The Presence of Sarcolipin Results in Increased Heat Production by Ca\(^{2+}\) -ATPase*

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Skeletal muscle sarcomplasmic reticulum of large mammals such as rabbit contains sarcolipin (SLN), a small peptide with a single transmembrane α-helix. When reconstituted with the Ca\(^{2+}\) -ATPase from skeletal muscle sarcomplasmic reticulum into sealed vesicles, the presence of SLN leads to a reduced level of accumulation of Ca\(^{2+}\). Heats of reaction of the reconstituted Ca\(^{2+}\) -ATPase with ATP were measured using isothermal calorimetry. The heat released increased linearly with time over 30 min and increased with increasing SLN content. Rates of ATP hydrolysis by the reconstituted Ca\(^{2+}\) -ATPase were constant over a 30-min time period and were the same when measured in the presence or absence of an ATP-regenerating system. The calculated values of heat released per mol of ATP hydrolyzed increased with increasing SLN content and fitted to a simple binding equation with a dissociation constant for the SLN-ATPase complex of 6.9 × 10\(^{-4}\) ± 2.9 × 10\(^{-4}\) in units of mol fraction per monolayer. It is suggested that the interaction between Ca\(^{2+}\) -ATPase and SLN in the sarcoplasmic reticulum could be important in thermogenesis by the sarcoplasmic reticulum.

The Ca\(^{2+}\) -ATPase of skeletal muscle sarcoplasmic reticulum (SR)

2 plays an important role in muscle contraction, pumping Ca\(^{2+}\) from the cytoplasm of the muscle cell into the lumen of the SR, leading to muscle relaxation and re-filling of the SR Ca\(^{2+}\) store (1). However, this is not the only important physiological role of the Ca\(^{2+}\) -ATPase since the principle source of heat during nonshivering thermogenesis in animals lacking brown adipose tissue is muscle, heat being produced by the hydrolysis of ATP by the SR Ca\(^{2+}\) -ATPase (2). de Meis et al. (3–6) has shown that the proportions of ATP used by the Ca\(^{2+}\) -ATPase for transport and for heat generation varies with conditions and that changes in work/heat output by the Ca\(^{2+}\) -ATPase are related to changes in the reaction cycle of the Ca\(^{2+}\) -ATPase.

The reaction cycle of the Ca\(^{2+}\) -ATPase is shown in simplified form in Scheme 1 (7). Transport of Ca\(^{2+}\) into the SR lumen involves binding of ATP and two Ca\(^{2+}\) ions from the cytoplasm to the E1 conformation of the Ca\(^{2+}\) -ATPase followed by phosphorylation of the Ca\(^{2+}\) -ATPase on Asp-351 (steps 1–3 in Scheme 1). On phosphorylation of the Ca\(^{2+}\) -ATPase, the two Ca\(^{2+}\) binding sites change to a state in which they are of low affinity and facing the lumen, so that Ca\(^{2+}\) dissociates from the phosphorylated Ca\(^{2+}\) -ATPase into the SR lumen (step 4). Dephosphorylation of the Ca\(^{2+}\) -ATPase then allows recycling to E1 (steps 5–7).

A number of processes compete with the transport process to reduce the net level of accumulation of Ca\(^{2+}\) below two per ATP molecule hydrolyzed. At high luminal and low cytoplasmic concentrations of Ca\(^{2+}\), when the concentration of ADP is high, the reaction cycle can be driven backwards, ADP binding to the phosphorylated ATPase, reforming ATP at the expense of moving two Ca\(^{2+}\) ions back across the membrane (steps 4 to 2) (4). The rate of this process is low when the concentration of ADP is low and when the concentration of Ca\(^{2+}\) in the cytoplasm is high (4). Two other processes can also reduce the efficiency of transport. The first is passive leak of Ca\(^{2+}\) out of the SR lumen down its concentration gradient (steps 8–10). Passive leak of Ca\(^{2+}\) has been observed from Ca\(^{2+}\)-loaded SR vesicles (8) and from vesicles reconstituted from purified Ca\(^{2+}\) -ATPase (9, 10), but the rate of this passive leak is slow when the cytoplasmic concentration of Ca\(^{2+}\) is sufficient to saturate the cytoplasmic binding sites for Ca\(^{2+}\) on the ATPase (10, 11). The second process is slippage, in which the phosphorylated, Ca\(^{2+}\)-bound intermediate (E2PCa\(_{a}\), in Scheme 1) releases its two Ca\(^{2+}\) ions to the cytoplasmic side of the membrane rather than to the luminal side (step 11) (8, 12, 13). Because during slippage there is no transport of Ca\(^{2+}\), all the energy derived from ATP hydrolysis will be converted into heat. Conditions favoring maximum heat production by the Ca\(^{2+}\) -ATPase, therefore, correspond to those that favor slippage (5).

The SR of fast- and slow-twitch skeletal muscles of large mammals such as rabbit contain a 31-residue transmembrane peptide sarcolipin (SLN), which is absent from atrial muscle (14). In contrast, in small mammals such as rat, SLN is present in the atria but absent from skeletal muscles (14). In reconstitution experiments with Ca\(^{2+}\) -ATPase from rabbit skeletal fast-twitch muscle, the presence of SLN led to a reduced level of accumulation of Ca\(^{2+}\) (7), and expression of SLN in rat slow-twitch muscle, a muscle that normally lacks SLN, also led to a decreased level of accumulation of Ca\(^{2+}\) by the SR (15). The effect of SLN on Ca\(^{2+}\) accumulation in reconstituted vesicles was consistent with interaction between SLN and the Ca\(^{2+}\) -ATPase, leading to an increased rate of slippage on the Ca\(^{2+}\) -
ATPase (7). This suggests that the presence of SLN might increase heat production by the Ca\(^{2+}\)-ATPase; here we show that this is indeed the case.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidic acid (DOPA) were obtained from Avanti Polar Lipids, and \(\beta\)-d-octyl glucoside and octa(ethylene glycol)-\(n\)-dodecyl ether (C\(_{12}\)E\(_8\)) were obtained from Sigma and CalBiochem, respectively. SR was prepared from rabbit skeletal muscle as described in Dalton et al. (13). Concentrations of ATPase were estimated using a specific absorption coefficient of 1.2 liters\(^{-1}\) cm\(^{-1}\) for a solution in 1% (v/v) SDS. Rabbit muscle SLN (MERSTRELCLNFTVVLITVILIWLLVRSYQY) was supplied by Peptide Protein Research Ltd. (Wickham, Hants, UK).

**Reconstitution into Sealed Vesicles**—SLN was dissolved in trifluoroethanol, and lipids were dissolved in chloroform. The solutions were mixed in the desired proportion, dried, and resuspended in buffer A (10 mM Pipes, 100 mM K\(_2\)SO\(_4\), pH 7.1) containing 40 mM \(\beta\)-d-octyl glucoside to give a final lipid concentration of 7.5 mM. The sample was then sonicated to optical clarity in a bath sonicator. SR was solubilized in buffer A containing C\(_{12}\)E\(_8\) (6 mg/ml) and 0.1 mM CaCl\(_2\). The solubilized SR was mixed with the lipid sample to give a 5000:1 molar ratio of lipid:ATPase. Detergent was removed by the addition of 80 mg of Bio-Beads (mesh size 20–50) followed after 1 h by a second addition of 80 mg of Bio-Beads. After a further hour the sample of reconstituted vesicles was removed from the Bio-Beads and kept on ice until use.

**Assay of Ca\(^{2+}\) Uptake**—Accumulation of Ca\(^{2+}\) by the reconstituted vesicles was measured at 35 °C using Antipyrylazo III to monitor the external Ca\(^{2+}\) concentration (13). The absorption difference 720–790 nm was recorded using an SLM Aminco dual wavelength spectrophotometer. The assay buffer used was 10 mM Pipes, 100 mM K\(_2\)SO\(_4\), 5 mM MgSO\(_4\), pH 7.1, containing 70 \(\mu\)M Antipyrylazo III with a protein concentration of 0.026 mg/ml. Carbonyl cyanide \(-\)trifluoromethoxyphenylhydrazone (FCCP) was added to a concentration of 0.25 \(\mu\)M to make the vesicles permeable to \(H^+\). The system was calibrated by the incremental addition of Ca\(^{2+}\) before the addition of ATP to initiate uptake.

**Assays of ATPase Activity**—ATPase activities were determined at 35 °C over a 2-min period using a coupled enzyme assay in a medium containing 40 mM Hepes, pH 7.2, 100 mM KCl, 5 mM MgSO\(_4\), 2.1 mM ATP, 1.1 mM EGTA, 0.41 mM phosphoenolpyruvate, 0.15 mM NADH, 7.5 units of pyruvate kinase, and 18 units of lactate dehydrogenase with 10 \(\mu\)g of ATPase. The reaction was initiated by the addition of an aliquot of a 25 mM CaCl\(_2\) solution to a cuvette containing the ATPase and the other reagents to give the required concentration of free Ca\(^{2+}\), typically 80 \(\mu\)M. The same coupled enzyme assay system was used to measure activities over a 30-min time period but with 2 \(\mu\)g of ATPase.

ATPase activities were also measured using a phosphate release assay using the Biomol green phosphate assay reagent from BioMol Research labs. Activities were measured in medium containing 40 mM Hepes, pH 7.2, 100 mM KCl, 5 mM MgSO\(_4\), 2.1 mM ATP, 1 mM EGTA, and 0.86 mM Ca\(^{2+}\), corresponding to a concentration of free Ca\(^{2+}\) of 80 \(\mu\)M. Assays were started by the addition of 5 \(\mu\)g of protein in a total volume of 2.5 ml. At the chosen time intervals, 20-\(\mu\)l samples were taken, added to 100 \(\mu\)l of 100 mM EGTA to stop the reaction, and stored on ice for up to 60 min. Phosphate levels were then determined by mixing with the Biomol reagent, and after a 30-min incubation at 25 °C, the absorbance at 620 nm was read. Free concentrations of Ca\(^{2+}\) were calculated by using the binding constants of Ca\(^{2+}\), Mg\(^{2+}\), and H\(^+\) for EGTA given by Godt (16).

**Heats of Reaction**—Heats of reaction were measured using an OMEGA isothermal titration calorimeter from Microcal Inc. using a procedure similar to that used by de Meis (4). The 1.5-ml calorimeter cell contained the reaction medium (40 mM Hepes, 100 mM KCl, 2.1 mM ATP, 5 mM MgCl\(_2\), 1 mM EGTA, 0.86 mM Ca\(^{2+}\), pH 7.1, and a free Ca\(^{2+}\) concentration of 80 \(\mu\)M), and the reference cell contained water. After equilibration at 35 °C for 20 min, the reaction was started by injecting vesicles (5 \(\mu\)g protein) into the reaction cell, and the heat change was recorded for 30 min. As described by de Meis (4), the heat change observed in the first 2 min of reaction was discarded since this includes a variety of artifacts, including heats of sample dilution. It was confirmed that vesicles were fully active after equilibration at 35 °C under the conditions used for the calorimetric measurements.

**RESULTS**

**ATPase Activities of Sealed Vesicles**—Vesicles of sarcoplasmic reticulum were prepared as described and contained typically 75% protein as Ca\(^{2+}\)-ATPase, the remainder being largely the luminal protein calsequestrin (7). The Ca\(^{2+}\)-ATPase was reconstituted into sealed vesicles containing SLN by mixing lipid and protein in detergent solution followed by removal of detergent with Bio-Beads (7). It was reported previously that levels of accumulation of Ca\(^{2+}\) by vesicles containing Ca\(^{2+}\)-ATPase were relatively low if the vesicles contained DOPC as the only lipid but increased considerably of the vesicles contained 10 mol % of an ionic lipid (13). Because the concentration of anionic lipid in the native SR membrane is also ~10 mol % (17), experiments were performed with vesicles containing 10 mol % of the anionic phospholipid DOPA.

The rate of ATP hydrolysis was measured over 3 min using a coupled enzyme assay at saturating concentrations of Ca\(^{2+}\). As shown in Table 1, activities of sealed vesicles approximately doubled on the addition of the detergent octa(ethylene glycol)-\(n\)-dodecyl ether to make the vesicles leaky to ATP. As described previously, this can be attributed to a close to random insertion of the Ca\(^{2+}\)-ATPase into the sealed vesicles, so that approximately half the ATPase molecules will be in the wrong orientation in the membrane to bind to ATP from the external...
TABLE 1  
**ATPase activities for reconstituted ATPase**

| Molar ratio SLN:ATPase | Assay                          | ATPase activity (IU/mg) |
|------------------------|-------------------------------|-------------------------|
| 0                      | Coupled enzyme assay (3 min)  | 2.2                     |
|                        | + C10E10                      | 5.8                     |
| 10:1                   | Coupled enzyme assay (30 min) | 2.2                     |
|                        | Phosphate release assay       | 2.3                     |
|                        | + C10E10                      | 5.4                     |
|                        | Coupled enzyme assay (30 min) | 3.2                     |
|                        | Phosphate release assay       | 2.9                     |

Effects of SLN on Accumulation of Ca\(^{2+}\)—As reported previously at 25 °C (7), the presence of SLN at 35 °C leads to a decreased level of accumulation of Ca\(^{2+}\) with the effect increasing with increasing molar ratios of SLN to ATPase (Fig. 2). Similar effects are seen for vesicles containing DOPC as the only phospholipid (data not shown). In these experiments FCCP was present to make the vesicles permeable to H\(^+\); in the absence of FCCP the presence of SLN again reduced the level of accumulation of Ca\(^{2+}\), but all the levels of accumulation of Ca\(^{2+}\) were lower than in the presence of FCCP (7).

Heat Production by Ca\(^{2+}\)-ATPase—The rate of heat release resulting from hydrolysis of ATP was measured for the Ca\(^{2+}\)-ATPase reconstituted into sealed vesicles containing 10 mol % DOPA as a function of the molar ratio of SLN to ATPase (Fig. 3). In all cases the heat released increased linearly with time, and the heat released increased with increasing SLN content. The measured heat released was found to be the same within 10% in buffer containing 0.86 mM Ca\(^{2+}\) and 1 mM EGTA, corresponding to a free Ca\(^{2+}\) concentration of 80 μM, and in buffer containing 30 or 100 μM Ca\(^{2+}\) in the absence of EGTA. Table 2 lists the ATPase activities of the reconstituted samples whose heat releases are shown in Fig. 3 together with the calculated heat released per mol of ATP hydrolyzed. The measurements of ATPase activity and heat produced were both performed in the absence of FCCP. Measurements were also made in the presence of FCCP to make the vesicles permeable to H\(^+\). It was found that the addition of FCCP resulted in changes in ATPase activity and heat produced of less than 10% (data not shown), showing that the observed effects of SLN could not be attributed to an effect on the permeability of the vesicles to H\(^+\). The heat released from reconstituted vesicles containing DOPC as the only phospholipid also increased linearly with time, giving calculated heats released per mol ATP hydrolyzed comparable with those observed for vesicles containing 10 mol % of DOPA (Table 2).

**DISCUSSION**

Of the 31 amino acid residues in SLN, about 22 are required to form a transmembrane α-helix (19). The structure of the transmembrane region of SLN is very similar to that of phospholamban (PLN), and SLN and PLN appear to bind to the same site on the Ca\(^{2+}\)-ATPase, located in a groove between transmembrane α-helices M2, M4, and M6 (19, 20). Binding of
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FIGURE 2. Effect of SLN on ATP-dependent accumulation of Ca$^{2+}$ by reconstituted vesicles. Shown is ATP-dependent accumulation of Ca$^{2+}$ by reconstituted vesicles containing the given molar ratios of SLN:ATPase. Accumulation of Ca$^{2+}$ was initiated by addition of 0.8 mM ATP. Samples contained 0.026 mg of ATPase/ml at a total phospholipid:ATPase molar ratio of 5000:1 with a 1:9 molar ratio of DOPA to DOPC. The buffer was 10 mM Pipes, 100 mM K$_2$SO$_4$, 5 mM MgSO$_4$, pH 7.1, containing 70 mM Arsenazo III, and the initial concentration of Ca$^{2+}$ was 120 mM.

FIGURE 3. Effect of SLN on heat released by reconstituted vesicles. Heat released (mcal/mg of protein) was measured as a function of time for reconstituted vesicles (5 μg of protein) in buffer (40 mM HEPES, 100 mM KCl, 5 mM MgCl$_2$) at 35 °C as described in the legend to Fig. 3. ATPase activities for the vesicles used for the heat release measurements were determined using a coupled enzyme assay. Vesicles contained either a 1:9 molar ratio of DOPA to DOPC or DOPC alone at a molar ratio of total phospholipid:ATPase of 5000:1 and the given molar ratios of SLN:ATPase.

| Molar ratio SLN:ATPase | Heat release | ATPase activity | ΔH (kcal/mole ATP hydrolyzed) |
|------------------------|-------------|----------------|-------------------------------|
|                        | kcal/mg/min | μmol ATP hydrolyzed/mg protein |                           |
| DOPA + DOPA (1:9 molar ratio) |            |                            |                              |
| 0                      | 13.1 ± 0.2  | 2.3 ± 0.1                  | 5.9 ± 0.1                     |
| 2:1                    | 18.7 ± 0.2  | 2.3 ± 0.1                  | 8.3 ± 0.2                     |
| 5:1                    | 25.2 ± 0.7  | 2.9 ± 0.1                  | 8.7 ± 0.2                     |
| 10:1                   | 31.6 ± 0.6  | 3.2 ± 0.1                  | 9.9 ± 0.2                     |
| 15:1                   | 32.7 ± 0.7  | 3.4 ± 0.1                  | 9.7 ± 0.2                     |
| 20:1                   | 26.4 ± 0.5  | 2.8 ± 0.1                  | 9.6 ± 0.2                     |
| DOPC                   | 19.3 ± 0.4  | 3.2 ± 0.1                  | 6.1 ± 0.1                     |
| 0:1                    | 21.3 ± 0.5  | 1.9 ± 0.1                  | 11.3 ± 0.2                    |

The transmembrane domain of PLN to the Ca$^{2+}$-ATPase results in a reduction in the apparent affinity of the Ca$^{2+}$-ATPase for Ca$^{2+}$ (21, 22). The presence of SLN has also been reported under some conditions to result in a modest decrease in the apparent affinity of the Ca$^{2+}$-ATPase for Ca$^{2+}$ (15, 19, 23), although under other conditions no significant effect on apparent affinity was observed (7, 15).

A major difference between SLN and PLN concerns the ways in which their interaction with the Ca$^{2+}$-ATPase can be modulated. Phosphorylation of PLN by protein kinases leads to a reversal of the effect of PLN on the Ca$^{2+}$-ATPase, therefore, linking Ca$^{2+}$-ATPase function to adrenergic activation (24). This kind of control is not possible for SLN because SLN cannot be phosphorylated (25). In muscles such as the atria of small mammals, where SLN is found together with PLN (20), complex interactions between SLN, PLN, and the Ca$^{2+}$-ATPase are possible because SLN and PLN form heterodimers that interact with the Ca$^{2+}$-ATPase, thus linking the effects of SLN on the Ca$^{2+}$-ATPase to phosphorylation of PLN (20). However, such interactions are clearly not possible in fast-twitch skeletal muscle that lacks PLN (26), and the role of SLN in such muscles is not clear.

The possibility explored here is that the presence of SLN in fast-twitch skeletal muscle of large mammals is related to the importance of SR in heat generation during nonshivering thermogenesis in animals lacking brown adipose tissue. de Meis et al. (3–6) has shown that ATP is hydrolyzed by SR Ca$^{2+}$-ATPase both to transport Ca$^{2+}$ and to generate heat (3–6). In the process of transport, two Ca$^{2+}$ ions are pumped from the cytoplasm to the lumen of the SR for each molecule of ATP hydrolyzed (Scheme 1), and the expected ratio of two Ca$^{2+}$ ions accumulated per ATP molecule hydrolyzed was observed during the first reaction cycle of the ATPase before the lumenal concentration of Ca$^{2+}$ had increased to a high level (27). However, when the lumenal concentration of Ca$^{2+}$ reached millimolar concentrations, the level of Ca$^{2+}$ accumulated became less than 2:1 with respect to ATP hydrolysis (8, 28) because high concentrations of luminal Ca$^{2+}$ led to a build up of E2PCa$^2+$ and E2PCa$^2+$ can dephosphorylate and release its bound Ca$^{2+}$ back to the cytoplasmic side of the membrane in a process of slippage (step 11 in Scheme 1). The process of slippage generates maximum heat from ATP hydrolysis since it corresponds to ATP hydrolysis without performing work. Thus, switching between transport and slippage by the Ca$^{2+}$-ATPase is “controlled” by the state of the system itself. When the concentration of Ca$^{2+}$ in the lumen of the SR is low, the level of E2PCa$^2+$ will be low, and there will be little slippage; ATP will be used mostly for transporting Ca$^{2+}$ into the SR lumen. The concentration of Ca$^{2+}$ in the SR lumen will build up as a result of this transport, leading to a build up of E2PCa$^2+$ and, thus, slippage and heat production. What this means physiologically is as follows. After the release of luminal Ca$^{2+}$ to cause muscle contraction, the level of luminal Ca$^{2+}$ will be low, and the Ca$^{2+}$-
ATPase will be used mainly to pump Ca\(^{2+}\) back into the lumen, lowering the cytoplasmic concentration of Ca\(^{2+}\) and leading to muscle relaxation. After the Ca\(^{2+}\) has been pumped back into the lumen, the lumenal Ca\(^{2+}\) concentration will be high, and now the Ca\(^{2+}\)-ATPase can be used for heat production since it is no longer required for muscle relaxation. Thus, switching of the Ca\(^{2+}\)-ATPase between pumping and heat production is controlled by the level of Ca\(^{2+}\) in the lumen of the SR.

Any factor that increases the rate of slippage of the Ca\(^{2+}\)-ATPase should increase heat generation by the Ca\(^{2+}\)-ATPase. In previous studies it was shown that incorporation of SLN into reconstituted vesicles of the Ca\(^{2+}\)-ATPase led to reduced levels of accumulation of Ca\(^{2+}\) at 25 °C, the effects of SLN being consistent with an increase in the rate of slippage of the Ca\(^{2+}\)-ATPase (7). Here we show that the presence of SLN also reduces the level of accumulation of Ca\(^{2+}\) at 35 °C (Fig. 2). As shown in Fig. 1, ATPase activities in the absence or presence of SLN are constant for up to 60 min, suggesting that concentrations of Ca\(^{2+}\) within the vesicle do not reach values high enough to significantly inhibit the Ca\(^{2+}\)-ATPase, since otherwise activities would decrease with increasing time. Activities measured in the presence and absence of an ATP-regenerating system are the same (Fig. 1, Table 1), showing that under the conditions of these experiments the concentrations of ADP generated in the absence of a regenerating system are not sufficient to inhibit ATP hydrolysis.

Isothermal calorimetry was used to measure directly the heat generated by the Ca\(^{2+}\)-ATPase as a result of ATP hydrolysis. As shown in Fig. 3 and Table 2, the presence of SLN did indeed result in increased heat production, the effect increasing with increasing SLN content. From the Ca\(^{2+}\) uptake data shown in Fig. 2, it can be estimated that the external free concentration of Ca\(^{2+}\) is greater than 10 μM at the end of the experiment, this being sufficiently high to inhibit passive leak of Ca\(^{2+}\) from the vesicles (10, 11) and to inhibit the back reaction of ADP with the phosphorylated ATPase (steps 4 to 2 in Scheme 1) (4). Thus, the heat produced per mol of ATP hydrolyzed can be calculated directly from the heat produced/mg of protein/min and the rate of ATP hydrolysis/mg of protein/min, giving the values listed in Table 2. A plot of heat produced per mol of ATP hydrolyzed against concentration of SLN, expressed as mole fraction per lipid monolayer to account for the fact that the lipids are arranged as a bilayer, fits to a simple binding equation, giving a value for the dissociation constant for the SLN-ATPase complex of 6.9 × 10\(^{-4}\) ± 2.9 × 10\(^{-4}\) in units of mole fraction (Fig. 4). The dissociation constant can also be expressed as 1.7 ± 0.7 in units of molar ratio of SLN to ATPase at a molar ratio of 5000 lipids:ATPase, but in molar ratio units the dissociation constant will vary with the molar ratio of lipid:protein in the membrane.

Although high molar ratios of SLN to ATPase are required for maximal effects of SLN in the reconstituted system (Fig. 4), this is simply the result of the high lipid content of the reconstituted system. The molar ratio of SLN:ATPase in rabbit extensor digitorum longus muscle (fast-twitch skeletal muscle) has been estimated by Vangheluwe et al. (14) to be ~0.4:1. The molar ratio of lipid:ATPase in skeletal muscle SR is ~90:1 (29), giving a mole fraction of SLN per lipid monolayer in the SR of ~0.01, so that with a dissociation constant of 6.9 × 10\(^{-4}\) mole

![Sarcolipin and Thermogenesis](image)

**FIGURE 4.** Concentration dependence of SLN effect on heat released by reconstituted vesicles. Heat released from hydrolysis of ATP (kcal/mol ATP hydrolyzed) are plotted as a function of mole fraction SLN per lipid monolayer for the ATPase reconstituted in vesicles of DOPC:DOPA at a molar ratio of 9:1. (C), experimental data; solid line, fit to a simple binding equation giving a value for Kd of 6.94 × 10\(^{-4}\) ± 2.9 × 10\(^{-4}\) in units of mole fraction. The inset shows a plot of the same data but plotted as a function of the molar ratio SLN:ATPase, giving a value for Kd of 1.73 ± 0.74 in units of mol ratio. The total molar ratio of lipid:ATPase was 5000:1, giving a molar ratio of lipid:ATPase of 2500:1 in each monolayer.
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