Physical Interactions between Phospholamban and Sarco(endo)plasmic Reticulum Ca\(^{2+}\)-ATPases Are Dissociated by Elevated Ca\(^{2+}\), but Not by Phospholamban Phosphorylation, Vanadate, or Thapsigargin, and Are Enhanced by ATP*  

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Previous co-immunoprecipitation studies (Asahi, M., Kimura, Y., Kurzydlowski, K., Tada, M., and MacLennan, D. H. (1999) J. Biol. Chem. 274, 32855–32862) revealed that physical interactions between phospholamban (PLN) and the fast-twitch skeletal muscle sarco(endo)plasmic reticulum Ca\(^{2+}\) ATPase (SERCA1a) were retained, even with PLN monoclonal antibody 1D11 bound to an epitope lying between PLN residues 7 and 17. Because the 1D11 antibody relieves inhibitory interaction between the two proteins, it was of interest to determine whether PLN phosphorylation or elevation of Ca\(^{2+}\), which also relieves inhibitory interactions between PLN and SERCA, would disrupt physical interactions. Co-immunoprecipitation was measured in the presence of increasing concentrations of Ca\(^{2+}\) or after phosphorylation of PLN by protein kinase A. Physical interactions were dissociated by elevated Ca\(^{2+}\) but not by PLN phosphorylation. The addition of ATP enhanced interactions between PLN and SERCA. The further addition of vanadate and thapsigargin, both of which stabilize the E\(_2\) conformation, did not diminish binding of PLN to SERCA. These data suggest that physical interactions between PLN and SERCA are stable when SERCA is in the Ca\(^{2+}\)-free E\(_2\) conformation but not when it is in the E\(_1\) conformation and that phosphorylation of PLN does not dissociate physical interactions between PLN and SERCA.

Muscle contraction is regulated by Ca\(^{2+}\) release from the sarcoplasmic reticulum through Ca\(^{2+}\) release channels and relaxation is regulated by the subsequent return of Ca\(^{2+}\) to the lumen of the sarcoplasmic reticulum through the action of Ca\(^{2+}\) pumps, referred to as sarco(endo)plasmic reticulum Ca\(^{2+}\) ATPases or SERCAs. SERCA molecules are 110-kDa transmembrane proteins that transport Ca\(^{2+}\) ions from the sarcoplasm to the lumen of the membrane system at the expense of ATP hydrolysis (1). These proteins contain 10 transmembrane helices, which form the Ca\(^{2+}\) binding and transport domain, and an elaborate cytoplasmic catalytic domain in which ATP is bound and hydrolyzed to energize the conformational changes that result in Ca\(^{2+}\) transport.

Phospholamban (PLN) is a 52-amino acid, integral membrane protein highly expressed in cardiac and slow-twitch skeletal muscle fibers that interacts with and, at low Ca\(^{2+}\) concentrations, reversibly inhibits the activity of SERCA2a by lowering its apparent affinity for Ca\(^{2+}\) (2). In its role as a modulator of the activity of the Ca\(^{2+}\) pump, PLN is a major regulator of the dynamics of cardiac contractility (3–5). PLN has been predicted to contain three domains. Domain IA, amino acids 1–20, is largely helical (6) and contains sites of regulatory phosphorylation by protein kinase A at Ser16 and by \(\alpha\)-calmodulin kinase at Thr17 (7, 8). Domain IB, amino acids 21–30, is also largely helical (9) and contains a high proportion of amidated residues. Domain II, amino acids 31–52, forms a transmembrane helix (2).

We have identified clusters of amino acids in both SERCA and PLN in which mutation diminishes the ability of PLN to inhibit SERCA molecules, suggesting that these residues form the sites through which SERCA2a and PLN interact. These sites of interaction include residues in cytosolic (10–12) and transmembrane domains (13–15). Although mutation of key cytosolic residues reduces PLN/SERCA2a inhibitory interactions, reconstitution with PLN domain IA/IB peptides has failed to show any affect on the affinity of SERCA2a for Ca\(^{2+}\) (16–19). By contrast, domain IB-domain II constructs, from which residues 1–27 were removed or replaced, retained the ability to lower the affinity of SERCA2a for Ca\(^{2+}\) (13, 16). On the basis of these results, we have proposed that PLN and SERCA2a interact via a four (or possibly six) base circuit through which long range inhibitory interactions might be propagated among a series of cytoplasmic and transmembrane interaction sites (14).

Molecular models of the mechanism by which PLN reversibly inhibits SERCA suggest that a reversible physical interaction occurs between the two proteins. James et al. (20) reconstituted purified PLN and SERCA2a and showed that covalent cross-links were formed between cytosolic domains of PLN and SERCA2a only in low Ca\(^{2+}\) and when PLN was dephosphorylated. In a later study, Negash et al. (21) showed that phosphodiaminonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; TBS, Tris-buffered saline; WT, wild-type.
ylation of PLN results in a nearly 2-fold reduction in rotational mobility of SERCA2a. These results suggest that PLN phosphorylation leads to altered interactions between PLN and SERCA2a. In this study, we have used co-immunoprecipitation with antibody 1D11 (22) against PLN to determine whether physical interactions are altered by elevated Ca\(^{2+}\), which activates PLN-inhibited SERCA, or by two inhibitors of SERCA, vanadate, and thapsigargin, which block SERCA in the E\(_2\) conformation (23, 24). We have also used co-immunoprecipitation with antibody 285, directed against a Ser\(^{16}\)-phosphorylated PLN peptide (25), to determine whether physical interactions between PLN and SERCA are retained following Ser\(^{16}\) phosphorylation of PLN.

**EXPERIMENTAL PROCEDURES**

**Materials**—Enzymes for DNA manipulation were obtained from New England Biolabs and Amersham Pharmacia Biotech. G-Sepharose and a chemiluminescence kit for measurement of co-immunoprecipitation were purchased from Pierce, vanadate was from Fisher Scientific, and thapsigargin was from Calbiochem.

**Oligonucleotide-directed Mutagenesis and Expression**—The preparation, expression, and assay of mutant PLN and SERCA1a or SERCA2a in microsomal fractions were described in earlier publications (10, 11, 15).

**Phosphorylation by Protein Kinase A**—Microsomes (16 \(\mu\)g of protein in 16 \(\mu\)l of 0.15 M KCl, 0.25 M sucrose, 10 mM Tris-HCl, pH 7.5, 20 \(\mu\)M CaCl\(_2\), 3 mM 2-mercaptoethanol) were phosphorylated by the addition of 2 \(\mu\)l of cAMP-dependent protein kinase (protein kinase A) catalytic subunit (Sigma) (3330 units/ml) and 1 \(\mu\)l of 0.5 mM ATP to yield a final ATP concentration of 25 \(\mu\)M. After 5 min at room temperature, 1 \(\mu\)l of 0.5 M NaF was added to bring the NaF concentration to 25 mM to inhibit dephosphorylation. To quantify the association between SERCA and wild-type or mutant phospho-PLN, an equal volume of lysis buffer, containing 40 mM HEPES-NaOH, pH 7.5, 300 mM NaCl, 2 mM EDTA, 4 mM phenylmethysulfonyl fluoride, and 1% Tween-20, was added to the reaction mix. Immunoprecipitation was carried out as described below and in a previous paper (15), except that antibody 285 (25) was used to form the immunoprecipitate instead of antibody 1D11. Antibody 285 is specific for the epitope between residues 657 to 672 in SERCA1a (27) or anti-SERCA2a monoclonal antibody 1D8F6 (28), as described under “Experimental Procedures.” The A52 antibody and the 1D8F6 antibody were then reacted with a horseradish peroxidase-conjugated anti-mouse IgG antibody (Promega), which was quantified using a Pierce chemiluminescence kit. The relative amount of SERCA and PLN in each lane in exposed films was quantified by scanning densitometry using a Kodak X-oanot Processor. Protein expression levels in all experiments were estimated by immunoblotting using antibodies A52, 1D8F6, and 1D11.

**RESULTS**

**Effects of elevated Ca\(^{2+}\) on the interaction between SERCA and PLN**—The elevation of Ca\(^{2+}\) to micromolar concentrations leads to activation of SERCA2a activity, independent of the phosphorylation state of PLN, the loss of the ability of PLN to form cross-links with SERCA2a (20), or the disaggregation of PLN-regulated SERCA2a complexes (29). The effects of elevated Ca\(^{2+}\) on physical interactions between PLN and SERCA1a and PLN and SERCA2a are shown in Fig. 1. Elevation of Ca\(^{2+}\) from pCa 8 to pCa 5 resulted in a concentration-dependent decrease in the amount of SERCA1a that was co-immunoprecipitated with antibody 1D11 directed against PLN (Fig. 1, A and B). At pCa 5, the amount of SERCA1a that was co-immunoprecipitated was reduced to about 27% of the amount co-immunoprecipitated at pCa 7. The same pattern of Ca\(^{2+}\)-dependent inhibition was observed for the co-immunoprecipitation of SERCA2a with PLN (Fig. 1, C and D).

The effects of PLN phosphorylation on co-immunoprecipitation of PLN-SERCA1a and PLN-SERCA2a complexes are shown in Fig. 2. In this case, antibody 285, which is specific for Ser\(^{16}\)-phosphorylated PLN, was used (25). The data presented in Fig. 2A show that Ser\(^{16}\)-phosphorylated PLN forms a complex with SERCA1a that is co-immunoprecipitated with antibody 285, and the data presented in Fig. 2B show that Ser\(^{16}\)-
phosphorylated PLN forms a complex with SERCA2a that is co-immunoprecipitated with antibody 285.

It is difficult to compare the amount of SERCA that is co-immunoprecipitated by antibody 285 to that which is co-immunoprecipitated with antibody 1D11. In previous studies, we used both PLN and SERCA mutants with a range of inhibitory properties to show that there is a reasonable correlation between the amount of wild-type or mutant SERCA1a protein that is co-immunoprecipitated with wild-type or mutant PLN by antibody 1D11 and the activity of the mutant PLN-SERCA complex (15). We have now carried out comparable experiments with phosphorylated forms of some of the mutant PLN proteins studied earlier. The data are presented in Table I where they are compared with earlier data from Asahi et al. (15). The results of experiments described in column 2, Parts A, B, and C, cannot be compared directly with those presented in columns 4 and 5, because the experiments were not carried out under identical conditions. Trends can be compared, however.

In Table I, Part A, column 2, the co-immunoprecipitation of SERCA1a by wild-type phosphorylated PLN is assigned a value of 1. Significantly different levels of co-immunoprecipitation of wild-type or mutant SERCA1a were observed with wild-type or mutant phosphorylated PLN, confirming that the level of co-immunoprecipitation of wild-type SERCA1a by wild-type phosphorylated PLN was not merely at a background level but lies in a range where variation, associated with different mutations, can be recorded. Co-immunoprecipitation following PLN phosphorylation was reduced compared with wild-type for mutants E2A, K3A, N27A, N30A, L31A, and N34A, but was increased compared with wild-type for mutant I40A. It is of particular interest that the fractional amount of SERCA1a co-immunoprecipitated with PLN was most strongly affected by phosphorylation for the superinhibitory, pentameric N27A and N30A mutants (column 2), when compared with the fractional amount co-immunoprecipitated by unphosphorylated PLN (column 4) (0.69 and 0.70 compared with 2.26 and 2.09). By contrast, the effect of phosphorylation on the fractional amount of SERCA1a precipitated by the superinhibitory, monomeric I40A mutant was less pronounced (1.76 versus 2.45). Data presented in Table I, Part B, column 2, show that co-immunoprecipitation of the loss of function SERCA1a mutant, L802A, with phosphorylated wild-type PLN was reduced compared with wild-type SERCA1 both before and after phosphorylation of PLN.

In Table I, part C, the effects of mutations in the cytosolic domain of SERCA2a on co-immunoprecipitation by wild-type phosphorylated and unphosphorylated PLN are described. These mutations were shown by Toyofuku et al. (11) to diminish PLN-SERCA2a interactions and led to the postulate that the sequence Lys397-Asp398-Asp399-Lys400-Pro401-Val402 is the binding site in SERCA2a for PLN. The double SERCA2a mutants, K397E/K400E and D398R/D399R, as well as the single mutant, P401L, all showed substantial reductions in co-immunoprecipitation by either phosphorylated or unphosphorylated wild-type PLN.

Effects of ATP, Vanadate, and Thapsigargin on Physical Interactions between PLN and SERCA—The conformation of SERCA is dependent on the ligands bound to the molecule (30). Ca\(^{2+}\) and ATP bind independently to SERCA, with Ca\(^{2+}\) driving the protein into the E\(_1\) conformation and ATP causing significant conformational changes (31). Both vanadate (23) and thapsigargin (24) drive SERCA molecules into a stable E\(_2\) conformation. Co-immunoprecipitation data, presented in Fig. 3, show that neither vanadate nor thapsigargin affected the ability of wild-type PLN to co-immunoprecipitate either wild-type SERCA1a or wild-type SERCA2a. The addition of 5 mM ATP, in the absence of Ca\(^{2+}\), increased the extent of co-immunoprecipitation of both SERCA1a and SERCA2a by wild-type PLN. The addition of either vanadate or thapsigargin did not diminish this enhanced level of co-immunoprecipitation.

**DISCUSSION**

In this study, we have examined the question of whether physical interactions are retained between PLN and SERCA2a under a variety of conditions that alter functional interactions between the two proteins or that alter the conformation of SERCA molecules. The question of whether Ca\(^{2+}\) disrupts physical interactions is relatively clear cut. The elevation of Ca\(^{2+}\) causes a reduction in the amount of either SERCA1a or SERCA2a that is co-immunoprecipitated with PLN to 27% of the amount that is co-immunoprecipitated in the presence of EDTA. This residual binding is probably a background level of interaction that is difficult to decrease under the conditions of the experiment. This result is consistent with earlier studies of James et al. (20) who showed that cross-linking between the cytoplasmic domains of PLN and SERCA2a was disrupted by elevated Ca\(^{2+}\) concentrations. The mechanism might be explained by the fact that PLN binds to M6 (15), a key transmembrane sequence in Ca\(^{2+}\) binding that contributes at least 3 of 6 known ligands to binding of 2 mol of Ca\(^{2+}\) in SERCA1a or SERCA2a (1, 32). M6 may undergo conformational changes in the presence of Ca\(^{2+}\) (33); a helical structure, extending above the Ca\(^{2+}\) binding cavity near the center of the transmembrane sequence in the E\(_2\) state, is converted to an extended conformation in the E\(_1\) state. Large conformational changes in M6, a key component of the transmembrane interaction site between SERCA and PLN, would provide the physical basis for dissociation of PLN from SERCA molecules in the presence of elevated Ca\(^{2+}\).

It is not so clear whether phosphorylation of PLN, leading to loss of functional interaction (34), also leads to physical dissociation. In the experiments of James et al. (20), phosphorylation of PLN diminished the formation of cross-links between PLN Lys\(^{397}\) and a sequence in SERCA2a that included Lys\(^{397}\) and Lys\(^{399}\). Our current results show that an antibody directed against Ser\(^{16}\)-phosphorylated PLN can immunoprecipitate SERCA1a and SERCA2a, indicating that physical interactions are retained between PLN and SERCA molecules in the ab-
to the SERCA protein with the phosphorylated PLN. We are not co-immunoprecipitating a background level of body 285 varies with a series of mutations. Thus we feel that or SERCA2a that is co-immunoprecipitated with PLN by anti-
ever, we have been able to show that the amount of SERCA1a
precipitates phosphorylated PLN (antibody 285) with the anti-
body that precipitates unphosphorylated PLN (1D11). How-

Since multiple cytosolic and transmembrane interaction sites between PLN and SERCA have been documented, it is reasonable to assume that phosphorylation might disrupt a key site in the circuit of functional interactions (14) without disrupting physical interactions at all sites. Thus phosphorylation at Ser16 may cause conformational changes in PLN that disrupt its ability to inhibit SERCA2a and the ability of PLN Lys3 to form cross-links to SERCA2a Lys397 or Lys400 but may not affect physical interactions at other sites in cytosolic or trans-
membrane domains of SERCA1a or SERCA2a.

The study of mutants highlights residues that might be involved in the interaction of SERCA with phosphorylated PLN. Mutation of the Lys397 to Pro401 sequence in SERCA2a disrupts its ability to inhibit SERCA2a and the ability of PLN Lys3 to form cross-links to SERCA2a Lys397 or Lys400 but may not affect physical interactions at other sites in cytosolic or transmembrane domains of SERCA1a or SERCA2a.

A. SERCA1a

B. SERCA2a

FIG. 3. Effects of ATP, vanadate, and thapsigargin on the in-
teraction between SERCA and PLN. (A) SERCA1a and (B) SERCA2a were co-expressed with PLN in HEK-293 cells. Microsomal fractions were incubated with either 400 μM sodium vanadate, 10 μM thapsigargin, or 10 mM CaCl₂ in the presence or absence of 5 mM ATP, as described under "Experimental Procedures," prior to the addition of Tween 20 and co-immunoprecipitation with anti-PLN monoclonal an-
tibody 1D11. Immunoprecipitates were treated as described in the
legend to Fig. 1.

sence of functional interactions. It has not been possible to
make a direct comparison of efficiency of the antibody that precipitates phosphorylated PLN (antibody 285) with the antibody that precipitates unphosphorylated PLN (1D11). However, we have been able to show that the amount of SERCA1a or SERCA2a that is co-immunoprecipitated with PLN by antibody 285 varies with a series of mutations. Thus we feel that we are not co-immunoprecipitating a background level of SERCA protein with the phosphorylated PLN.

By contrast, the monomeric, superinhibitory, transmembrane domain mutant, I40A, lost only 28% of its enhanced physical interaction when phosphorylated. By contrast, the monomeric, superinhibitory mutants, N27A and N30A, which gain both

| TABLE I |

| Table 1: Effects of phosphorylation of PLN on co-immunoprecipitation of SERCA1a or SERCA2a with PLN |

| A. PLN          | Co-immunoprecipitation relative to phosphorylated wild type PLN and wild type SERCA | Co-immunoprecipitation relative to nonphosphorylated wild type PLN and wild type SERCA | n | ΔK₉ca
|---------------|----------------------------------|----------------------------------|---|------|
| E2A           | 0.38 ± 0.13**                    | 0.55 ± 0.12*                     | 3 | −0.34b |
| K3A           | 0.46 ± 0.09**                    | 1.15 ± 0.26                      | 3 | −0.35b |
| N27A          | 0.69 ± 0.05**                    | 2.26 ± 0.17                      | 4 | −1.06d |
| N30A          | 0.70 ± 0.12*                     | 2.09 ± 0.13                      | 4 | −0.62f |
| L31A          | 0.65 ± 0.08*                     | 0.27 ± 0.12                      | 4 | 0.01e  |
| N34A          | 0.62 ± 0.09*                     | 0.35 ± 0.10                      | 4 | 0.78e  |
| I40A          | 1.76 ± 0.17**                    | 2.45 ± 0.30                      | 4 | 0.44e  |

ΔK₉ca is the Ca²⁺ concentration at which half-maximal Ca²⁺ uptake rates were observed. The ΔK₉ca values on the abscissa are negative relative to the K₉ca for SERCA1a alone, since the apparent affinity of SERCA1a for Ca²⁺ is decreased in the presence of PLN.

a Results abstracted from Ref. 10.
b Results abstracted from Ref. 14.
c Results abstracted from Ref. 12.
d Results abstracted from Ref. 15.
e Results abstracted from Ref. 11.
f Results abstracted from Ref. 10.

A. PLN

|        | Co-immunoprecipitation relative to phosphorylated wild type PLN | Co-immunoprecipitation relative to nonphosphorylated wild type PLN | n | ΔK₉ca
|--------|---------------------------------------------------------------|---------------------------------------------------------------|---|------|
| E2A    | 0.38 ± 0.13**                                                 | 0.55 ± 0.12*                                                 | 3 | −0.34b |
| K3A    | 0.46 ± 0.09**                                                 | 1.15 ± 0.26                                                   | 3 | −0.35b |
| N27A   | 0.69 ± 0.05**                                                 | 2.26 ± 0.17                                                   | 4 | −1.06d |
| N30A   | 0.70 ± 0.12*                                                 | 2.09 ± 0.13                                                   | 4 | −0.62f |
| L31A   | 0.65 ± 0.08*                                                 | 0.27 ± 0.12                                                   | 4 | 0.01e  |
| N34A   | 0.62 ± 0.09*                                                 | 0.35 ± 0.10                                                   | 4 | 0.78e  |
| I40A   | 1.76 ± 0.17**                                                 | 2.45 ± 0.30                                                   | 4 | 0.44e  |

B. SERCA1a

|        | Co-immunoprecipitation relative to phosphorylated WT SERCA1a | Co-immunoprecipitation relative to nonphosphorylated WT SERCA1a | n | ΔK₉ca
|--------|---------------------------------------------------------------|---------------------------------------------------------------|---|------|
| E2A    | 0.38 ± 0.13**                                                 | 0.55 ± 0.12*                                                 | 3 | −0.34b |
| K3A    | 0.46 ± 0.09**                                                 | 1.15 ± 0.26                                                   | 3 | −0.35b |
| N27A   | 0.69 ± 0.05**                                                 | 2.26 ± 0.17                                                   | 4 | −1.06d |
| N30A   | 0.70 ± 0.12*                                                 | 2.09 ± 0.13                                                   | 4 | −0.62f |
| L31A   | 0.65 ± 0.08*                                                 | 0.27 ± 0.12                                                   | 4 | 0.01e  |
| N34A   | 0.62 ± 0.09*                                                 | 0.35 ± 0.10                                                   | 4 | 0.78e  |
| I40A   | 1.76 ± 0.17**                                                 | 2.45 ± 0.30                                                   | 4 | 0.44e  |

C. SERCA2a

|        | Co-immunoprecipitation relative to phosphorylated WT SERCA2a | Co-immunoprecipitation relative to nonphosphorylated WT SERCA2a | n | ΔK₉ca
|--------|---------------------------------------------------------------|---------------------------------------------------------------|---|------|
| E2A    | 0.38 ± 0.13**                                                 | 0.55 ± 0.12*                                                 | 3 | −0.34b |
| K3A    | 0.46 ± 0.09**                                                 | 1.15 ± 0.26                                                   | 3 | −0.35b |
| N27A   | 0.69 ± 0.05**                                                 | 2.26 ± 0.17                                                   | 4 | −1.06d |
| N30A   | 0.70 ± 0.12*                                                 | 2.09 ± 0.13                                                   | 4 | −0.62f |
| L31A   | 0.65 ± 0.08*                                                 | 0.27 ± 0.12                                                   | 4 | 0.01e  |
| N34A   | 0.62 ± 0.09*                                                 | 0.35 ± 0.10                                                   | 4 | 0.78e  |
| I40A   | 1.76 ± 0.17**                                                 | 2.45 ± 0.30                                                   | 4 | 0.44e  |

ΔK₉ca is the Ca²⁺ concentration at which half-maximal Ca²⁺ uptake rates were observed. The ΔK₉ca values on the abscissa are negative relative to the K₉ca for SERCA1a alone, since the apparent affinity of SERCA1a for Ca²⁺ is decreased in the presence of PLN.

a Results abstracted from Ref. 10.
b Results abstracted from Ref. 14.
c Results abstracted from Ref. 12.
d Results abstracted from Ref. 15.
e Results abstracted from Ref. 11.
direct effect on PLN interaction with SERCA. Thus stabilization of the E_2 conformation is the common theme for the stabilization of the interaction of SERCA with PLN by thapsigargin and vanadate. By contrast, elevation of Ca^{2+} to as high as 10 mM (data not shown), driving SERCA into the E_1 conformation, led to a dramatic reduction in the amount of SERCA that was co-immunoprecipitated with PLN.

We also investigated the effects of ATP on physical interactions between PLN and SERCA. Surprisingly, ATP, in the absence of Ca^{2+}, increased the amount of SERCA that was co-immunoprecipitated with dephospho-PLN, suggesting that it caused conformational changes in SERCA that were beneficial to the PLN/SERCA interaction. It is unlikely that these were changes driving the enzyme into the E_1 conformation, because PLN binding is destabilized in the E_1 conformation.

In summary, our findings provide new insights into the complexity of the physical interactions that occur between PLN and SERCA. We have shown that elevated Ca^{2+} is very effective in disrupting PLN/SERCA physical interactions, probably through its effect on the conformation of M6, the major component in PLN/SERCA transmembrane interactions. Phosphorylation disrupts functional interactions more effectively than it disrupts physical interactions, and differences in the effects of phosphorylation on interactions between different mutant proteins might lead to a better understanding of the interaction sites that are affected following phosphorylation. Agents that stabilize the E_2 conformation of SERCA, such as thapsigargin and vanadate, also stabilize physical interactions between PLN and SERCA molecules. ATP, in the absence of Ca^{2+}, also enhances PLN/SERCA interactions, but the mechanism is unknown.

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