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The Ca^{2+} -ATPase facilitates bidirectional proton transport across the sarco/endoplasmic reticulum

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Abstract

Ca^{2+} transport across the sarco/endoplasmic reticulum (SR) plays an essential role in intracellular Ca^{2+} homeostasis, signalling, cell differentiation and muscle contractility. During SR Ca^{2+} uptake and release, proton fluxes are required to balance the charge deficit generated by exchange of Ca^{2+} and other ions across the SR. During Ca^{2+} uptake by the SR Ca^{2+} -ATPase (SERCA), two protons are counter transported from the SR lumen to the cytosol, thus partially compensating for the charge moved by Ca^{2+} transport. Studies have shown that protons are also transported from the cytosol to the lumen during Ca^{2+} release, but a transporter that facilitates proton transport into the SR lumen has not been described. In this article we propose that SERCA contains the pathways that facilitate bidirectional proton transport across the SR. We describe the location and structure of water-filled pores in SERCA that form cytosolic and luminal pathways for protons to cross the SR membrane. Based on this structural information, we suggest mechanistic models for proton translocation to the cytosol during active Ca^{2+} transport, and into the SR lumen during SERCA inhibition by endogenous regulatory proteins. Finally, we discuss the physiological consequences of SERCA-mediated bidirectional proton transport across the SR membrane of muscle and non-muscle cells.

Graphical Abstract

We propose that the Ca^{2+} -ATPase pump contains the pathways that facilitate bidirectional proton transport across the sarco/endoplasmic reticulum.

Introduction

The sarco/endoplasmic reticulum (SR) regulates Ca^{2+} ion concentrations in the cytosol. The SR serve as a storage as well as a source for intracellular Ca^{2+} , thus playing a central role in Ca^{2+} homeostasis,¹ signaling,^{2–4} development of epithelial junctions,⁵ cell differentiation,⁶ and muscle contractility.⁷ Ca^{2+} is released from the SR to the cytosol through a variety of receptor/channels such as the ryanodine receptor (RyR),⁸ the IP3 receptor,⁹ and leak channels.¹⁰ Removal of Ca^{2+} from the cytosol occurs primarily through the SR Ca^{2+} -ATPase (SERCA), which pumps Ca^{2+} back into the SR lumen at the expense of ATP hydrolysis.¹¹

Ca^{2+} exchange across the SR requires concomitant flux of ions to prevent significant charge build-up across the membrane.¹² Ca^{2+} release from the SR has been associated with a significant uptake of Mg^{2+} and K^{+} to maintain the membrane potential near zero.^{13, 14} For instance, trimeric intracellular cation channels, a family of K^{+} transporters in the SR, provide a counter balancing K^{+} flux that reduces SR membrane depolarization to maintain efficient Ca^{2+} release and uptake mechanisms.¹⁵ Although K^{+} and Mg^{2+} play a major role in electrically compensating the charge deficit generated by SR Ca^{2+} uptake and release, the concentrations of these ions are insufficient to account for the necessary total charge compensation.^{13, 16} Therefore, H^{+} fluxes exist to balance the relatively small charge deficit induced by ion exchange across the SR.¹⁷

Measurements of charge transfer upon addition of Ca^{2+} or ATP to microsomal vesicles adsorbed on a solid supported membrane system show that $\text{Ca}^{2+}/\text{H}^{+}$ exchange occurs through SERCA, where H^{+} ions are actively transported from the SR lumen to the cytosol.¹⁸ It has also been suggested that H^{+} ions are transported into the SR lumen, which contribute 5–10% of the necessary charge compensation during SR Ca^{2+} release.^{19, 20} These studies indicate that the SR is permeable to H^{+} in both directions of the SR, but a transporter that

facilitates H^+ movement from the cytosol into the SR lumen has not been described. In this article we propose that SERCA contains the pathways that facilitate bidirectional H^+ transport across the SR membrane.

SERCA-mediated H^+ movement from the SR lumen to the cytosol during active Ca^{2+} transport

SERCA uses the energy produced by ATP hydrolysis to pump two Ca^{2+} ions from the cytosol into the SR lumen, and two H^+ from the SR lumen to the cytosol (Figure 1).^{21, 22} At the beginning of its catalytic cycle, SERCA populates a high- Ca^{2+} affinity state (E1), and binds two Ca^{2+} ions from the cytosol to the transmembrane (TM) transport sites and one molecule of ATP in the nucleotide-binding domain. The ATP-bound E1·2 Ca^{2+} complex populates a structural arrangement that is suitable for ATP hydrolysis and the formation of a phosphorylated intermediate, E1~P·2 Ca^{2+} ·ADP.²³ SERCA autophosphorylation then induces a structural transition toward a phosphorylated low- Ca^{2+} affinity E2~P·2 Ca^{2+} state. Two Ca^{2+} ions then dissociate from the transport sites into the SR lumen. Following Ca^{2+} release, two luminal H^+ bind to the transport sites to neutralize the negative charge in the TM domain and stabilize a protonated, low Ca^{2+} -affinity intermediate E2~P.^{24–27} SERCA is then dephosphorylated^{28, 29} to populate a Ca^{2+} -free, protonated E2 state.^{23, 30, 31} At normal intracellular pH (7.1–7.2),^{32, 33} two H^+ spontaneously translocate from the transport sites to the cytosol,^{24, 34, 35} thus completing H^+ transport from the lumen to the cytosol. H^+ release to the cytosol induces an E2-to-E1 transition of SERCA required for the next Ca^{2+} -pumping cycle.^{30, 31}

Extensive pK_a calculations and molecular dynamics (MD) simulations have shown that transport site residues Glu309, Glu771, and Glu908 play a central role in sequestering two H^+ from the lumen and releasing them to the cytosol.^{25, 39} In this section, we describe the pathways used by SERCA to release two luminal H^+ occluded in the transport sites during the transition from E2~P·2 Ca^{2+} to E2~P (Figure 1).

The N-terminal H^+ pathway

At the beginning of the E2-to-E1 transition, a luminal H^+ occluded by Glu309 is released to the cytosol. Glu309 is located in the N-terminal region of SERCA near the cytosolic side of the membrane. It has been suggested that proton shuttling from Glu309 to the cytosol occurs through a transition from a high- pK_a , lumen-facing orientation to a low- pK_a , cytosol-facing orientation of the carboxylic moiety.^{24, 34} However, microsecond MD simulations have shown that H^+ shuttling to the cytosol occurs primarily through a water-filled pore connecting the side chain of Glu309 with the cytosol (Figure 2).⁴⁰ Helices M1, M2 and M4 form the narrow (0.55 nm in diameter) N-terminal H^+ pathway. Residues Glu309, Val62, Leu65, and Leu98 delineate this pathway. In this pathway, three water molecules form hydrogen-bonded water wires in two different arrangements (Figure 2); both water-wire configurations are stabilized by residue Asn101. H^+ translocation from Glu309 to the cytosol through this pore occurs through a Grotthuss shuttling because the length of the water wires is much shorter (< 1nm) and forms within the 100-ps lifetime span required for proton translocation.^{41, 42} The absence of a H^+ donor (e.g., Asp or Glu) at the cytosolic side

of the pore (Figure 2) suggest that the N-terminal H^+ pathway exclusively transports a single H^+ from Glu309 to the cytosol.

The C-terminal H^+ pathway

Following release of a proton from Glu309 through the N-terminal pathway, the transport sites rapidly bind metal ions (e.g., K^+ , Ca^{2+}) from the cytosol, which induces the release of a proton from either Glu771 or Glu908. This H^+ may be released through the N-terminal pathway, but this situation is problematic because the second H^+ has to compete for passage with incoming metal ions from the cytosol. Therefore, an alternative cytosolic pathway must exist for H^+ translocation from Glu771 or Glu908 to the cytosol.

To test this hypothesis, Bublitz et al. obtained a 2.2-Å structure of SERCA in the AlF_4^- -bound transition state of E2-P dephosphorylation.⁴³ This crystal structure revealed a narrow, water-filled pore located between transmembrane helices M5, M6, M7, M8 and M10 (Figure 2). Residues Tyr763, Ser766, Ser767, Arg836, Tyr837, Asn911, Asn914, Asn920, and Asp981 form this pore (Figure 2). These residues form a hydration path leading to cytosol, but not all water molecules were visible in the crystal structure. To complement these observations, we analyzed microsecond-long MD simulations previously reported by our group.^{39, 40} Analysis of the trajectories show that stable (>100 ps) water wires form in between transport site residue Glu908 and the cytosol (Figure 2). These continuous water wires are supported by electrostatic interactions with residues Ser767, Arg836, Tyr837, Asn911, and Asn914, in excellent agreement with x-ray crystallography.⁴³ We also found that in the presence of a water wire, Glu908 has a $pK_a < 7.0$, thus supporting the hypothesis that shuttling of the second H^+ to the cytosol likely occurs through the C-terminal pathway. Release of the second H^+ to the cytosol and metal ion occlusion are coupled: metal ion binding to the transport sites induce structural changes in the transport sites that facilitate H^+ shuttling from Glu771 to Glu908 and then to the cytosol.

SERCA also contains a pathway to transport H^+ from the cytosol into the SR lumen

Three research groups reported crystal structures of SERCA bound to endogenous regulators phospholamban (PLB)⁴⁴ and sarcolipin (SLN).^{36, 37} Extensive computational studies showed that at physiological conditions, binding of either PLB or SLN to SERCA populates a Ca^{2+} -free E1 state protonated at residues Glu771 and Glu908.^{39, 45} This intermediate serves as a kinetic trap that prevents the formation of the $E1 \cdot 2Ca^{2+}$ intermediate shown in Figure 1.^{39, 45} Based on these findings, it was proposed that upon relief of SERCA inhibition, Ca^{2+} binding to the transport sites and H^+ release from Glu771 must occur rapidly, so a cytosolic H^+ pathway must exist in the C-terminus of the pump to allow for expedited H^+ release.

To test this hypothesis, an exhaustive search of potential H^+ pathways was performed using crystal and MD structures of the E1 state bound to Ca^{2+} , unphosphorylated PLB, and unphosphorylated SLN.⁴⁶ These studies showed that the C-terminal pathway (Figure 2) is closed and shielded from water molecules. Surprisingly, microsecond MD simulations

revealed PLB-bound SERCA contains a water-filled pore that directly connects the transport sites with the SR lumen.⁴⁶ Quantitative comparison with SLN- and Ca^{2+} bound crystal structures of the E1 state showed that this luminal pore exist only in the Ca^{2+} -free E1 state populated by unphosphorylated PLB or SLN. This narrow pore (0.5–0.6 nm in diameter) is transiently formed in a hydrophobic cavity between transmembrane helices M6, M8 and M9 (Figure 3). This luminal pore accommodates two types of hydrogen-bonded water wires: a water wire consisting of four water molecules connecting the Thr799 and His944, and a wire of five water molecules linking Glu908 with Trp798 and His944 (Figure 3).⁴⁶ Based on the structural and physicochemical properties of the pore, it was concluded that this pathway facilitates unidirectional H^+ shuttling from the transport sites to the lumen. Multi-scale reactive MD simulations support this hypothesis and show that this luminal pore is permeable to H^+ from residue Glu908 to the SR lumen (Gregory Voth, personal communication).

These studies suggest that this pore serves as a H^+ exit pathway during $\text{Ca}^{2+}/\text{H}^+$ exchange upon relief of SERCA inhibition by PLB and SLN phosphorylation, but we ask whether this pore functions as a pathway for H^+ into the SR lumen during Ca^{2+} release to the cytosol. Studies have shown that at physiological pH and in the absence of Ca^{2+} , PLB or SLN binding primarily populates an inhibited Ca^{2+} -free E1 state of SERCA.^{36, 37, 39, 44, 45} The luminal pore is open during PLB- and SLN-inhibition, so we propose that cytosolic H^+ collected by “sponges” of water molecules are funneled to residue Glu908 and then shuttled to the lumen through this luminal pore. The pathway leading to Glu908 (e.g., via Glu309, Ala305 and Glu771) is virtually metal ion-free,^{39, 45} thus reducing the electrostatic barrier to proton translocation from the water sponges to Glu908. An increase in cytosolic Ca^{2+} concentration relieves SERCA inhibition closes the luminal H^+ pathway and blocks SERCA-mediated H^+ transport into the SR lumen.⁴⁶ This mechanistic model explains the coupling between Ca^{2+} release to the cytosol and SERCA-mediated H^+ uptake into the SR lumen during a contraction–relaxation cycle in myocytes.^{20, 47}

Physiological consequences of SERCA-mediated bidirectional H^+ transport across the SR

SERCA activity is tightly coupled to pH^{35} and cytosolic Ca^{2+} concentrations,⁴⁸ so characterization of SERCA pathways is important in understanding the physiological role of bidirectional H^+ flux across the SR membrane. First, we examine the functional roles of SERCA-mediated H^+ transport from the SR lumen to the cytosol. Studies have suggested that PLB and SLN oligomers can serve as selective channel pore to transport ions across the SR membrane. It has been shown that PLB and SLN form ion-selective channels in lipid bilayers; PLB oligomers are permeable to various metal ions,^{49–51} whereas SLN oligomers are permeable to small inorganic anions such as chloride, phosphate, or sulfate.⁵² Despite the differences in ion permeability, voltammetry and electrochemical impedance spectroscopy experiments have shown that both PLB and SLN oligomers become permeable to monovalent metal ions (e.g., K^+) following a small decrease in cytosolic pH.⁵³ SERCA-mediated $\text{Ca}^{2+}/\text{H}^+$ exchange decreases the pH of the cytosol,⁴⁷ so it is likely that SERCA-mediated H^+ transport to the cytosol contributes to modulating ion selectivity of PLB and

SLN oligomers. We propose that this H⁺-induced change in ion selectivity of PLB and SLN increases the cation transport capacity of the SR that is necessary to balance the charge deficit generated during SR Ca²⁺ uptake. SERCA-mediated H⁺ transport to the cytosol might also play a role in regulating ion exchange through the plasma membrane. For instance, the cytosolic pH of skeletal muscle can fluctuate between 6.2 and 7.0 during repeated exercise.⁵⁴ These changes in pH are sufficient to alter the function of the Na⁺/Ca²⁺ exchanger, which is primarily inhibited by H⁺ at pH<7.^{55–57}

Additional members of the SERCA-inhibitory micropeptide family have been identified across diverse cell types,⁵⁸ so it is likely that SERCA-mediated H⁺ transport into the SR lumen is a widespread process. Therefore, we also examine the physiological consequences of SERCA-mediated H⁺ transport into the SR lumen. Luminal pH values between 5.5–6.0 activate calcium release rates,⁵⁹ but pH<5.5 alters the interaction between RyR and luminal proteins such as calsequestrin, thus inhibiting RyR-Ca²⁺ release channels.⁶⁰ Changes in luminal pH also regulate Ca²⁺ uptake into the SR through controlling Ca²⁺/ATP coupling ratios of SERCA. For example, a decrease in the luminal H⁺ concentration uncouples Ca²⁺ transport from ATP hydrolysis, thus allowing Ca²⁺ backflux to the cytosol.⁶¹ Based on this evidence, we propose that SERCA-mediated H⁺ transport into the SR lumen contributes to regulating Ca²⁺ handling in muscle and non-muscle cells.

While there is no direct evidence of the role of H⁺ transport across the endoplasmic reticulum (ER) of non-muscle cells, disease-causing mutations occur in the H⁺ pathways of non-muscle SERCA isoforms. Mutations associated with Darier disease, a skin disorder characterized by loss of adhesion between epidermal cells and abnormal keratinization, are found in both cytosolic and luminal H⁺ pathways of the pump. Disease-causing mutations Ser766Leu^{62, 63} and His944Arg (His943Arg in SERCA2b)^{63, 64} are located in cytosolic and luminal H⁺ pathways of the pump, respectively. These mutations probably alter the structure and stability of the water wires, hence partially or completely blocking H⁺ flow through these pores. Changes in H⁺ translocation through SERCA probably alter either Ca²⁺/ATP coupling⁶¹ or the structural stability of the pump during active Ca²⁺ transport,^{24, 26} thus explaining impaired Ca²⁺ homeostasis in Darier keratinocytes.⁶⁵ We propose that SERCA-mediated H⁺ transport also contributes to normal ER Ca²⁺ handling in non-muscle cells.

Conclusion

We have shown that SERCA contains three water-filled pores that form cytosolic and luminal pathways. These pores are filled with continuous hydrogen-bonded water wires that connect the transport sites of SERCA with either the cytosol or the SR lumen. Based on the location and structural arrangement of water molecules in these pores, we propose that they are suitable for bidirectional H⁺ shuttling across the SR membrane. We propose mechanistic models for proton translocation to the cytosol during active Ca²⁺ transport, and into the SR lumen during SERCA inhibition by endogenous regulatory proteins PLB and SLN. These models explain the coupling between Ca²⁺ cycling and SERCA-mediated H⁺ transport across the SR membrane. Our analysis provides novel hypotheses that can be tested by complementary functional mutagenesis, fluorescence experiments and computer simulations to measure SERCA-mediated H⁺ transport at physiologically relevant conditions. Finally,

we discuss the physiological consequences of SERCA-mediated bidirectional H^+ transport across the SR on muscle and non-muscle cells. This analysis has important implications for understanding the role of SR/ER H^+ transport in health and disease.

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Figure 1.

Schematic representation of the catalytic cycle of SERCA. The biochemical intermediates in the cycle correspond to those proposed by x-ray crystallography, biochemical assays, spectroscopic experiments and computer simulations. The box indicates the steps in the catalytic cycle associated with H⁺ transport from the SR lumen to the cytosol during active SR Ca²⁺ uptake. Low-Ca²⁺ affinity (E2) and high-Ca²⁺ affinity (E1) states of SERCA are shown in blue and red, respectively. The asterisk indicates the Ca²⁺-free E1 intermediate proposed by x-ray crystallography studies (E1·Mg²⁺)^{36, 37} and molecular dynamics simulations (E1·2K⁺).³⁸

Figure 2.

The pathways in the E2 state of SERCA that facilitate H^+ release to the cytosol (Partially adapted from Ref. ⁴⁰). The schematic shows the location and structure of the N- and C-terminal H^+ transport pathways reported in Ref. ⁴⁰ and ⁴³. The blue and red boxes show the location of the cytosolic N- and C-terminal H^+ pathways, respectively; water is shown as a green surface. We show the structure of the water wires linking Glu309 with the cytosol (N-terminal pathway) and Glu908 with the cytosol (C-terminal pathway). Water molecules are shown as spheres; SERCA residues that form the water pore are shown as sticks. The arrows indicate the direction of H^+ translocation. The structure of N- and C-terminal water wires was determined using MD trajectories of the E2 state of SERCA.^{39, 40}

Figure 3.

The pore in the PLB-bound E1 state of SERCA that facilitates H^+ uptake into the SR lumen. The schematic shows the location and structure of the luminal H^+ transport pathway (Adapted from Ref. ⁴⁶). The black box shows the location of water-filled luminal pathway (red surface); transmembrane helices M6, M8, and M9 are shown in blue, green, and yellow, respectively. The water-wire configurations = reported in Ref. ⁴⁶ are shown on the right; residues that form the water pore as well as water molecules are shown as sticks. In both cases, the water wires directly connect transport site residue Glu908 with the SR lumen. The arrows indicate the direction of H^+ translocation.