How phospholamban could affect the apparent affinity of Ca^{2+}-ATPase for Ca^{2+} in kinetic experiments

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Abstract Binding of phospholamban (PLN) to the Ca^{2+}-ATPase of muscle sarcoplasmic reticulum results in a decrease in apparent affinity for Ca^{2+} without affecting the true binding constant for Ca^{2+} determined in equilibrium binding experiments. It is shown that this can be explained by a scheme in which the ATPase shows two modes of binding for PLN, one of high and one of low affinity; the proposed scheme is not dependent on the kinetic model assumed for the Ca^{2+}-ATPase.

Key words: Phospholamban; Ca^{2+}-ATPase; Calcium binding; Sarcoplasmic reticulum; Kinetic simulation

1. Introduction

The activity of the Ca^{2+}-ATPase in cardiac muscle sarcoplasmic reticulum is regulated by interaction with a 52-residue integral membrane protein, phospholamban (PLN) [1]. Interaction of PLN with the Ca^{2+}-ATPase results in a decrease in the apparent affinity of the Ca^{2+}-ATPase for Ca^{2+}, determined from plots of the rates of hydrolysis of ATP or accumulation of Ca^{2+} as a function of Ca^{2+} concentration [1]. This decrease in apparent affinity for Ca^{2+} results in a decrease in activity for the Ca^{2+}-ATPase in the physiologically relevant Ca^{2+} concentration range from 100 nM to 1 μM. The effect of PLN is reversed by phosphorylation during β-adrenergic stimulation, resulting in increased Ca^{2+}-ATPase activity, enhancing cardiac muscle relaxation rates and contractility [1].

PLN consists of a hydrophilic N-terminal domain linked to a hydrophobic C-terminal domain forming a transmembrane α-helix. PLN forms pentamers in the membrane but the monomeric form is the active form [2–5]. Although not expressed in skeletal muscle sarcoplasmic reticulum, PLN has the same effect on the apparent affinity for Ca^{2+} of the fast-twitch Ca^{2+}-ATPase isoform (SERCA1) as on the cardiac muscle isoform (SERCA2a) in coexpression or reconstitution experiments [3,6–9]. The transmembrane domain of PLN, PLN(25–52), is sufficient to cause the shift in apparent affinity for Ca^{2+} of the Ca^{2+}-ATPase [3,7,10]. The crystal structure of SERCA1 in its Ca^{2+}-free form shows the presence of a cleft formed by transmembrane α-helices M2, M4, M6 and M9 into which the transmembrane α-helix of PLN can be modelled, with the N-terminal domain interacting with the nucleotide binding domain of SERCA1 [11], consistent with mutagenesis experiments [11,12]. The cleft is large and so does not form a tight binding site for PLN [13]. The cleft is closed in the structure of the Ca^{2+}-bound form of SERCA1 [14]. The transmembrane α-helix of PLN has therefore been suggested to interact with the surface of the bundle of transmembrane α-helices in the Ca^{2+}-bound form of the Ca^{2+}-ATPase, again with the N-terminal domain of PLN interacting with the nucleotide binding domain of Ca^{2+}-ATPase [11].

Surprisingly, despite the effect of PLN on the apparent affinity for Ca^{2+} measured in kinetic experiments, binding of PLN to Ca^{2+}-ATPase results in no change in the affinity for Ca^{2+} measured in equilibrium experiments [3,15]. Here it is shown that all the known experimental facts about the effect of PLN can be accounted for if PLN has two different modes of binding to the Ca^{2+}-ATPase.

2. Materials and methods

Kinetics of the Ca^{2+}-ATPase were simulated using FACSIMILE (UES Software); FACSIMILE is a program for solving sets of differential equations. The mechanism of the Ca^{2+}-ATPase is usually described in terms of the E1–E2 scheme developed by de Meis and Vianna [16] and shown in modified form in Fig. 1A. The Ca^{2+}-ATPase transports two Ca^{2+} ions for each ATP molecule hydrolysed. Binding of Ca^{2+} from the cytoplasmic side of the membrane to the E1 conformation gives $E1Ca_2$ that can then bind ATP and be phosphorylated to $E2PCa_2$. $E2PCa_2$ can release Ca^{2+} on the luminal side of the membrane to give E2P. Hydrolysis of E2P then regenerates E2. The scheme also includes a conformational change between binding the first and second Ca^{2+} ions (step 3) and a conformational change following binding of ATP and before phosphorylation (step 6) [17,18]. Rate constants describing the E2–E1 step, Ca^{2+} binding to the ATPase (steps 1–4), binding of ATP and phosphorylation of the ATPase (steps 5–8) and dissociation of Ca^{2+} from the phosphorylated protein and dephosphorylation (steps 9–11) were set at the values used in previous simulations (Table 1) [17,19].

Because of the complexity of the full reaction scheme for the Ca^{2+}-ATPase it is convenient initially to describe the effects of PLN on the apparent Ca^{2+} affinity of the Ca^{2+}-ATPase using the highly simplified scheme shown in Fig. 1B. This represents binding of the two Ca^{2+} ions to the Ca^{2+}-ATPase as a single step, followed by a single irreversible step representing the binding of ATP and its hydrolysis to ADP. The effect of PLN on the Ca^{2+}-ATPase was then simulated in terms of the full reaction scheme shown in Fig. 1A.

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Abbreviations: PLN, phospholamban; PLN(25–52), the transmembrane domain of PLN; PLN^{Cys-}, PLN with the three Cys residues replaced by Ala

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3. Results and discussion

Any kinetic model for the effect of PLN on the Ca^{2+}-ATPase has to be able to explain the following observations:

1. Binding of PLN affects the apparent affinity for Ca^{2+} measured from the concentration dependence of the effect of Ca^{2+} on the rate of ATP hydrolysis without affecting the true binding constants for Ca^{2+} [3,15].
2. Binding of PLN reduces the rate of phosphorylation of the Ca^{2+}-ATPase when the Ca^{2+}-free enzyme is mixed simultaneously with ATP and low concentrations of Ca^{2+} [15].
3. Binding of PLN does not affect the rate of dissociation of Ca^{2+} from the Ca^{2+}-bound form $E1Ca_2$ [3].
4. Binding of PLN does not affect the equilibrium between E1 and E2 [3].
5. The effect of PLN on apparent Ca^{2+} affinity saturates at high molar ratios of PLN to Ca^{2+}-ATPase [3,20].

Observations 1 and 5 mean that binding of PLN and Ca^{2+} to the Ca^{2+}-ATPase is not simple competitive binding. Any model in which PLN binds to the Ca^{2+}-free form of the ATPase but not to the Ca^{2+}-bound form must result in a decrease in the affinity for Ca^{2+} measured in an equilibrium binding experiment, and since this is not seen experimentally [3,15] PLN must bind with comparable affinity to both Ca^{2+}-free and Ca^{2+}-bound forms of the Ca^{2+}-ATPase. In contrast, Asahi et al. [23] have shown that, when solubilised in Tween-20, Ca^{2+}-ATPase and PLN are co-immunoprecipitated by an antibody against PLN in the absence of Ca^{2+} but not in the presence of Ca^{2+}. This implies that, in micelles of Tween-20, PLN and Ca^{2+} are in competition for binding to the Ca^{2+}-ATPase, a result very different from that seen for PLN and Ca^{2+}-ATPase in a lipid bilayer environment.

A decrease in the rate constant for one of the Ca^{2+} binding steps for the PLN-bound form of the ATPase could result in a decrease in the apparent affinity for Ca^{2+}, and if there was an equivalent decrease in the rate constant describing Ca^{2+} dissociation this would not result in any decrease in true affinity for Ca^{2+}. Thus Cantilina et al. [15] have shown that the relatively small shifts in pCa value caused by PLN in native cardiac sarcoplasmic reticulum can be simulated assuming 10-fold decreases in the forward and backward rate constants for the conformation change between binding the first and second Ca^{2+} ions (step 3 in Fig. 1A). However, to simulate the larger shifts in pCa value seen at higher molar ratios of PLN to Ca^{2+}-ATPase [3,20] requires larger decreases in the forward and backward rate constants for step 3 which then become partly rate-limiting, slowing the overall rate of ATP hydrolysis at saturating concentrations of Ca^{2+}, as shown in Fig. 2, and this is not observed experimentally [3]. Further, any significant decrease in the backward rate constant for step 3 results in a decrease in the rate of dissociation of Ca^{2+} from the Ca^{2+}-bound ATPase, as shown in Fig. 2, and this also is not seen experimentally [3]. A shift in E1–E2 equilibrium (step 1 in Fig. 1A) towards E2 on binding PLN would result in a decrease in apparent affinity for Ca^{2+} but would also result in a decrease in the true affinity for Ca^{2+} and a decrease in the equilibrium constant E1/E2, neither of which are observed [3]. Equal decreases in the forward and backward rate constants for step 1 simply result in a decrease in the rate of ATP hydrolysis with no effect on the apparent affinity for Ca^{2+} as shown in Fig. 2.

Observations 1–5 are therefore not compatible with a single mode of binding of PLN to the Ca^{2+}-ATPase but are compatible with a scheme of the type shown in Fig. 1B with two modes of binding. In the scheme PLN can bind with high affinity to the Ca^{2+}-ATPase to give EN_H and can also bind with lower affinity to give a second form EN_L where N represents bound PLN. The binding constant for the step giving EN_H was chosen so that the effect of PLN increases with increasing molar ratio of PLN/ATPase but has saturated by a molar ratio of 3:1 [20], as described in more detail below. For simplicity, rate constants were put high for the PLN binding steps so that only the equilibrium constants for these steps affect the kinetics; the rate constant for PLN binding to give

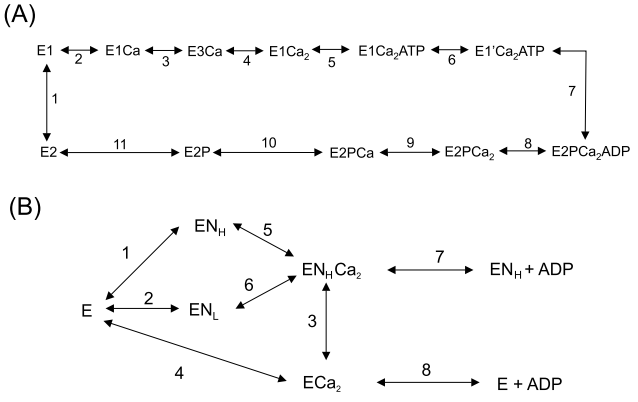

Fig. 1. Reaction scheme for the Ca^{2+}-ATPase. A: The modified E1–E2 model for the Ca^{2+}-ATPase. Following binding of the first Ca^{2+} ion from the cytoplasmic side of the sarcoplasmic reticulum membrane to the E1 conformation of the Ca^{2+}-ATPase (step 2) a conformation change to the E3Ca conformation (step 3) allows the binding of a second Ca^{2+} ion (step 4) to give $E1Ca_2$. Binding of ATP (step 5) is then followed by a conformation change (step 6) before the phosphorylation step (step 7), forming the intermediate $E2PCa_2ADP$, from which ADP then dissociates (step 8) [17,18]. Loss of Ca^{2+} to the luminal side of the membrane (steps 9 and 10) followed by dephosphorylation (step 11) gives E2 that can then recycle to E1 (step 1). B: A highly simplified reaction scheme for the Ca^{2+}-ATPase. Binding of PLN with high or low affinity gives EN_H and EN_L respectively.

Table 1
Rate constants used to simulate the Ca^{2+}-ATPase and the effect of PLN using the schemes shown in Figs. 1A and 4

Step	Forward rate constant	Reverse rate constant	Equilibrium constant
1	15.4 s^{-1}	57.6 s^{-1}	0.267
2	5.57×10^{7} M^{-1} s^{-1}	35.7 s^{-1}	1.56×10^{6} M^{-1}
3	162 s^{-1}	162 s^{-1}	1.0
4	1.99×10^{9} M^{-1} s^{-1}	31.1 s^{-1}	6.4×10^{7} M^{-1}
5	2×10^{6} M^{-1} s^{-1}	20 s^{-1}	1.0×10^{5} M^{-1}
6	220 s^{-1}	100 s^{-1}	2.2
7	5.0×10^{3} s^{-1}	5.0×10^{3} s^{-1}	1.0
8	1.3×10^{3} s^{-1}	1.73×10^{6} M^{-1} s^{-1}	7.5×10^{-4} M
9	60 s^{-1}	1.8×10^{4} M^{-1} s^{-1}	3.3×10^{-3} M
10	30 s^{-1}	9.0×10^{3} M^{-1} s^{-1}	3.3×10^{-3} M
11	21 s^{-1}	–	–
12	7.0×10^{9} M^{-1} s^{-1}	1.0×10^{3} s^{-1}	7.0×10^{6} M^{-1}
13	5.07×10^{6} M^{-1} s^{-1}	3.25 s^{-1}	1.56×10^{6} M^{-1}
14	7.0×10^{6} M^{-1} s^{-1}	1.0×10^{3} s^{-1}	7.0×10^{3} M^{-1}
15	5.57×10^{10} M^{-1} s^{-1}	35.7 s^{-1}	1.56×10^{9} M^{-1}

For simplicity, the rate constants for the PLN binding and dissociation steps (steps 12 and 14) have been set high so that only the equilibrium constants for these steps affect the kinetics of the Ca^{2+}-ATPase, although the rate constant for forward step 14 must be less than that for forward step 12, as described in the text.

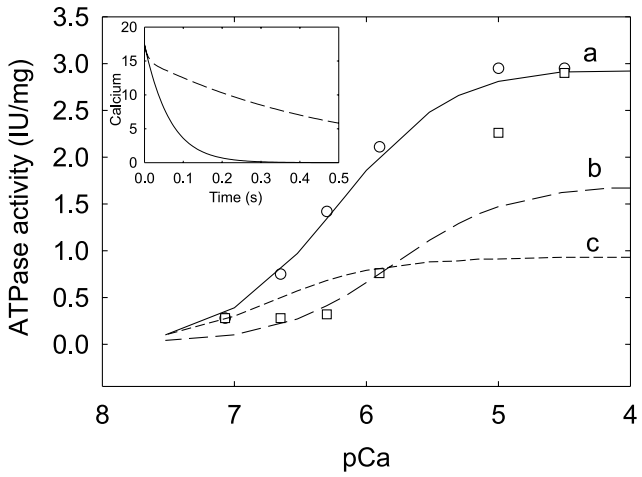

Fig. 2. The effects of changes in the rates of the E1Ca–E3Ca step (step 3 in Fig. 1A) and E2–E1 step (step 1 in Fig. 1A) on the Ca^{2+} dependence of ATPase activity. The experimental data from Hughes et al. [3] show the activity of the Ca^{2+}-ATPase in the absence (○) or presence (□) of PLN(25–52) at a molar ratio of PLN(25–52): phospholipid:ATPase: of 100:2000:1. The solid line (a) shows a simulation of the data in the absence of PLN(25–52) using the parameters in Table 1. The broken line (b) shows the effect of reducing both the forward and reverse rate constants for step 3 in Fig. 1A ($E1Ca \leftrightarrows E3Ca$) by a factor of 22. The dotted line (c) shows the effect of reducing both the forward and reverse rate constants for step 1 in Fig. 1A ($E2 \leftrightarrows E1$) by a factor of 7. The decreases in rates for steps 3 and 1 in simulations b and c respectively were chosen to match the experimentally determined rate in the presence of PLN(25–52) at pCa = 5.9. The inset shows the time dependence of Ca^{2+} dissociation from $E1Ca_2$ using the parameters in Table 1 (solid line) and with both the forward and reverse rate constants for step 3 reduced by a factor of 22 (broken line); the units for bound Ca^{2+} are nmoles Ca^{2+}/mg protein.

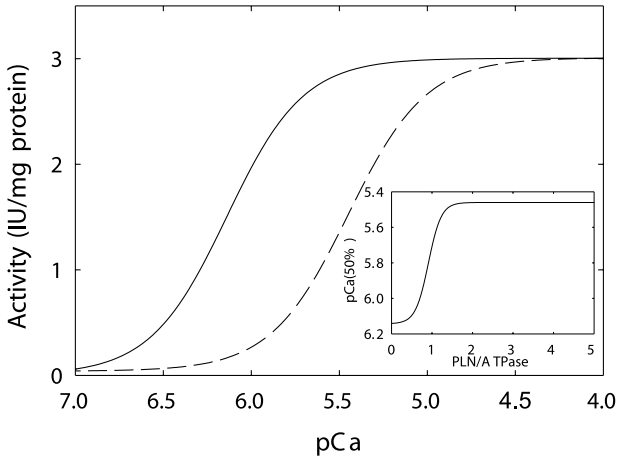

Fig. 3. The effect of PLN on the Ca^{2+} dependence of ATPase activity, simulated in terms of the scheme shown in Fig. 1B, with the parameters listed in Table 2. Solid line, no PLN; broken line, 3 μM PLN, with an ATPase concentration of 1 μM. The inset shows a plot of pCa value giving 50% maximal ATPase activity, as a function of the molar ratio of PLN/ATPase, with an ATPase concentration of 1 μM.

EN_L (step 2) was set lower than that giving EN_H (step 1) to ensure that binding of PLN followed pathway 1 to EN_H rather than pathway 2 to EN_L (Table 2). Binding of two Ca^{2+} ions to E was described by a single rate constant, chosen to give 50% binding of Ca^{2+} at a Ca^{2+} concentration of 0.7 μM with a rate for dissociation of Ca^{2+} from the Ca^{2+}-bound form of 14 s^{-1}, as determined experimentally [3]. The equilibrium constant for binding of Ca^{2+} to EN_H was put equal to that for E so that the presence of PLN would not affect the true binding constant for Ca^{2+}, but the rate of Ca^{2+} binding to EN_H was set slower than that to E, resulting in a decrease in the apparent affinity for Ca^{2+} derived from plots of ATPase activity against Ca^{2+} concentration. A slow rate of Ca^{2+} binding to EN_H with no change in equilibrium constant results in a slow rate for the step $EN_HCa_2 \rightarrow EN_H + 2\ Ca^{2+}$ (step 5) but the rate of the step $EN_HCa_2 \rightarrow EN_L + 2\ Ca^{2+}$ (step 6) was set equal to that of the step $ECa_2 \rightarrow E + 2\ Ca^{2+}$ (step 4) so that the presence of PLN would have no effect on the net rate of Ca^{2+} dissociation from EN_HCa_2. The rate constant for production of ADP was chosen to match the observed rate of ATP hydrolysis by the Ca^{2+}-ATPase, which is typically 3 μmoles ADP/mg protein/min under standard assay conditions at 25°C [3]; it was assumed that binding of PLN did not affect the rate constant for this step.

The results of a simulation using the parameters in Table 2 are shown in Fig. 3. With the forward rate constant for step 5 set at 4.4×10^{11} M^{-2} s^{-1} the concentration of Ca^{2+} giving 50% maximal activity increases from a pCa value of 6.14 in the absence of PLN to a maximum of 5.46 in the presence of PLN, in agreement with experimental data [3,20]. The pCa value giving 50% binding of Ca^{2+} is, using the parameters in Table 2, 6.24 and is unaffected by the presence of PLN and the presence of PLN does not affect the rate of dissociation of Ca^{2+} from the Ca^{2+}-bound ATPase.

This same model for the effect of PLN was then applied to a full reaction scheme for the Ca^{2+}-ATPase (Fig. 4), allowing

Table 2
Rate constants used to simulate the effect of PLN using the scheme shown in Fig. 1B

Step	Forward rate constant	Backward rate constant	Equilibrium constant
1	6×10^{10} M^{-1} s^{-1}	100 s^{-1}	6×10^{8} M^{-1}
2	6×10^{7} M^{-1} s^{-1}	100 s^{-1}	6×10^{5} M^{-1}
3	6×10^{10} M^{-1} s^{-1}	100 s^{-1}	6×10^{8} M^{-1}
4	4×10^{13} M^{-2} s^{-1}	14 s^{-1}	2.9×10^{12} M^{-2}
5	4.4×10^{11} M^{-2} s^{-1}	0.15 s^{-1}	2.9×10^{12} M^{-2}
6	4.1×10^{16} M^{-2} s^{-1}	14 s^{-1}	2.9×10^{15} M^{-2}
7	5.75 s^{-1}	–	–
8	5.75 s^{-1}	–	–

For simplicity, the rate constants for the PLN binding and dissociation steps (steps 1, 2 and 3) have been set high so that only the equilibrium constants for these steps affect the kinetics of the Ca^{2+}-ATPase, although the rate constant for forward step 2 must be less than that for forward step 1, as described in the text. EN_H and EN_L are assumed to be in fast equilibrium; the direct conversion $EN_H \leftrightarrows EN_L$ has not been included in the simulation because the rapid rates assumed for binding and dissociation of PLN ensure rapid equilibrium between EN_H and EN_L by the pathway $EN_H \leftrightarrows E \leftrightarrows EN_L$.

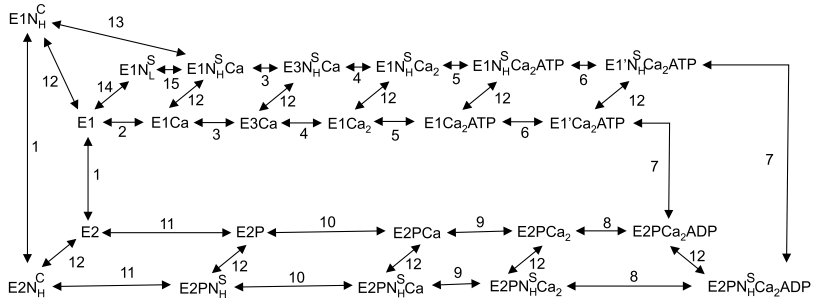

Fig. 4. A full reaction scheme for the effect of PLN on Ca^{2+}-ATPase. Steps 1–11 represent the normal reaction scheme for the ATPase in the absence of PLN. The E1 conformation can bind PLN in the cleft formed by transmembrane α-helices M2, M4, M6 and M9 with high affinity to give $E1N_H^C$ or can bind PLN on the surface with low affinity to give $E1N_L^S$. The E2 conformation can bind PLN with high affinity in the cleft to give $E2N_H^C$ and all other states of the ATPase can bind PLN with high affinity on the surface to give the corresponding N_H^S state.

a direct fit to experimental data on the effects of PLN. Following Fig. 1B, the scheme proposes that PLN can bind in two ways to E1, with different affinities. In terms of the model of Toyoshima et al. [11] the high affinity state ($E1N_H^C$) could correspond to PLN bound within the cleft on the Ca^{2+}-ATPase and the low affinity state ($E1N_L^S$) could correspond to PLN bound to the surface of the transmembrane α-helical bundle. Similarly, PLN can bind with high affinity within the cleft in E2 to give $E2N_H^C$; PLN could also bind with low affinity to E2 on the surface giving $E2N_L^S$ but formation of such a state would have no effect on the ATPase and so has not been included in the scheme shown in Fig. 4. In the presence of Ca^{2+} the surface bound state becomes a state of high affinity for PLN ($E1N_H^SCa$, etc.), the change in affinity following from the rearrangement of the transmembrane α-helices in the Ca^{2+}-ATPase on binding Ca^{2+}, which forces PLN out of the cleft and could make surface binding more favourable [11,13]. For simplicity, it was assumed that all other forms of the ATPase bind PLN with high affinity. In the scheme presented in Fig. 4 conversion from surface bound to groove-bound PLN occurs at the time of dephosphorylation ($E2PN_H^S \rightarrow E2N_H^C$) but could occur at any other step following binding of Ca^{2+} and before formation of E2, depending on whether or not PLN is assumed to affect the v_{max} value for the Ca^{2+}-ATPase, a point of some controversy [1,3,22].

The binding constant for the step $E1 \rightarrow E1N_L^S$ (step 14) was set at a factor of 1000 less than that for the step $E1 \rightarrow E1N_H^C$ (step 12) so that the PLN-bound forms of the Ca^{2+}-ATPase will be predominantly a mixture of $E1N_H^C$ and $E2N_H^C$ and PLN will have a negligible effect on the E1/E2 equilibrium, in agreement with experiment [3]. The equilibrium constants for Ca^{2+} binding to $E1N_H^C$ and E1 were set equal, but the rate constant for binding of Ca^{2+} to $E1N_H^C$ to give $E1N_H^SCa$ was set less than that for binding of Ca^{2+} to E1, explaining the decrease in apparent affinity for Ca^{2+} in the presence of PLN, with no effect on the true binding constant for Ca^{2+}. This step could be slow because the step involves the movement of PLN from the cleft to the surface of the transmembrane α-helical bundle. The rate constants for the Ca^{2+} dissociation steps between $E1N_H^SCa_2$ and $E1N_L^S$ are the same as those for the corresponding steps between $E1Ca_2$ and E1 (steps 4 to 2) so that the rate of dissociation of Ca^{2+} from the Ca^{2+}-bound ATPase is unaffected by the presence of PLN. The binding constant for PLN was chosen to fit the data on the apparent Ca^{2+} affinity as a function of PLN concentration (see below). The forward rate constant for the step $E1N_L^S \rightarrow E1N_H^SCa$ was calculated from the other rate constants, as required by microscopic reversibility. The maximum shift in pCa value giving 50% maximal activity calculated using the parameters in Table 1 is 0.8, in good agreement with the experimental data of Brittsan et al. [20] for transgenic cardiac muscle (Fig. 5A) and the calculated shift in pCa value as a function of PLN concentration also agrees well with experiment (Fig. 5A). Comparison with data for reconstituted systems has to ac-

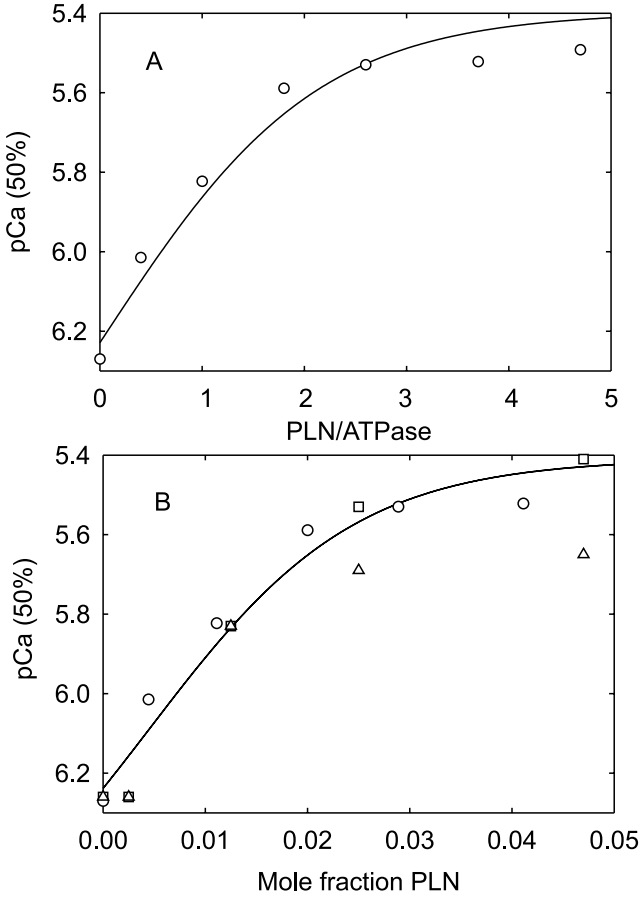

Fig. 5. pCa value giving 50% maximal ATPase activity as a function of PLN concentration, simulated using the reaction scheme shown in Fig. 4, with the parameters listed in Table 1. The pCa value giving 50% maximal ATPase activity is plotted against the molar ratio of PLN/ATPase (A) and against the concentration of PLN expressed as mole fraction in the membrane, calculated as described in the text (B). ○, data of Brittsan et al. [20] with pCa values shifted by −0.73; □, data of Hughes et al. [3] for reconstitution with PLN(25–52); △, data of Hughes et al. [3] for reconstitution with PLN^{Cys-}.

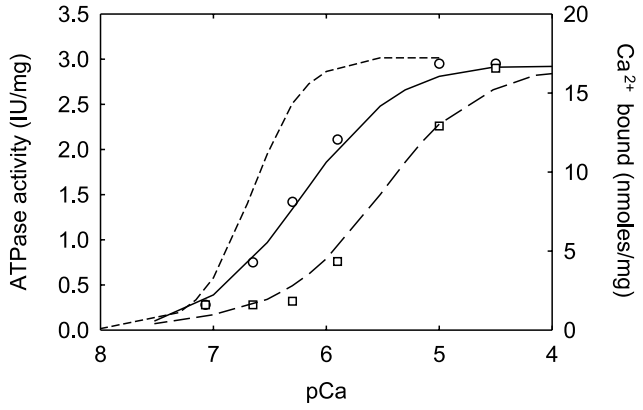

Fig. 6. The effect of PLN on the Ca^{2+} dependence of ATPase activity, simulated using the reaction scheme shown in Fig. 4, with the parameters listed in Table 1. Solid line, no PLN(25–52); broken line, 2.3 μM PLN(25–52), with an ATPase concentration of 1 μM. ○, experimental data from Hughes et al. [3] for Ca^{2+}-ATPase in the absence of PLN(25–52) and □, reconstituted with PLN(25–52) at a molar ratio of PLN(25–52):phospholipid:ATPase: of 100:2000:1, equivalent to a mole fraction of correctly oriented PLN(25–52) in the membrane of 0.025. The concentrations of 2.3 μM PLN and 1 μM ATPase used in the simulation correspond to a mole fraction of 0.025 PLN in the membrane, assuming a molar ratio of lipid:ATPase of 90:1. Also shown is the calculated equilibrium binding curve for Ca^{2+} (dotted line); binding curves in the absence and presence of PLN are identical.

count for the high lipid concentrations used in the reconstitution experiments, since the effect of PLN on the Ca^{2+}-ATPase decreases with increasing lipid content [3]. It is therefore convenient to express the concentration of PLN in terms of mole fraction within the membrane. In the experiments of Hughes et al. [3] the molar ratio of lipid:ATPase was 2000:1 and, since the reconstitution process results in a random distribution of Ca^{2+}-ATPase and PLN molecules between the two sides of the membrane, only half the PLN molecules to which a Ca^{2+}-ATPase molecule is exposed will be in the correct orientation. Plots of the pCa value giving 50% activity for either PLN(25–52) or the Cys-free mutant PLN^{Cys-} as a function of the mole fraction of correctly oriented PLN molecules are very similar to those calculated from the data of Brittsan et al. [20] assuming a molar ratio of phospholipid to Ca^{2+}-ATPase in sarcoplasmic reticulum of 90:1 [21], and are in good agreement with the simulations (Fig. 5B). Simulations of ATPase activity as a function of Ca^{2+} concentration also agree well with the experimental data for PLN(25–52) (Fig. 6). As shown in Fig. 6, the presence of PLN has no effect on the calculated equilibrium binding of Ca^{2+} to the ATPase. The pCa value giving 50% binding of Ca^{2+} in equilibrium experiments is 0.36 greater than that required for 50% maximal activity in the absence of PLN, in good agreement with the shift of c. 0.33 observed experimentally by Cantilina et al. [15]. Finally, slow binding of Ca^{2+} in the presence of PLN results in a slow rate of phosphorylation when the Ca^{2+}-free enzyme is mixed simultaneously with ATP and low concentrations of Ca^{2+}, as observed experimentally by Cantilina et al. [15].

The model proposed here is therefore capable of explaining the experimental data for the effect of PLN on the apparent affinity of the Ca^{2+}-ATPase for Ca^{2+}. In structural terms, the model simply proposes that PLN can bind to the ATPase in the absence of Ca^{2+} in one of two ways, with high affinity binding in a cleft on the ATPase formed by transmembrane α-helices M2, M4, M6 and M9, as modelled by Toyoshima et al. [11], or with low affinity on the surface. Binding of Ca^{2+} to the Ca^{2+}-ATPase closes the cleft with PLN now binding to the surface of the rearranged Ca^{2+}-ATPase with high affinity. The rate of Ca^{2+} binding to the Ca^{2+}-ATPase when PLN is bound in the cleft will be slow because of the requirement to displace the bound PLN to the surface.

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