Dephosphorylation of the Calcium Pump Coupled to Counterion Occlusion

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Outline

- Brief review of P-type ATPases
- Introduction to calcium pump – structure, function, and importance
- Detailed description of ATPase structure with bound calcium
- Mechanism of dephosphorylation coupled to counterion occlusion
P-Type ATPases

- Actively transport ions up a concentration gradient in order to maintain the gradient.
- Active transport - energy is required to make this transportation possible.
- P-Type indicates that the protein is phosphorylated during the transport process.
- Important proteins belonging to this class include the Na\(^+\)/K\(^+\)-ATPase, as well as the Ca\(^{2+}\) ATPase.
P-Type ATPases

- ATP binds to the P-Type ATPases in at least one location. Binding sites for ATP are always located on the cytosolic side of the membrane.
- ATP is not hydrolyzed into ADP and P\textsubscript{i} unless ions are simultaneously being transported.
- Maintenance of the electrochemical gradient is very expensive energetically. For example, up to 25% of ATP produced in nerve and kidney cells is required for functioning of their pumps.
Calcium Pump

- As with other ions, a pump is required to maintain the calcium gradient across the membrane.
- The plasma membrane of each cell contains a calcium pump to maintain the calcium gradient across the membrane by pumping two calcium ions for each molecule of ATP hydrolyzed.
- \([\text{Ca}^{2+}]_{\text{cytosol}} = 0.2 \, \mu\text{M}\)
- \([\text{Ca}^{2+}]_{\text{blood}} = 1.8 \, \text{mM}\)
Muscle Calcium Pump

- Muscle cells contain a second calcium pump located on the membrane of the sarcoplasmic reticulum (SR), and transports calcium from the cytosol to the SR.
- Known as SERCA – Sarco/Endoplasmic Reticulum Ca²⁺-ATPase.
As with almost any protein, the structure and function of the SERCA protein are highly intertwined.

A basic understanding of the protein’s structure and its mechanism of action are necessary prior to a detailed description.
Importance of Calcium Pump

- Vital in muscle contraction and relaxation.
- When the nerve impulse reaches the muscle cell, $\text{Ca}^{2+}$ is released from the SR lumen, raising the cytosolic $[\text{Ca}^{2+}]$ from approximately $10^{-7}$ M to $10^{-6}$ M, and thus causing muscle contraction.
Importance of Calcium Pump

- Upon release of the calcium from the SR, the SERCA protein kicks into gear, pumping the Ca$^{2+}$ back into the SR, and thus causing muscle relaxation.
The protein consists of 994 amino acids and has a molecular weight of approximately 110 kDa. Made up of 10 transmembrane helices, as well as 3 cytosolic domains.
At the most basic level, the SR calcium pump functions in four steps as shown above.

First, two cytosolic Ca\(^{2+}\) ions bind to separate high-affinity sites on SERCA in its E1 conformation (step 1).
Secondly, ATP binds to its specified location within the cytosolic domain. ATP is hydrolyzed to ADP, resulting in the phosphorylation of Asp351 (step 2).
As this occurs, the protein changes from the E1 to the E2 conformation as the calcium ions are transported across the membrane (step 3) and released into the lumen of the sarcoplasmic reticulum (step 4).
The cycle is complete when the Asp351 is dephosphorylated through hydrolysis of the bond. This deactivates the lumenal $\text{Ca}^{2+}$ binding sites and reactivates the cytosolic $\text{Ca}^{2+}$ binding sites while shifting the conformation of the protein back to the E1 state.
Structure of SERCA (reference 3)

- The figure shown depicts a calcium-bound state of SERCA.
- The transmembrane helices are commonly labelled M1-M10.
- The cytosolic domains are labelled A (actuator), P (phosphorylation), and N (nucleotide-binding).
Structure – Arrangement of Transmembrane Helices

- The transmembrane helices are shown below.
- Loops connect all transmembrane helices. These regions are generally quite short, except for the approximately 35 amino acid long loop connecting helices M7 and M8.

Toyoshima
Structure of the Ca\textsuperscript{2+} Binding Sites

- There exist two Ca\textsuperscript{2+} binding sites within the protein.
- Several different helices are involved in the binding of each calcium ion. The binding is coordinated by both side chain and main chain oxygen atoms.
Phosphorylation (P) Domain

- Contains Asp 351, the site of phosphorylation.
- Made up of two different sections, separated in the sequence by approximately 250 amino acids.
- These sections form a seven-stranded parallel beta sheet, with 8 short surrounding helices.
- Contains a K$^+$ binding site that is important for E2-P dephosphorylation.

Toyoshima
Nucleotide-Binding (N) Domain

- Molecular weight of 27 kDa, making it the largest of the cytosolic domains.
- Comprised of approximately 244 amino acids, arranged as two helical bundles flanking a seven-stranded antiparallel beta sheet
- Physically located between the two sections that form the phosphorylation domain.
Actuator (A) Domain

- Smallest of the three cytosolic domains, with a molecular weight of approximately 16 kDa (3)
- Located between the M2 and M3 helices
- Forms a jelly roll structure, and also contains the 40 amino acids at the N-terminal end of the protein
- Contains the Thr-Gly-Glu-Ser (TGES) sequence, amino acids 181-184 (main article). This sequence is highly conserved in P-type ATPases, and plays an important role in the dephosphorylation of the E2-P phosphoenzyme.
- Links the changes at the phosphorylation site of the cytoplasmic region to the transmembrane ion conducting pathway.
Throughout the cycle, it has been found that conformational changes occur in SERCA, which are essential to its function.

The above diagram (reference main paper) shows the overall differences between 3 different states.
Overall Mechanism

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Experimental Goal/Methods

- Olesen *et al.* had the goal of gaining an understanding of the mechanism of dephosphorylation of P-Type ATPases.

- To do this, SERCA was complexed in its E2 conformation to E2-AlF$_4^-$, which mimics the transition state of hydrolysis of the E2-P enzyme. SERCA remained in the presence of native lipids, and thapsigargin.

- Thapsigargin is an inhibitor of SERCA, which blocks its ability to sequester calcium.
Conformational change occurs, changing SERCA from its E1~P state to its E2-P state in an energetically downhill reaction.

While ADP is bound, the N and P cytosolic domains are stuck together. However, upon transfer of the phosphoryl group, the ADP is released back into the SR lumen and movement of both subunits can occur.
Structural Changes Following ADP Release

- The N domain (red) rotates 50° relative to the P domain (blue).
- Allows the A domain (yellow cartoon) to rotate 108° and shift 8 A around the P domain. This step is required to line up the TGES loop with the phosphorylation site.
Structural Changes Following ADP Release

- As a result of these conformational changes, the membrane Ca\(^{2+}\) binding sites are destabilized slightly.
- However, Ca\(^{2+}\) is unable to dissociate to the cytoplasm due to the relative orientation of some of the transmembrane helices.
Ca$^{2+}$ release/H$^+$ binding

- In the Ca$_2$E2-P state, SERCA still has low-affinity Ca$^{2+}$ binding site on the lumenal side. So, the calcium ions are deposited into the SR lumen with partial exchange of protons.

- As the Ca$^{2+}$ dissociate, four side chain carboxyl groups that had previously been involved in binding the calcium ions (E309, E771, D800, E908) are buried in the membrane without any positively charged ions attached or nearby amino acid residues.
Counterion Occlusion

- As can be seen here, the positively charged residues (lysines and arginines) are not near enough the glutamate and aspartate residues to interact.

- This is energetically unfavourable: D and E residues are charged and polar, and prefer a hydrophilic environment. The transmembrane helices, however, are quite hydrophobic.
Counterion Occlusion

- To counter this, the D and E residues bind positive countercations, in this case protons.
- Therefore, protons must be bound to those residues to provide a favourable environment before hydrolysis can occur, dephosphorylating the enzyme and returning it to its E2 conformation as the protons travel into the cytoplasm.
The hypothesis of proton dependent transition is supported by the fact that alkaline pH reduces the rate of dephosphorylation.

The transition state (E2-P) has a low affinity to Ca$^{2+}$, which contrasts with E2.

Lumenal Ca$^{2+}$ is electrostatically repelled by the protonated ion conducting pathway and charged amino acids near the lumen surface. This prevents lumenal calcium from approaching the transmembrane domain and attempting to cross back into the cytosol.
On the left is shown a cross section of the membrane of the E2-AlF4- structure. The carboxylate residues with bound protons, shown in red, are buried in the membrane as they are now neutrally charged, preferring a hydrophobic environment.
On the right is shown a cross section of the membrane of the E1~P structure, mimicked by the Ca$_2$E1~P:ADP-AlF$_4^-$ complex. The carboxylate residues in this case are not binding protons, and are therefore charged and interacting with calcium ions. Notice the much more open conformation.
Dephosphorylation

- Coupled to proton occlusion.
- Shown here are the conformational changes occurring as SERCA is dephosphorylated.
Conformational changes During Dephosphorylation

- The cyan molecule is \( \text{Mg}^{2+} \).
- Magenta \( \text{K}^+ \) ion stabilizes this region of the protein.

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Conformational changes during dephosphorylation

- The wheat coloured structure is bound to E2 whereas the blue structure binds E2-AlF$_4^-$ (represents the transition state, E2-P).
- This picture shows the overall conformational changes occurring in the 5 helix cluster of the P domain during dephosphorylation.
Conformational Change
Summary

- Ca$^{2+}$ transport from the cytosol to the lumen is coupled to phosphorylation.
- Proton countertransport from the lumen to the cytosol is coupled to dephosphorylation.
- Conformational changes of the subunits relative to each other, and within the subunits themselves, make the process possible.
Summary

- Phosphoryl transfer can’t be initiated unless the SERCA protein is in a very specific spatial conformation.

- This requires that Ca$^{2+}$ are bound at the ion occluding pathway during phosphorylation, and that H$^+$ are bound at that pathway during dephosphorylation.
References


