Published in final edited form as:

Biochemistry. 2011 November 29; 50(47): 10367–10374. doi:10.1021/bi200759y.

ACTIVATING AND DEACTIVATING ROLES OF LIPID BILAYERS ON THE Ca^{2+} -ATPASE/PHOSPHOLAMBAN COMPLEX

Martin Gustavsson¹, Nathaniel J. Traaseth¹, and Gianluigi Veglia^{1,2,*}

[†]Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN 55455

²Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

Abstract

The physicochemical properties of the lipid bilayer shape the structure and topology of membrane proteins and regulate their biological function. Here, we investigated the functional effects of various lipid bilayer compositions on the sarcoplasmic reticulum (SR) Ca^{2+} -ATPase (SERCA) in the presence and absence of its endogenous regulator, phospholamban (PLN). In the cardiac muscle, SERCA hydrolyzes one ATP molecule to translocate two Ca^{2+} ions into the SR membrane per enzymatic cycle. Unphosphorylated PLN reduces SERCA's affinity for Ca^{2+} and affects the enzymatic turnover. We varied bilayer thickness, head group, fluidity, and found that both the maximal velocity (V_{max}) of the enzyme and its apparent affinity for Ca^{2+} (K_{Ca}) are strongly affected. Our results show that a) SERCA V_{max} has a biphasic dependence on bilayer thickness, reaching its maximum with 22-carbon lipid chain length, b) phosphatidylethanolamine (PE) and phosphatidylserine (PS) decrease K_{Ca} , and c) monounsaturated lipids afford higher SERCA V_{max} and Ca^{2+} -affinity than diunsaturated lipids. The presence of PLN removes the activating effect of PE and shifts SERCA's activity profile, with a maximal activity reached in bilayers with 20-carbon lipid chain length. Our results in synthetic lipid systems compare well with those carried out in native SR lipids. Importantly, we found that specific membrane compositions closely reproduce PLN effects (V_{max} and K_{Ca}) found in living cells, reconciling an ongoing controversy regarding the regulatory role of PLN on SERCA function. Taken with the physiological changes occurring in the SR membrane composition, these studies underscore a possible allosteric role of the lipid bilayers on the SERCA/PLN complex.

Sarcoplasmic reticulum (SR) Ca^{2+} -ATPase (SERCA) is a multi-domain membrane-spanning enzyme that regulates muscle relaxation by Ca^{2+} translocation from the cytosol into the SR lumen (1, 2). SERCA is a P-type ATPase and transports two Ca^{2+} ions per hydrolyzed ATP (3). Phospholamban (PLN) is the main endogenous inhibitor of SERCA activity in cardiomyocytes and its inhibition is relieved upon phosphorylation at Ser 16 or Thr 17 of PLN (4, 5, 6, 7). Since SERCA and PLN are the main regulators of the cardiac output, they are targets for treatment of cardiac disease (8, 9).

SERCA function has been measured in several different systems including mammalian cell lines (10), insect cell microsomes (11), and reconstituted lipid vesicles (12). In a reconstituted system, the bilayer lipid composition can be tightly controlled, making it ideal for studying the effects of lipids on SERCA function. Specifically, the effects of bilayer thickness, head group, and membrane fluidity have all been previously investigated. It was

*CORRESPONDING AUTHOR FOOTNOTE: Prof. Gianluigi Veglia, Department of Biochemistry, Molecular Biology & Biophysics, University of Minnesota – 321 Church Street SE, Minneapolis, MN 55455, Phone: (612) 625 0758, Fax: (612) 626 7541, vegli001@umn.edu.

found that SERCA activity directly depends on bilayer thickness, with a maximum activity found for a lipid chain length of 18 carbons and a gradual decrease in activity for either thicker or thinner bilayers (13, 14). Later data from Cornea and Thomas support these results and, more importantly, showed that the effect of bilayer thickness is correlated to the degree of SERCA oligomerization (15). The biochemical results were supported by molecular dynamics simulations, which show that SERCA can adapt to the thickness of the bilayer through small conformational changes but that this adaptation is not efficient in thin (14-carbon) bilayers (16). Moreover, Squier and co-workers showed that the zwitterionic phosphatidylethanolamine (PE), which represents 16% and 27% of the skeletal and cardiac SR membrane lipid, respectively (17), increases SERCA's maximal activity ($V_{\max}$) (18). A similar activating effect was seen for the anionic lipids phosphatidylserine (PS) and phosphatidylinositol (PI) phosphate which are present at lower concentrations in the SR membrane (19). In addition, SERCA requires fluid, liquid crystalline bilayers to function properly (i.e., more active in 18:1 phosphocholine (PC) than 18:0 PC) (20). All of these SERCA activity studies based their conclusions on the maximum ATPase activity or the activity at a single Ca^{2+} concentration. Also, the functional effects of lipids on the SERCA/PLN complex have not been investigated.

Here, we analyzed the effects of bilayer thickness, head group composition and fluidity for SERCA and the SERCA/PLN complex in reconstituted lipid vesicles, monitoring the ATPase activity using enzyme-coupled assays. We show how apparent Ca^{2+} affinity (K_{Ca}) and $V_{\max}$ are strongly dependent on the nature of the lipid bilayer. Importantly, our data reveal how the lipid bilayer composition influences the regulation of SERCA by PLN. Our conclusions on model membranes are supported by activity measurements in lipid membranes extracted from rabbit skeletal muscle.

EXPERIMENTAL PROCEDURES

All synthetic lipids (Table 1) were purchased from Avanti Polar Lipids (Alabaster, AL). Skeletal SR lipids were extracted using an acidified solution of 2:1 chloroform:methanol (17).

SERCA1a was purified from rabbit skeletal muscle using a reactive red affinity column (21) and was reconstituted for functional assays according to a well established protocol (12, 22, 23, 24, 25, 26, 27, 28). Briefly, the lipids solubilized in chloroform were dried under N_2 (g), desiccated for several hours, and resuspended in a buffer containing 20 mM Imidazole, 0.1 M KCl, 5 mM MgCl_2 , 10% (v/v) Glycerol and octaethylene glycol monododecyl ether (C_{12}E_8) at 4:1 (w/w) C_{12}E_8 :lipid ratio. SERCA (~1 mg/mL in 0.1% w/v C_{12}E_8) was added to the suspension to a final lipid:SERCA (mol/mol) ratio of 700:1. To remove the detergent, the samples were incubated with Biobeads SM2 (75:1 (w/w) Biobeads: C_{12}E_8) for three hours at 30°C (for experiments where the bilayer thickness or lipid chain saturation was varied) or 20°C (for all other experiments). A temperature of 30°C was chosen to ensure that lipids with relatively high gel-liquid crystal transition temperature (T_m) (i.e. 24:1 PC) were in the liquid crystalline phase during the incubation period. A coupled enzyme assay was used to monitor SERCA activity (hydrolysis of ATP) at 37 °C as a function of calcium concentration (12). The rate of enzyme activity was measured as a decrease of NADH absorption at 340 nm using a Spectramax plate reader (Molecular Devices). Data were fit using the Hill equation:

$$V = V_{\max} \frac{[\text{Ca}^{2+}]^n}{[\text{Ca}^{2+}]^n + K_{\text{Ca}}^n}$$

to extract the maximum activity ($V_{\max}$), Hill coefficient (n), and calcium concentration needed to achieve half maximal activity (K_{Ca}).

To monitor the inhibition of SERCA by PLN, we chose a monomeric mutant of PLN (AFA-PLN^{N27A}) that induces a larger shift in SERCA's apparent Ca^{2+} affinity than wild-type PLN (PLN^{wt}). Recombinant AFA-PLN^{N27A} and PLN^{wt} were expressed and purified as described previously (29). The co-reconstitution of SERCA and PLN was identical to the free SERCA samples with the exception that PLN (AFA-PLN^{N27A} or PLN^{wt}) dissolved in a 4:1 (v/v) mixture of trifluoroethanol/chloroform was dried together with the lipids at a molar ratio of 10:1 or 5:1 PLN:SERCA.

RESULTS

Effects of lipid chain structure on SERCA activity

The lipid chain structure is the main determinant of the fluidity as well as the thickness of the lipid bilayer. To evaluate the effects of bilayer thickness on SERCA activity, we conducted ATPase assays in *cis*-mono unsaturated PC bilayers of varied lipid chain length. PC lipids were utilized since they constitute the major component of the SR membrane (17). In our experience, SERCA $V_{\max}$ varies between different rabbits and preparations (2.9 ± 1.4 I.U, average $\pm$ standard deviation for four preparations in 4:1 (w/w) 18:1 Δ^9 -*Cis* PC (DOPC)/18:1 Δ^9 -*Cis* PE (DOPE) bilayers), while K_{Ca} is highly reproducible (0.47 ± 0.01 μ M). Thus, we express SERCA $V_{\max}$ as a relative value to our standard bilayers (DOPC or 4:1 (w/w) DOPC/DOPE) while K_{Ca} is reported as raw values. In Figure 1A and B, we show that SERCA activity is highest in bilayers with a chain length of 22 carbons (22:1 bilayers) with a $V_{\max}$ value ~40-fold higher than in 14:1 bilayers, which had the lowest $V_{\max}$. Previous studies, however, found that 18:1 bilayers support the highest SERCA activity. (13, 15). The discrepancy with our results might be due to the higher Ca^{2+} -concentration used in the previous studies (10-fold excess relative to this study), which relied on measuring SERCA activity at only one Ca^{2+} concentration. Since our activity assays were measured for several Ca^{2+} concentrations (complete Ca^{2+} dependence curves), the fits to the Hill equation provide an accurate estimate of $V_{\max}$. From the fitting of the activity curves, we also measured the SERCA K_{Ca} for each lipid length. Figure 1C and D show that SERCA affinity for Ca^{2+} is biphasic, with the minimum K_{Ca} (highest apparent Ca^{2+} affinity) in 20:1 bilayers. Taken together, these data indicate that SERCA has the highest $V_{\max}$ in relatively thick bilayers (22:1), while bilayers with a thickness similar to the native SR membrane (average chain length of 18.1 carbons in cardiac SR and 17.8 carbons in skeletal SR)(17) give the highest affinity for Ca^{2+} . Therefore, the native SR lipid composition seems tuned to maximize the apparent Ca^{2+} affinity of SERCA.

Bilayer fluidity is another important physical property of biological membranes and SERCA requires a fluid bilayer to function (20). To test the dependence of SERCA on bilayer fluidity, we reconstituted SERCA into 18:1 Δ^9 -*Trans* PC (similar bilayer thickness but less fluid than DOPC as determined by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH)) (14, 30) and 18:2 Δ^9,Δ^{12} -*Cis* PC (similar bilayer thickness but more fluid than DOPC) (14, 30). In 18:1 Δ^9 -*Trans* PC, SERCA $V_{\max}$ is slightly higher than in DOPC, while in 18:2 Δ^9,Δ^{12} -*Cis* PC $V_{\max}$ is significantly lower (Figure 2A). However, in both 18:2 Δ^9,Δ^{12} -*Cis* PC and 18:1 Δ^9 -*Trans* PC bilayers K_{Ca} is significantly higher than in DOPC (Figure 2B). In the cardiac SR, the average number of unsaturated (*cis*) bonds per lipid chain is 1.6 (17), which is intermediate between that of DOPC and 18:2 Δ^9,Δ^{12} -*Cis* PC. Thus, both the thickness and fluidity of the SR bilayer are probably optimized for K_{Ca} rather than $V_{\max}$.

An inherent shortcoming of our studies is that bilayer thickness and fluidity cannot be varied independently (i.e 18:1 Δ^9 -*Trans* PC bilayers are thicker than DOPC bilayers). Therefore it is

important to emphasize that a change in SERCA activity cannot be explained by one single characteristic of the lipid bilayer. Nevertheless, the difference in bilayer thickness between DOPC and 18:1 Δ^9 -Trans PC is only ~ 1 Å (14) compared to the difference between for example 20:1 PC and 18:1 PC (~ 3 Å) (14). Thus, the difference in SERCA $V_{\max}$ and K_{Ca} between 18:1 Δ^9 -Trans PC and DOPC can be mainly attributed to the difference in fluidity between the two bilayers.

Effects of phosphoethanolamine (PE) on the activity of the SERCA/PLN complex

Since the two major components of the native SR membrane are PC and PE lipids (17), 4:1 (w/w) DOPC/DOPE lipid bilayers preparations have been used to study SERCA activity (12, 31). To determine the effects of PE on SERCA function, we carried out functional assays at different molar ratios of DOPC:DOPE. Since pure PE membranes form hexagonal phases and inhibit SERCA activity (32), we limited the PE content to 60% (w/w) of the total lipid bilayer composition to retain the liquid crystalline phase.

When SERCA was reconstituted in the absence of PLN, we found that increased PE content steadily increases $V_{\max}$ at PE ratios greater than 20% (w/w), with no significant effects observed at PE content $< 20\%$ (w/w) (Figure 3A and B), in agreement with previous studies (18, 32). Also, we found that the DOPC:DOPE bilayers have a significantly lower K_{Ca} compared to pure DOPC bilayers (Figure 3D, E). Thus, PE has an activating role on SERCA function, increasing both the apparent Ca^{2+} affinity and $V_{\max}$.

PE is a curvature-forming non-bilayer lipid (33), which can affect membrane protein function by inducing curvature and changing the lateral pressure in the membrane (34, 35). To test if this change in bilayer property could explain the effects of PE on SERCA function we measured SERCA activity in the presence of phosphoserine (PS) lipids, which make up $\sim 10\%$ of the total SR membrane lipid content (17) and does not induce membrane curvature. In this case, we found that the 4:1 (w/w) PC:PS bilayers decreased SERCA $V_{\max}$ relative to both PC and 4:1 (w/w) PC:PE bilayers (Figure 3C). However, K_{Ca} values were slightly lower than those obtained in PC:PE (Figure 3F). The effects on K_{Ca} and $V_{\max}$ are present also at a physiological 9:1 (w/w) PC:PS ratio. These data show that PS increases the apparent Ca^{2+} affinity of SERCA and reveal a possible activating role of PS similar to PE lipids. This also suggests that the activation of SERCA by PE is not due to increased bilayer curvature.

In situ and *in vitro* experiments have shown that SERCA's apparent Ca^{2+} affinity is lowered in the presence of PLN (11, 12, 23). To determine the effects of bilayer composition on SERCA inhibition by PLN, we co-reconstituted a monomeric, superinhibitory mutant of PLN (AFA-PLN^{N27A}) (12, 36) with SERCA into lipid vesicles composed of DOPC or DOPC/DOPE mixtures. We found that AFA-PLN^{N27A}'s effects on SERCA are the same either in the absence or presence of 20% PE (w/w). In both cases AFA-PLN^{N27A} increases both $V_{\max}$ and K_{Ca} (Figure 4). Note that in the presence of AFA-PLN^{N27A}, SERCA activity is insensitive to a change in PE content. Therefore, the presence of PLN removes the activating effects of PE on SERCA Ca^{2+} affinity.

PLN regulation of SERCA and bilayer thickness

To analyze how PLN regulation of SERCA depends on the bilayer thickness, we co-reconstituted SERCA and AFA-PLN^{N27A} into bilayers of varied chain length and with 20% (w/w) DOPE to maximize the effects on SERCA activity. From the data in Figure 5 it is clear that the effects of AFA-PLN^{N27A} on SERCA function are dependent on the bilayer composition. In 18:1 bilayers, AFA-PLN^{N27A} binding increases SERCA $V_{\max}$ and decreases its Ca^{2+} affinity, as previously noted (12, 23). However, in 22:1 bilayers AFA-

PLN^{N27A} does not alter the enzyme's $V_{\max}$ but lowers its Ca^{2+} affinity in a manner that is strikingly similar to the behavior of the SERCA/PLN complex in co-expression systems (11). These results imply that differences in bilayer composition may account for some of the discrepancies seen between *in vitro* and *in vivo* studies. In addition, AFA-PLN^{N27A} shifts the highest $V_{\max}$ of SERCA to thinner bilayers (from 24:1 to 20:1). The slight difference in maximum $V_{\max}$ in the absence of PLN from that measured in pure PC bilayers (Figure 1) is probably attributable to the DOPE (18:1) lipids, which may reduce the thickness of 20:1, 22:1 and 24:1 bilayers. As shown in Figure 4, AFA-PLN^{N27A} removes the activating effects of PE on SERCA. Also, AFA-PLN^{N27A} attenuates the differences in K_{Ca} found for bilayers of different chain length (*i.e.*, 5-fold vs 1.5-fold difference between 20:1 and 24:1 in the absence and presence of AFA-PLN^{N27A}, respectively). Thus, PLN binding alters the effects of head group composition and chain length on SERCA activity.

Lipid effects on the SERCA/PLN complex at physiological protein to lipid ratios

A lipid-to-protein ratio of 700:1 is typically used in the current literature for the ATPase assays (12, 31). To determine if the effects of lipids is preserved under more physiological lipid-to-protein ratios, we measured SERCA activity using 150:1 lipid:SERCA molar ratio. We found that under these conditions both $V_{\max}$ (35% increase) and K_{Ca} ($0.63 \pm 0.02 \mu\text{M}$ vs $0.47 \pm 0.01 \mu\text{M}$) are slightly higher than in 700:1 bilayers. Figure 6A shows that incorporation of 20% (w/w) PE leads to an increase of $V_{\max}$, which is also seen in 700:1 bilayers but at slightly higher PE concentrations. Also, 20% (w/w) PE leads to lower K_{Ca} (Figure 6B) and thus a higher apparent Ca^{2+} affinity in agreement with the data obtained with 700:1 lipid-to-protein ratio (Figure 3). Therefore, the conclusions obtained in bilayers with a 700:1 lipid:SERCA molar ratio are also valid for 150:1 bilayers, which mimic the SR composition.

In the cardiac SR membrane, SERCA is surrounded by a ~5-fold molar excess of PLN (37). Wild type PLN (PLN^{wt}) forms a pentamer (38, 39), but de-oligomerizes into monomers to form a 1:1 complex with SERCA (40, 41). To supplement the results with the superinhibitory, monomeric AFA-PLN^{N27A} mutant we co-reconstituted PLN^{wt} with SERCA at a 5:1 PLN^{wt}:SERCA molar ratio into bilayers containing 150 lipid molecules per SERCA, mimicking the conditions of the native SR membrane. As for the superinhibitory monomeric PLN, addition of PLN^{wt} increases K_{Ca} and raises $V_{\max}$ of SERCA (Figure 6). Importantly, in the presence of PLN^{wt}, PE head groups do not have an effect on the K_{Ca} or $V_{\max}$ of SERCA in analogy to the results with AFA-PLN^{N27A} at a 700:1 lipid:SERCA molar ratio (Figure 4). Thus, we conclude that the effects of lipids are similar for pentameric and monomeric PLN and that our results with AFA-PLN^{N27A} are representative also for other PLN variants.

SERCA function and regulation by PLN in native lipids

Previous studies have shown discrepancies in SERCA function and PLN regulation ($V_{\max}$) between different membrane mimicking systems (11, 12, 23). To confirm that our conclusions in model membranes apply to native SR lipids, we reconstituted SERCA and AFA-PLN^{N27A} into lipids extracted from rabbit hind-leg skeletal muscle. While these extracted lipids cannot fully mimic all properties of the SR membrane (*i.e.*, asymmetric distribution of lipids between bilayer leaflets or presence of lipid rafts) they allowed us to test SERCA activity and PLN regulation in a fully physiological bilayer composition. In SR lipids, SERCA $V_{\max}$ ($V_{\max,\text{SR}}/V_{\max,\text{DOPC}} = 1.3$) and K_{Ca} ($0.81 \mu\text{M}$) are slightly higher than in the 4:1 (w/w) DOPC:DOPE bilayers used to mimic the SR composition (Figure 7). Addition of AFA-PLN^{N27A} leads to an increase in $V_{\max}$ ($V_{\max,+ \text{PLN}}/V_{\max-\text{PLN}} = 2.6$) and a shift in K_{Ca} (to $2.0 \mu\text{M}$). These effects are similar to those obtained in synthetic lipid preparations (Figure 4). Interestingly, addition of AFA-PLN^{N27A} increases the degree of

Ca^{2+} binding cooperativity (Hill coefficient). This effect has been seen in SR vesicles(42) and reconstituted lipid bilayers consisting of a mixture of natural egg yolk PC and PA (phosphatidic acid) lipids(23) but was not detected in synthetic model membranes (Figure 4). Nevertheless, the differences between the native lipids and the DOPC:DOPE bilayers are relatively minor considering the sensitivity of SERCA to the bilayer thickness and the nature of the lipid head group.

DISCUSSION

The importance of lipid bilayer composition for protein function is well established for SERCA (43, 44) as well as for other membrane bound enzymes (45, 46). In the present study, we show that SERCA $V_{\max}$ and K_{Ca} are significantly affected by the lipid bilayer composition. Thus, lipids with different chain length and head groups have activating or inhibitory effects on the enzyme. Since PE and PS lipids increase the apparent Ca^{2+} affinity of SERCA (Figure 3), we believe these to be *activating* with respect to PC lipids. This activation could be a result either of specific protein-lipid interactions (47) or non-specific effects on bilayer properties (48). Previously, it has been shown that addition of PE to PC bilayers does not alter lipid chain dynamics or the overall SERCA rotational correlation time (18), and that change in lipid head group composition has marginal effect on bilayer thickness (49). Thus, the SERCA activation by PE head groups is likely due to specific protein-lipid interactions rather than macroscopic changes of the lipid bilayer properties. A possible explanation for such interactions is that in contrast to PC, PE and PS can function as hydrogen bond donors and potentially form hydrogen bonds with sites on SERCA in agreement with other membrane proteins (47). In fact, lipid molecules have been detected in SERCA crystal structures (3) with the bound lipids modeled as PE and located in the groove between helices M2, M4 and M6. These lipids could possibly be responsible for the activating effects of PE on SERCA. Interestingly, this lipid binding site overlaps with the proposed binding site for PLN (50). Here, we showed that in the presence of PLN, SERCA activity is independent of PE (Figure 4). Therefore, the inhibitory PLN and the activating PE lipids might compete for the same binding site, which could explain part of the inhibitory effect that PLN has on SERCA.

It is widely accepted that PLN inhibits SERCA by lowering its apparent Ca^{2+} affinity. However, contrasting studies have reported that PLN increases (12, 23) or does not affect $V_{\max}$ (11). The increase of $V_{\max}$ has mainly been observed in reconstituted systems, but vanishes at high lipid:SERCA ratios (12). Based on our results in model membranes, PLN increases (16:1, 18:1, 20:1), does not change (22:1), or decreases (24:1) the enzyme's $V_{\max}$ in bilayers of different thickness (Figure 5). In 22:1 bilayers AFA-PLN^{N27A} lowers the Ca^{2+} affinity of SERCA without affecting $V_{\max}$. The similarity of these data with those from co-expression systems (11) raises the question whether SERCA and PLN preferentially localize to thicker regions of the bilayer *in vivo*. Another possibility is that PLN changes the thickness of the lipid bilayers, affecting $V_{\max}$. In the absence of PLN (and in the presence of 20% (w/w) DOPE), SERCA reaches its maximum activity in 24:1 lipid bilayers (Figure 5A). In contrast, when co-reconstituted with AFA-PLN^{N27A}, SERCA reaches its maximum activity in 20:1 lipid bilayers (Figure 5A). This result may be rationalized by a thickening of the bilayer caused by PLN. Under diluted conditions (high lipid:AFA-PLN^{N27A} molar ratios), the effects of AFA-PLN^{N27A} on $V_{\max}$ vanishes (12). It is possible that under these conditions AFA-PLN^{N27A} concentration is too low to affect the thickness of the bilayer, thus losing its ability to increase $V_{\max}$. In fact, depleting membrane proteins from bilayers can change the thickness by $\sim 5 \text{ \AA}$ (51), which corresponds to a difference in chain length of ~ 3 carbons. In the native SR membrane, the lipid:SERCA molar ratio is $\sim 100:1$ (52), and thus significantly lower than the 700:1 ratio used in our measurements. Even if additional proteins, lipids, and other biomolecules are present *in vivo*, PLN-induced changes to the

lipid bilayer could be important for regulation of SERCA in the muscle cell. Previous studies have shown that the effect of bilayer thickness on SERCA $V_{\max}$ is correlated to reversible aggregation (15). In light of those results, SERCA aggregation is another possible mechanism for regulation of the enzyme by PLN. Also, PLN is in conformational equilibria between different conformational states (31, 53, 54), which are sensitive to the composition of the lipid bilayer (55, 56). Furthermore, negatively charged lipids influence the ability of a soluble peptide corresponding to residues 1–23 of PLN to regulate SERCA activity (57). Further studies are needed to determine if a shift in the conformational equilibrium of PLN is responsible for the effects of lipid composition on the SERCA/PLN complex.

Cardiac and skeletal SR membranes have an average lipid chain length of ~18, and an average of 1.6 unsaturated bonds per lipid chain. They are mainly composed of lipids with PC head groups (~53% (cardiac), ~68% (skeletal)) with smaller amounts of PE (~27%, ~16%), PS (~10%, 10%), and PI (~1%, ~2%) lipids (17). Our data show that in bilayers with this chain length (Figure 1), fluidity (Figure 2) and head group content (Figure 3) SERCA's apparent apparent Ca^{2+} affinity is optimal in while $V_{\max}$ is higher in thicker, less fluid bilayers. Thus, the SR membrane appears to be tuned to maximize the apparent Ca^{2+} affinity of SERCA. Our data also show that a PE concentration 20% (w/w) raises SERCA $V_{\max}$ and K_{Ca} is significantly lowered at PE concentrations of less than 20% (Figure 3). Therefore, at physiological PE concentrations, small changes in the PE head group content of the SR could significantly alter intracellular calcium cycling. The same is true for changes in bilayer thickness or lipid saturation.

Changes to the bilayer composition in mice have been shown to occur with muscular dystrophy (58) and aging (59). In addition, diet can also alter the distribution of lipids, as shown in another mouse study where increased levels of unsaturated lipids occurred from *exclusive* fish intake. These mice had lower Ca^{2+} uptake into the SR (60). In agreement with this finding, our activity assays using model membranes show that SERCA $V_{\max}$ is significantly lower in polyunsaturated 18:2 Δ^9, Δ^{12} -Cis PC than in monounsaturated DOPC (Figure 2). This implies that differences in SERCA function between bilayers with different lipid chain structure could be explained by differences in lipid bilayer thickness. Also, a recent study showed that obesity-induced ER stress can significantly raise the PC:PE ratio of the ER (61). Interestingly, this change in head group composition was correlated to a decreased SERCA activity which is in excellent agreement with our *in vitro* studies.

In the cardiac SR, PLN is present at a five-fold excess molar concentration with respect to SERCA (37). Our data show that SERCA activity is significantly less sensitive to alterations of the lipid thickness of the PC:PE bilayer in the presence of AFA-PLN^{N27A}. Thus, PLN may have a *buffering effect* for SERCA in response to any changes in membrane structure due to pathology or diet.

In summary, we have shown that SERCA activity and its regulation by PLN are sensitive to bilayer thickness, fluidity and head group content with maximum activity in bilayers that are slightly thicker than the native SR membrane. SERCA's apparent Ca^{2+} affinity, however, is optimized in bilayers mimicking the native SR membrane. The presence of PLN decreases the calcium affinity, removes the activating effects of PE and attenuates the effects on SERCA activity in lipids of varying chain length. These new findings for Ca^{2+} translocation underline the importance of lipids for SERCA function and its regulation by PLN and could reconcile discrepancies between reconstituted and co-expression systems.

Acknowledgments

The authors thank Kim Ha, Raffaello Verardi and Rahel Woldeyes for excellent technical assistance and Razvan Cornea for critically reading the manuscript.

This work was supported by grants to GV from the National Institutes of Health (GM64742) and a pre-doctoral fellowship to MG from the American Heart Association (10PRE3860050)

ABBREVIATIONS

SERCA	Sarcoplasmic reticulum Ca ²⁺ -ATPase
PLN	Phospholamban
PE	Phosphatidylethanolamine
PC	Phosphatidylcholine
PS	Phosphoserine
C₁₂E₈	octaethylene glycol monododecyl ether

References

1. Bers DM. Cardiac Excitation-Contraction Coupling. *Nature*. 2002; 415:198–205. [PubMed: 11805843]
2. Brini M, Carafoli E. Calcium Pumps in Health and Disease. *Physiol. Rev.* 2009; 89:1341–1378. [PubMed: 19789383]
3. Obara K, Miyashita N, Xu C, Toyoshima I, Sugita Y, Inesi G, Toyoshima C. Structural Role of Countertransport Revealed in Ca(2+) Pump Crystal Structure in the Absence of Ca(2+). *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:14489–14496. [PubMed: 16150713]
4. Simmerman HK, Collins JH, Theibert JL, Wegener AD, Jones LR. Sequence Analysis of Phospholamban. Identification of Phosphorylation Sites and Two Major Structural Domains. *J. Biol. Chem.* 1986; 261:13333–13341. [PubMed: 3759968]
5. Wegener AD, Simmerman HK, Lindemann JP, Jones LR. Phospholamban Phosphorylation in Intact Ventricles. Phosphorylation of Serine 16 and Threonine 17 in Response to Beta-Adrenergic Stimulation. *J. Biol. Chem.* 1989; 264:11468–11474. [PubMed: 2544595]
6. MacLennan DH, Kranias EG. Phospholamban: A Crucial Regulator of Cardiac Contractility. *Nat. Rev. Mol. Cell Biol.* 2003; 4:566–577. [PubMed: 12838339]
7. Traaseth NJ, Ha KN, Verardi R, Shi L, Buffy JJ, Masterson LR, Veglia G. Structural and Dynamic Basis of Phospholamban and Sarcolipin Inhibition of Ca(2+)-ATPase. *Biochemistry*. 2008; 47:3–13. [PubMed: 18081313]
8. Kaye DM, Hoshijima M, Chien KR. Reversing Advanced Heart Failure by Targeting Ca²⁺ Cycling. *Annu. Rev. Med.* 2008; 59:13–28. [PubMed: 18186701]
9. Inesi G, Prasad AM, Pilankatta R. The Ca²⁺ ATPase of Cardiac Sarcoplasmic Reticulum: Physiological Role and Relevance to Diseases. *Biochem. Biophys. Res. Commun.* 2008; 369:182–187. [PubMed: 18068669]
10. Vandecaetsbeek I, Trekels M, De Maeyer M, Ceulemans H, Lescrinier E, Raeymaekers L, Wuytack F, Vangheluwe P. Structural Basis for the High Ca²⁺ Affinity of the Ubiquitous SERCA2b Ca²⁺ Pump. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:18533–18538. [PubMed: 19846779]
11. Mahaney JE, Autry JM, Jones LR. Kinetics Studies of the Cardiac Ca-ATPase Expressed in Sf21 Cells: New Insights on Ca-ATPase Regulation by Phospholamban. *Biophys. J.* 2000; 78:1306–1323. [PubMed: 10692318]
12. Reddy LG, Cornea RL, Winters DL, McKenna E, Thomas DD. Defining the Molecular Components of Calcium Transport Regulation in a Reconstituted Membrane System. *Biochemistry*. 2003; 42:4585–4592. [PubMed: 12693956]
13. Froud RJ, Earl CR, East JM, Lee AG. Effects of Lipid Fatty Acyl Chain Structure on the Activity of the (Ca²⁺ + Mg²⁺)-ATPase. *Biochim. Biophys. Acta.* 1986; 860:354–360. [PubMed: 2943317]
14. Caffrey M, Feigenson GW. Fluorescence Quenching in Model Membranes. 3. Relationship between Calcium Adenosinetriphosphatase Enzyme Activity and the Affinity of the Protein for

- Phosphatidylcholines with Different Acyl Chain Characteristics. *Biochemistry*. 1981; 20:1949–1961. [PubMed: 6452902]
15. Cornea RL, Thomas DD. Effects of Membrane Thickness on the Molecular Dynamics and Enzymatic Activity of Reconstituted Ca-ATPase. *Biochemistry*. 1994; 33:2912–2920. [PubMed: 8130205]
 16. Sonntag Y, Musgaard M, Olesen C, Schiott B, Moller JV, Nissen P, Thogersen L. Mutual Adaptation of a Membrane Protein and its Lipid Bilayer during Conformational Changes. *Nat. Commun.* 2011; 2:304. [PubMed: 21556058]
 17. Bick RJ, Buja LM, Van Winkle WB, Taffet GE. Membrane Asymmetry in Isolated Canine Cardiac Sarcoplasmic Reticulum: Comparison with Skeletal Muscle Sarcoplasmic Reticulum. *J. Membr. Biol.* 1998; 164:169–175. [PubMed: 9662560]
 18. Hunter GW, Negash S, Squier TC. Phosphatidylethanolamine Modulates Ca-ATPase Function and Dynamics. *Biochemistry*. 1999; 38:1356–1364. [PubMed: 9930998]
 19. Szymanska G, Kim HW, Kranias EG. Reconstitution of the Skeletal Sarcoplasmic Reticulum Ca²⁺(+)-Pump: Influence of Negatively Charged Phospholipids. *Biochim. Biophys. Acta.* 1991; 1091:127–134. [PubMed: 1825285]
 20. Lee AG. Lipid-Protein Interactions in Biological Membranes: A Structural Perspective. *Biochim. Biophys. Acta.* 2003; 1612:1–40. [PubMed: 12729927]
 21. Stokes DL, Green NM. Three-Dimensional Crystals of CaATPase from Sarcoplasmic Reticulum. Symmetry and Molecular Packing. *Biophys. J.* 1990; 57:1–14. [PubMed: 2137017]
 22. Afara MR, Trieber CA, Ceholski DK, Young HS. Peptide Inhibitors use Two Related Mechanisms to Alter the Apparent Calcium Affinity of the Sarcoplasmic Reticulum Calcium Pump. *Biochemistry*. 2008; 47:9522–9530. [PubMed: 18702513]
 23. Trieber CA, Afara M, Young HS. Effects of Phospholamban Transmembrane Mutants on the Calcium Affinity, Maximal Activity, and Cooperativity of the Sarcoplasmic Reticulum Calcium Pump. *Biochemistry*. 2009; 48:9287–9296. [PubMed: 19708671]
 24. Trieber CA, Douglas JL, Afara M, Young HS. The Effects of Mutation on the Regulatory Properties of Phospholamban in Co-Reconstituted Membranes. *Biochemistry*. 2005; 44:3289–3297. [PubMed: 15736939]
 25. Levy D, Gulik A, Bluzat A, Rigaud JL. Reconstitution of the Sarcoplasmic Reticulum Ca²⁺(+)-ATPase: Mechanisms of Membrane Protein Insertion into Liposomes during Reconstitution Procedures Involving the use of Detergents. *Biochim. Biophys. Acta.* 1992; 1107:283–298. [PubMed: 1387003]
 26. Young HS, Reddy LG, Jones LR, Stokes DL. Co-Reconstitution and Co-Crystallization of Phospholamban and Ca²⁺(+)-ATPase. *Ann. NY. Acad. Sci.* 1998; 853:103–115. [PubMed: 10603940]
 27. Reddy LG, Jones LR, Cala SE, O'Brian JJ, Tatulian SA, Stokes DL. Functional Reconstitution of Recombinant Phospholamban with Rabbit Skeletal Ca²⁺(+)-ATPase. *J. Biol. Chem.* 1995; 270:9390–9397. [PubMed: 7721863]
 28. Young HS, Rigaud JL, Lacapere JJ, Reddy LG, Stokes DL. How to make Tubular Crystals by Reconstitution of Detergent-Solubilized Ca²⁺(+)-ATPase. *Biophys. J.* 1997; 72:2545–2558. [PubMed: 9168030]
 29. Buck B, Zamoon J, Kirby TL, DeSilva TM, Karim C, Thomas D, Veglia G. Overexpression, Purification, and Characterization of Recombinant Ca-ATPase Regulators for High-Resolution Solution and Solid-State NMR Studies. *Protein Expr. Purif.* 2003; 30:253–261. [PubMed: 12880775]
 30. Roach C, Feller SE, Ward JA, Shaikh SR, Zerouga M, Stillwell W. Comparison of Cis and Trans Fatty Acid Containing Phosphatidylcholines on Membrane Properties. *Biochemistry*. 2004; 43:6344–6351. [PubMed: 15147219]
 31. Ha KN, Traaseth NJ, Verardi R, Zamoon J, Cembran A, Karim CB, Thomas DD, Veglia G. Controlling the Inhibition of the Sarcoplasmic Ca²⁺(+)-ATPase by Tuning Phospholamban Structural Dynamics. *J. Biol. Chem.* 2007; 282:37205–37214. [PubMed: 17908690]

32. Starling AP, Dalton KA, East JM, Oliver S, Lee AG. Effects of Phosphatidylethanolamines on the Activity of the Ca(2+)-ATPase of Sarcoplasmic Reticulum. *Biochem. J.* 1996; 320(Pt 1):309–314. [PubMed: 8947502]
33. Dowhan, W.; Bogdanov, M.; Mileyskovskaya, E. Functional Roles of Lipids in Membranes. In: Vance, DE.; Vance, JE., editors. *Biochemistry of Lipids, Lipoproteins and Membranes*. 5th ed.. Elsevier B.E; 2008. p. 11-37.
34. Moe P, Blount P. Assessment of Potential Stimuli for Mechano-Dependent Gating of MscL: Effects of Pressure, Tension, and Lipid Headgroups. *Biochemistry*. 2005; 44:12239–12244. [PubMed: 16142922]
35. Scarlata S, Gruner SM. Role of Phosphatidylethanolamine Lipids in the Stabilization of Protein-Lipid Contacts. *Biophys. Chem.* 1997; 67:269–279. [PubMed: 9397529]
36. Kimura Y, Asahi M, Kurzydowski K, Tada M, MacLennan DH. Phospholamban Domain Ib Mutations Influence Functional Interactions with the Ca²⁺-ATPase Isoform of Cardiac Sarcoplasmic Reticulum. *J. Biol. Chem.* 1998; 273:14238–14241. [PubMed: 9603928]
37. Ferrington DA, Yao Q, Squier TC, Bigelow DJ. Comparable Levels of Ca-ATPase Inhibition by Phospholamban in Slow-Twitch Skeletal and Cardiac Sarcoplasmic Reticulum. *Biochemistry*. 2002; 41:13289–13296. [PubMed: 12403631]
38. Cornea RL, Jones LR, Autry JM, Thomas DD. Mutation and Phosphorylation Change the Oligomeric Structure of Phospholamban in Lipid Bilayers. *Biochemistry*. 1997; 36:2960–2967. [PubMed: 9062126]
39. Verardi R, Shi L, Traaseth NJ, Walsh N, Veglia G. Structural Topology of Phospholamban Pentamer in Lipid Bilayers by a Hybrid Solution and Solid-State NMR Method. *Proc. Natl. Acad. Sci. U.S.A.* 2011; 108:9101–9106. [PubMed: 21576492]
40. Robia SL, Campbell KS, Kelly EM, Hou Z, Winters DL, Thomas DD. Forster Transfer Recovery Reveals that Phospholamban Exchanges Slowly from Pentamers but Rapidly from the SERCA Regulatory Complex. *Circ. Res.* 2007; 101:1123–1129. [PubMed: 17975108]
41. Kimura Y, Kurzydowski K, Tada M, MacLennan DH. Phospholamban Inhibitory Function is Activated by Depolymerization. *J. Biol. Chem.* 1997; 272:15061–15064. [PubMed: 9182523]
42. Hicks MJ, Shigekawa M, Katz AM. Mechanism by which Cyclic Adenosine 3':5'-Monophosphate-Dependent Protein Kinase Stimulates Calcium Transport in Cardiac Sarcoplasmic Reticulum. *Circ. Res.* 1979; 44:384–391. [PubMed: 216505]
43. Lee AG, East JM. The Effects of Phospholipid Structure on the Function of a Calcium Pump. *Biochem. Soc. Trans.* 1998; 26:359–365. [PubMed: 9765879]
44. Lee AG. How Lipids Interact with an Intrinsic Membrane Protein: The Case of the Calcium Pump. *Biochim. Biophys. Acta.* 1998; 1376:381–390. [PubMed: 9804995]
45. Lee AG. How Lipids Affect the Activities of Integral Membrane Proteins. *Biochim. Biophys. Acta.* 2004; 1666:62–87. [PubMed: 15519309]
46. Farias RN, Bloj B, Morero RD, Sineriz F, Trucco RE. Regulation of Allosteric Membrane-Bound Enzymes through Changes in Membrane Lipid Composition. *Biochim. Biophys. Acta.* 1975; 415:231–251. [PubMed: 167865]
47. Hakizimana P, Masureel M, Gbaguidi B, Ruysschaert JM, Govaerts C. Interactions between Phosphatidylethanolamine Headgroup and LmrP, a Multidrug Transporter: A Conserved Mechanism for Proton Gradient Sensing? *J. Biol. Chem.* 2008; 283:9369–9376. [PubMed: 18234676]
48. Bogdanov M, Heacock P, Guan Z, Dowhan W. Plasticity of Lipid-Protein Interactions in the Function and Topogenesis of the Membrane Protein Lactose Permease from *Escherichia Coli*. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:15057–15062. [PubMed: 20696931]
49. Rand RP, Fuller N, Parsegian VA, Rau DC. Variation in Hydration Forces between Neutral Phospholipid Bilayers: Evidence for Hydration Attraction. *Biochemistry*. 1988; 27:7711–7722. [PubMed: 3207702]
50. Toyoshima C, Asahi M, Sugita Y, Khanna R, Tsuda T, MacLennan DH. Modeling of the Inhibitory Interaction of Phospholamban with the Ca²⁺ ATPase. *Proc. Natl. Acad. Sci. U.S.A.* 2003; 100:467–472. [PubMed: 12525698]

51. Mitra K, Ubarretxena-Belandia I, Taguchi T, Warren G, Engelman DM. Modulation of the Bilayer Thickness of Exocytic Pathway Membranes by Membrane Proteins rather than Cholesterol. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:4083–4088. [PubMed: 15016920]
52. Tada M, Yamamoto T, Tonomura Y. Molecular Mechanism of Active Calcium Transport by Sarcoplasmic Reticulum. *Physiol. Rev.* 1978; 58:1–79. [PubMed: 23557]
53. Gustavsson M, Traaseth NJ, Karim CB, Lockamy EL, Thomas DD, Veglia G. Lipid-Mediated Folding/Unfolding of Phospholamban as a Regulatory Mechanism for the Sarcoplasmic Reticulum Ca(2+)-ATPase. *J. Mol. Biol.* 2011
54. Karim CB, Zhang Z, Howard EC, Torgersen KD, Thomas DD. Phosphorylation-Dependent Conformational Switch in Spin-Labeled Phospholamban Bound to SERCA. *J. Mol. Biol.* 2006; 358:1032–1040. [PubMed: 16574147]
55. Gustavsson M, Traaseth NJ, Veglia G. Probing Ground and Excited States of Phospholamban in Model and Native Lipid Membranes by Magic Angle Spinning NMR Spectroscopy. *Biochim. Biophys. Acta.* 2011
56. Masterson LR, Yu T, Shi L, Wang Y, Gustavsson M, Mueller MM, Veglia G. CAMP-Dependent Protein Kinase A Selects the Excited State of the Membrane Substrate Phospholamban. *J. Mol. Biol.* 2011
57. Hughes E, Clayton JC, Middleton DA. Cytoplasmic Residues of Phospholamban Interact with Membrane Surfaces in the Presence of SERCA: A New Role for Phospholipids in the Regulation of Cardiac Calcium Cycling? *Biochim. Biophys. Acta.* 2009; 1788:559–566. [PubMed: 19059204]
58. Mrak RE, Fleischer S. Lipid Composition of Sarcoplasmic Reticulum from Mice with Muscular Dystrophy. *Muscle Nerve.* 1982; 5:439–446. [PubMed: 7133039]
59. Krainev AG, Ferrington DA, Williams TD, Squier TC, Bigelow DJ. Adaptive Changes in Lipid Composition of Skeletal Sarcoplasmic Reticulum Membranes Associated with Aging. *Biochim. Biophys. Acta.* 1995; 1235:406–418. [PubMed: 7756351]
60. Taffet GE, Pham TT, Bick DL, Entman ML, Pownall HJ, Bick RJ. The Calcium Uptake of the Rat Heart Sarcoplasmic Reticulum is Altered by Dietary Lipid. *J. Membr. Biol.* 1993; 131:35–42. [PubMed: 8381872]
61. Fu S, Yang L, Li P, Hofmann O, Dicker L, Hide W, Lin X, Watkins SM, Ivanov AR, Hotamisligil GS. Aberrant Lipid Metabolism Disrupts Calcium Homeostasis Causing Liver Endoplasmic Reticulum Stress in Obesity. *Nature.* 2011; 473:528–531. [PubMed: 21532591]

FIGURE 1.

SERCA activity in bilayers of varying thickness. A, C.) Representative curves of raw and normalized ATPase activity as a function of calcium concentration in 14:1 Δ^9 -Cis, 16:1 Δ^9 -Cis, DOPC, 20:1 Δ^{11} -Cis, 22:1 Δ^{13} -Cis and 24:1 Δ^{15} -Cis PC bilayers. Each point represents the average and standard error from three measurements. B.) $V_{\max}$ of SERCA relative to DOPC ($V_{\max}/V_{\max, \text{DOPC}}$) as a function of bilayer chain length. Error bars represent the standard error from at least three separate reconstitutions. D.) K_{Ca} in bilayers of varying thickness. Each bar was determined from the average of 3 reconstitutions and error bars show the standard error of the mean.

FIGURE 2.

SERCA activity in C18 bilayers with different fluidity. A, C.) Raw and normalized ATPase activity in PC bilayers with differences in geometry around the unsaturated bond (18:1 Δ^9 -trans PC vs DOPC (18:1 Δ^9 -cis PC)) but identical length of the aliphatic chains. B, D.) ATPase activity in PC bilayers with differences in chain saturation (DOPC vs 18:2 $\Delta^9,12$ -cis PC) but identical length of the aliphatic chains. Error bars correspond to the standard error of three measurements. Averaged K_{Ca} and relative V_{max} are shown as insets, with the error bars representing the standard error of three or more separate reconstitutions.

FIGURE 3.

Effects of PE and PS head groups on SERCA activity. A,D.) Representative curves of raw and normalized SERCA activity in bilayers composed of DOPC and different mole fractions of 18:1 DOPE as indicated in figure. Error bars represent an average of triplicate measurements and are for some points smaller than the symbol. B.) $V_{\max}$ of SERCA in bilayer with varied amount of PE head group. $V_{\max}$ values are shown relative to the $V_{\max}$ in 0% PE (DOPC). C.) $V_{\max}$ relative to $V_{\max,DOPC}$ in 4:1 (w/w) DOPC:DOPE and 4:1 or 9:1 (w/w) DOPC:DOPS. E.) K_{Ca} of SERCA in different amounts of PE. ΔK_{Ca} corresponds to $K_{Ca,X\% PE} - K_{Ca,0\% PE}$ where X is the mole fraction of PE in the bilayer. F.) K_{Ca} in DOPC, 4:1 (w/w) DOPC:DOPE and 4:1 or 9:1 (w/w) DOPC:DOPS. Error bars in B,C,E and F represent the standard error of three or more separate reconstitutions. For points where error bars are not visible they are smaller than the symbol.

FIGURE 4.

Inhibition of SERCA by AFA-PLN^{N27A} in bilayers with different head group composition. A,C.) Representative curves of raw and normalized SERCA activity in DOPC and 4:1 (w/w) DOPC:DOPE in the absence and presence of a 10-fold molar excess of AFA-PLN^{N27A}. Each point shows the average and standard error of three measurements, for some points the error bar is smaller than the symbol. B.) Average $V_{\max}$ in DOPC and 4:1 (w/w) DOPC:DOPE in the presence and absence of AFA-PLN^{N27A}. Values are reported relative to the value in DOPC. D.) Averaged K_{Ca} values. Error bars in B.) and D.) represent the standard error of three or more separate reconstitutions.

FIGURE 5.

Effects of AFA-PLN^{N27A} on SERCA $V_{\max}$ and K_{Ca} in bilayers of varying thickness composed of 4:1 (w/w) 14:1 – 20:1 Δ^9 -Cis PC:DOPE. A, B.) $V_{\max}$ values relative to 4:1 (w/w) DOPC:DOPE in the absence and presence of AFA-PLN^{N27A}. C.) SERCA $V_{\max}$ change caused by AFA-PLN^{N27A} measured as $V_{\max,+AFA-PLN^{N27A}} / V_{\max,-AFA-PLN^{N27A}}$. D, E.) K_{Ca} values in the absence and presence of AFA-PLN^{N27A}. F.) K_{Ca} change due caused by AFA-PLN^{N27A} and measured as $\Delta K_{Ca} = K_{Ca,+AFA-PLN^{N27A}} - K_{Ca,-AFA-PLN^{N27A}}$. The error bars represent standard error for 3 separate reconstitutions.

FIGURE 6.

Lipid effects on SERCA and PLN^{wt} at a physiological lipid concentration. SERCA activity in samples reconstituted with a lipid:PLN^{wt}:SERCA molar ratio of 150:5:1. A.) Average $V_{\max}$ in the presence and absence of PLN^{wt} in DOPC and 4:1 (w/w) DOPC:DOPE. $V_{\max}$ is relative to the value in DOPC. B.) Averaged K_{Ca} values. All error bars represent the standard error of three separate reconstitutions.

FIGURE 7.

SERCA and PLN in native lipids. SERCA ATPase activity and AFA-PLN^{N27A} regulation with proteins reconstituted into lipids extracted from rabbit skeletal muscle SR. A.) Raw activity. B.) Normalized values. Error bars display the standard error of three measurements and are smaller than the symbol for several of the data points.

TABLE 1

Synthetic lipids used for reconstitutions.

Lipid	Chemical name
14:1Δ^9-Cis PC	1,2-di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine
16:1Δ^9-Cis PC	1,2-di-(9Z-hexadecenoyl)-sn-glycero-3-phosphocholine
18:1Δ^9-Cis PC (DOPC)	1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine
18:1Δ^9-Trans PC	1,2-di-(9E-octadecenoyl)-sn-glycero-3-phosphocholine
18:2Δ^9,Δ^{12}-Cis PC	1,2-di-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphocholine
20:1Δ^{11}-Cis PC	1,2-di-(11Z-eicosenoyl)-sn-glycero-3-phosphocholine
22:1Δ^{13}-Cis PC	1,2-di-(13Z-docosenoyl)-sn-glycero-3-phosphocholine
24:1Δ^{15}-Cis PC	1,2-di-(15Z-tetracosenoyl)-sn-glycero-3-phosphocholine
18:1Δ^9-Cis PE (DOPE)	1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine
18:1Δ^9-Cis PS (DOPS)	1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phospho-L-serine