11A). We found that this motion is captured by the fourth and first PC in the trajectories of protonated and deprotonated E2 SERCA, respectively (Figure 11B–C). However, the contribution of this motion to the essential phase space is much smaller in the protonated than in the unprotonated state (3.5% vs. 41%, Figure 11B–C). Additional analysis of C α root mean square fluctuation (RMSF) for this motion shows that deprotonation substantially increases the magnitude of the N-A domain counter-rotation (Figure 11D). These findings indicate that directionality of the functional N-A domain counter-rotation is preserved in the protonated E2 state, and deprotonation only induces changes in the dominance and amplitude of this motion.

Discussion

We used microsecond MD simulations to determine the effects of transport site protonation on the structural dynamics of the E2 state in solution. Our simulations showed that the protonated E2 state has structural and functional characteristics that are similar to those observed in crystal structures of this state, including the inability to bind metal ions in the transport sites. Deprotonation of E2 is accompanied by a rapid binding of a Na⁺ ion and formation of a competent transport site I. Na⁺ The formation of the transport site I in the E2 state occurs independently of Ca²⁺ binding, in agreement with previous studies showing that K⁺ and Mg²⁺ populate competent or partially competent transport site geometries.^{46, 55, 62} Weak Na⁺ binding in site I suggests that Ca²⁺ exchange in the transport sites will occur at a later stage in the cycle, e.g., during a transient rise in cytosolic Ca²⁺ concentration.

H⁺-Na⁺ exchange in the transport site I disrupts the α -helical periodicity of M5 at Ile765-Asn768 and induces a 53° kink in M5. Serine has been identified as a factor for the formation of kinks in proteins,^{63–65} and kink formation in the M5 helix has also been identified in the Na⁺,K⁺-ATPase.⁶⁶ We found that kink formation in M5 plays a functional role in SERCA: this structural change in M5 propagates to helix M3 and the A-M3 linker to modulate the structural dynamics of helix M5 and the A-M3 linker. These findings agree with mutagenesis experiments showing that the cytosolic segment of M3 (up to residue Gly257) plays a direct role in the mechanism for inhibitor-mediated control of structural transitions between the E2 and E1 states.^{67, 68} Our simulations also agree with mutagenesis experiments showing that changes in the flexibility of the A-M3 linker controls the rate of E2-E1 transition: an increase in the flexibility of the A-M3 linker shifts the equilibrium toward the E2 state, whereas a strain of the A-M3 shifts the equilibrium toward the E1 state.⁶⁹

We ask how protonation controls the large motions of the cytosolic headpiece that drive the E2-to-E1 transition of the pump. Previous studies have suggested that the catalytic cycle of SERCA has elements of a Brownian ratchet, where biochemical states have an effect on the random thermal oscillations of an intrinsically flexible cytosolic domain.⁷⁰ cPCA of available crystallographic structures showed that counter-rotation of the N-A domains about

the membrane normal axis, and not random thermal oscillations, is a primary structural mechanism driving the transition between E2 and E1 states. The time scale available for our studies is insufficient to observe the full E2-to-E1 transition of SERCA, but we found this was sufficient to observe the substantial differences between the effects of protonation and deprotonation on the cytosolic headpiece. Indeed, cPCA analysis of the MD trajectories revealed that the PC describing the counter-rotation motion of the N-A domains exists in both protonated and deprotonated E2 states, but the dominance and amplitude of motion is modulated by transport site protonation to shift the equilibrium between the E1 and E2 states. This mechanistic model transition helps explain how thapsigargin (TG), an inhibitor of the E2-to-E1 transition,^{71–73} alters the structural transitions of SERCA: the high-affinity interaction between TG and M3/M5 does not have a direct effect on the rate of E2 deprotonation.¹⁷ Instead, TG stabilizes the M3–M5 interface⁷⁴ and blocks the signal transmission to the cytosolic headpiece, thus altering the rate at which SERCA populates the E1 state. Our findings suggest that physically controlled processes, such as counter-rotation of the N-A domains, are rate limiting for the overall SERCA reaction.^{75, 76}

Our simulations also help reconcile crystallographic and spectroscopic data, since it supports the biochemistry²² and crystallography-based¹⁸ model that metal-ion binding and transport site protonation control the structural arrangement of the cytosolic domains, while supporting FRET-based conclusion that the headpiece of SERCA in the E2 state is inherently mobile in solution.⁵⁸ While we simulated both protonated and deprotonated states in solution, previous studies have shown that SERCA is primarily deprotonated at physiological pH.^{17, 20} Our MD simulations indicate that at physiological pH, the E2 state is substantially more disordered than previously suggested by x-ray crystallography,¹⁷ so the crystal structures of the E2 state represent a configuration that is populated transiently at physiological pH. Our simulations also support the FRET observation that the structural transitions of SERCA associated with the E2-to-E1 transition are the result of conformational selection: domain motions preexist in the E2 state and transport site protonation shifts the equilibrium between E1 and E2 states.

Finally, there is extensive data showing that the structure and function of other P-type ATPases, such as the Na⁺,K⁺-ATPase and the H⁺,K⁺-ATPase, are very similar to that of SERCA.¹⁸ These ATPases operate through a similar E2-E1 model: the ion-binding sites, which are located within the TM domain, are exposed to the cytosol in the E1 conformation, and exposed to the extracellular side of the membrane. It is likely that other P-type ATPases for which their structural mechanisms are less known, such as the Zn²⁺- and Cu⁺-transporting ATPases,^{77, 78} use a similar E2-E1 mechanism to couple structural changes with ion transport.⁷⁹ We propose that preexisting domain motions that underlie the E2-E1 structural transition described in this study are a common theme in ligand-induced structural shifts associated with function of the P-type ATPase superfamily.

Conclusions

We used atomistic MD simulations to determine the structural mechanisms for protonation-dependent structural transitions of SERCA in solution. These simulations revealed that protonation of the E2 state stabilizes a structure that is similar to that observed in crystal

structures of E2. Deprotonation of the E2 state induces substantial changes in the structural dynamics of the pump. Upon deprotonation, a Na⁺ ion binds to the transport site I, which induces a kink in M5, disrupts the M3–M5 interface, and increases the mobility of cytosolic region of M3 and the A-M3 linker. These protonation-dependent structural changes in the TM domain propagate to the cytosolic headpiece and control the hierarchical structure and amplitude of a preexisting counter-rotation motion of N and A domains about the membrane normal axis. Comparison with available crystallographic data showed that this motion plays a primary role in the transition between E2 and E1 states. We conclude that preexisting rigid-body domain motions underlie protonation-dependent E2-to-E1 transition, where the functionally important directionality is preserved while the dominance and amplitude is modulated by transport site protonation to shift the equilibrium between the E1 and E2 states. We propose that ligand-induced control of the hierarchical structure and amplitude of preexisting domain motions is likely a common theme in functional transitions of P-type ATPases.

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Figure 1.

Effects of transport site protonation on the structural dynamics of E2. (A) Crystal and MD structures at the end of the trajectories of the protonated and deprotonated E2 states. SERCA is colored according to its four functional domains: N (green), P (blue), A (red), and TM (gray). RMSD evolution of SERCA domains in the (B) protonated and (C) deprotonated states of the pump. The RMSD of the TM domain of SERCA was calculated using backbone alignment for TM helices of the pump. The RMSD of N, P, and A domains was calculated by aligning the backbone of the cytosolic headpiece with the structure at $t=0$ μs of each trajectory.

Figure 2. Structural arrangement of the transport sites. (A) Structure of the transport sites at the end of the MD trajectories of protonated and deprotonated E2 states. The TM helices are shown in gray, and transport site residues are shown as sticks. The Na^+ ion bound to the transport sites of the deprotonated E2 state is shown as a yellow sphere. (B) Time-dependent evolution of the interaction between transport site residue pairs Val304^O-Glu309^{C γ} and Glu771^{C γ} -Asn796^{C γ} .

Figure 3.

Na⁺ ion interactions in the transport sites of the deprotonated E2 state. (A) Location of the three different positions occupied by Na⁺ (yellow spheres) in the transport sites of the deprotonated E2 state. The dashed circles show the approximate location of the Ca²⁺-binding site in the transport site I. (B) Time-dependent changes in the location of Na⁺ in the transport site I. The plot shows the changes in the RMSD of Na⁺ relative to the configuration of the bound ion at the beginning of the MD trajectory (Position 1). The shaded areas correspond to the Na⁺ configurations shown in panel (A).

Figure 4. Protonation-dependent structural changes in the TM helices of SERCA. (A) Average changes in the RMSD of each TM helix in the trajectories of protonated and deprotonated E2 state. Data are presented as the mean $\pm$ SD. The location of each TM helix is shown in the right panel as a cartoon representation. (B) Evolution of the secondary structure of the TM helix M5 in the 3.6 μ s trajectory of protonated and deprotonated E2 SERCA. Secondary structure is colored as α -helix (pink), turn (cyan), and coil (white).

Figure 5.

Kink formation in TM helix M5 induced by deprotonation of the transport sites. (A) Structure of M5 at the end of the trajectories of protonated and deprotonated E2 SERCA. The TM helices are shown as gray cartoons; M5 is shown in orange. Residues Ile765 through Asn768 are shown in magenta. (B) Time-dependent changes in the kink angle of M5.

Figure 6.

Na^+ binding to site I induces kink of M5. (A) Representation of the structural arrangement of M5 between residues Ser767 and Glu771; transport site protonation stabilizes a backbone hydrogen bond between Ser767-Glu771, and H^+ - Na^+ exchange in this site destabilizes it. M5 is shown as orange cartoon, Ser767 and Glu771 as sticks, and Na^+ as a yellow sphere. (B) Time-dependent changes in the distance between atoms Ser767^O and Glu771^N. The shadowed area corresponds to the formation of M5 kink described in Figure 5.

Figure 7.

Effects of transport site protonation on the M3–M5 interface. (A) Structure of the M3–M5 interface at the end of the trajectories of protonated and deprotonated E2 SERCA. TM helices M3 and M5 are shown as purple and orange cartoons, respectively. The ovals show the approximate location of the binding site for the inhibitor thapsigargin. Residues Phe256 and Ile765, which play a role in binding and stabilization of thapsigargin, are shown as spheres. (B) Evolution of the native contacts between M3 and M5 in the trajectories of protonated and deprotonated E2 SERCA.

Figure 8.

Effects of transport site protonation on the mobility of M3 and the A-M3 linker. (A) Location of the M3 and A-M3 linker. For clarity, the luminal and cytosolic regions of M3 are shown as pink and blue cartoons, respectively. The A-M3 linker and the cytosolic A domain are shown in lime and red, respectively. (B) Mean square displacement of M3 and the A-M3 linker calculated in the MD trajectories of protonated and deprotonated E2 SERCA. The shaded areas indicate the location of cytosolic/luminal segments of M3, and the A-M3 linker.

Figure 9.

Time-dependent structural transitions of the cytosolic headpiece. (A) MD trajectories of protonated and deprotonated E2 state were used to compute the time-dependent C α -C α distances between residues Thr171 and Lys515 (N and A domains, residues Lys515 and Glu680 (N and P domains) and residues Thr171 and Glu680 (A and P domains). For comparison, discrete distances for the same pairs of residues were calculated from crystal structures of E2. (B) Location of the residues used to calculate interdomain distances (cyan spheres). N, A and P domains are colored in green, red and blue, respectively.

Figure 10.

Probability distributions for the first 10 PCs extracted in the trajectories of (A) protonated and (B) deprotonated E2 state. These histograms show examples of a non-Gaussian distribution (e.g., PC 1), a distribution that maintains some non-Gaussian features (e.g., PCs 2–4) and purely Gaussian distributions (e.g., PC 10). The r^2 values shown inside each plot corresponds to the coefficient of determination obtained after fitting each histogram to a one-Gaussian distribution by least squares analysis.

Figure 11.

Preexisting domain motions of the cytoplasmic headpiece detected by cPCA. (A) Counter-rotation motion of the N and A domains along the E2-to-E1 transition of SERCA detected by cPCA of ensembles of x-ray structures of E1 and E2 states. The counter-rotation motion of the N-A domains is present in both (B) protonated and (C) deprotonated E2 states. The contribution of the PC corresponding to this motion is shown in parentheses. (D) C α RMSF for PC1 and PC4 of deprotonated and protonated E2, respectively. The shaded areas indicate the location of A and N domains in the sequence of the protein.