Role of Cysteine Residues in Structural Stability and Function of a Transmembrane Helix Bundle*  

To study the structural and functional roles of the cysteine residues at positions 36, 41, and 46 in the transmembrane domain of phospholamban (PLB), we have used Fmoc (N-(9-fluorenyl)methoxycarbonyl) solid-phase peptide synthesis to prepare α-amino-n-butyric acid (Abu)-PLB, the analogue in which all three cysteine residues are replaced by Abu. Whereas previous studies have shown that replacement of the three Cys residues by Ala (producing Ala-PLB) greatly destabilizes the pentameric structure, we hypothesized that replacement of Cys with Abu, which is isosteric to Cys, might preserve the pentameric stability. Therefore, we compared the oligomeric structure (from SDS-polyacrylamide gel electrophoresis) and function (inhibition of the Ca-ATPase in reconstituted membranes) of Abu-PLB with those of synthetic wild-type PLB and Ala-PLB. Molecular modeling provides structural and energetic insight into the different oligomeric stabilities of these molecules. We conclude that 1) the Cys residues of PLB are not necessary for pentamer formation or inhibitory function; 2) the steric properties of cysteine residues in the PLB transmembrane domain contribute substantially to the pentameric stability, whereas the polar or chemical properties of the sulfhydryl group play only a minor role; 3) the functional potency of these PLB variants does not correlate with oligomeric stability; and 4) acetylation of the N-terminal methionine has neither a functional nor a structural effect in full-length PLB.

Phospholamban (PLB) is a 52-residue integral membrane protein that regulates the enzymatic activity of the Ca-ATPase in cardiac sarcolemmal reticulum (1). PLB is predominantly a homopentamer, with a small fraction of monomer, as assayed by SDS-PAGE (2), electron paramagnetic resonance spectroscopy (3–5), and fluorescence energy transfer (6, 7). It has been suggested, however, that the less predominant monomeric form of PLB is primarily responsible for inhibition of the Ca-ATPase (3, 4, 7–9). The residues presumed responsible for stabilizing the pentameric structure of PLB are located in the hydrophobic transmembrane domain (10–12). These transmembrane domain residues are largely composed of Leu and Ile, but this arrangement is punctuated with three cysteines in a five-residue repeat (Fig. 1). Mutation of these cysteines (at positions 36, 41, and 46 to Ser, Ala, or Phe, respectively) induces changes in the oligomeric stability of PLB (13). The mutation Cys-41 to Phe shows the strongest effect, decreasing the apparent pentameric stability. We have found that Cys-41 is unreactive and is located at a crucial site for the maintenance of the pentameric structure (5). Based on these results, a structural model for the PLB pentamer has been proposed, in which each pair of subunits is stabilized by interhelical interactions between leucines 37, 44, and 51 with isoleucines 40 and 47 to form a Leu/Ile zipper (5, 11, 12). The transmembrane Cys residues do not appear to be involved in intermolecular disulfide bonding (2, 15). To evaluate the role of the chemical and steric packing properties of the cysteines in the pentameric structure of PLB as well as their function, we have used Fmoc solid-phase peptide synthesis to design a stERICally identical PLB derivative. In the present study, we replaced all cysteine residues in PLB with α-amino-n-butyric acid (Abu), which is isosteric to cysteine (16). Like many eukaryotic proteins, PLB is “capped” at the N-terminal methionine by posttranslational acetylation (17). The N-terminal cytoplasmic portion of WT-PLB has a net charge of +3 with the posttranslational acetylation, but this charge increases to +4 in the absence of the acetyl group. It is not known whether this acetyl group in the full-length PLB is necessary for pentameric stabilization and interaction with the Ca-ATPase. No effect of nonacetylated PLB was reported, and only acetylated peptide Ac-PLB showed inhibition of the Ca-ATPase (18). To clarify this subject, we determined the oligomeric states of acetylated and nonacetylated Abu-PLB in detergent solution by SDS-PAGE. The synthetic peptides were then co-reconstituted with the Ca-ATPase in lipid vesicles, and Ca-ATPase inhibition assays were performed in comparison to WT-PLB.

EXPERIMENTAL PROCEDURES

Peptide Synthesis and Purification of Abu-PLB—Materials, solvents, instrumentation, and general methods of solid-phase peptide synthesis were essentially as described in our previous publications (15, 16, 20–22). We used acetic anhydride for the acetylation of the N-terminal amino group (23). First, an Fmoc removal step was carried out on 200 mg of peptide resin (15), followed by treatment with 0.5 M acetic anhydride in 10 ml of N,N-dimethylformamide. After 2 h, the acetylated peptide resin was filtered and then used for cleavage and purification (15). Fractions containing peptides were lyophilized to yield 26 mg of Abu-PLB (Fig. 1) (12% yield based on starting resin).

Synthetic Wild-type PLB—Edward McKenna (Merck Research Lab-

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‡ The abbreviations used are: PLB, phospholamban; WT, wild-type; Ala-PLB, replacement of Cys-36, -41, -46 in PLB with alanine; Abu, α-amino-n-butyric acid; DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; Fmoc, 9-fluorenylmethoxycarbonyl; NADH, β-nicotinamide adenine dinucleotide; PAGE, polyacrylamide gel electrophoresis; SR, sarcoplasmic reticulum.

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oratories), who synthesized the protein using an Applied Biosystems 430A synthesizer, graciously provided WT-PLB.

Expressed Wild-type PLB—WT-PLB was expressed in SF21/baculovirus insect cell system and purified by monoclonal antibody affinity chromatography as described previously (7, 12).

SR Vesicles—SR vesicles were prepared from the fast-twitch skeletal muscle of New Zealand White rabbits (24). The Ca-ATPase from the SR vesicles was purified using a reactive-red affinity column (25).

Analysis of Peptide Size and Composition—SDS-PAGE was performed using 16.5% Tris/Trisine gel (Bio-Rad) (15). The peptide samples from the stock methanol/chloroform 2:1 solution were dried overnight. 20 μl of 1% SDS was added to the samples that contained 5 μg of Abu-PLB and WT-PLB. For SDS-PAGE, samples contained 20 μl of Tricine sample buffer (26) with a final SDS concentration of 1.5%. The temperature was controlled during electrophoresis by using a recirculating water bath. For the quantitation of Abu-PLB and WT-PLB monomers, the gels were scanned by a densitometer using the transmittance mode, and then the bands were quantitated using the volume (area × density) analysis method (27).

Mass spectral data was acquired with a Bruker Biflex III matrix-assisted laser desorption/ionization time of flight mass spectrometer, which is equipped with an N_2-laser (337 nm, 3-ns pulse length) and a microchannel plate detector. The data was collected in the linear mode, positive polarity, with an accelerating potential of 19 kV. Each spectrum is the accumulation of 100–400 laser shots. The samples were co-crystallized with the matrix 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid).

Ca-ATPase/PLB Co-reconstitution—The method used for the functional reconstitution of Ca-ATPase with PLB has been described (28). In short, 33 μg of Abu-PLB or its analogues was dried and solubilized in 240 μl of chloroform containing 2.4 mg of lipids (DOPE/DOPE, 4:1). The dried film of lipid and PLB was hydrated with 120 μl of 25 mM imidazole, pH 7.0, by vortexing followed by a brief sonication. The resulting vesicles were diluted to 20 mM imidazole, pH 7.0, 0.1 M KCl, 5 mM MgCl_2, 10% glycerol. Then, 4.8 μg of β-octyl glucoside was added, followed by 60 μg of purified Ca-ATPase. The final volume was adjusted to 300 μl with buffer. The detergent was then removed by incubation with 120 μg of hydrated Biobeads for 3 h at room temperature. The Ca-ATPase/PLB lipid vesicles were separated from Biobeads and assayed immediately. All Ca-ATPase/PLB co-reconstitution in the present study used a fixed molar ratio of 10 PLB/Ca-ATPase. As shown previously (27), this ratio gives substantial effects, comparable to those in cardiac SR.

ATPase Activity Measurements—Ca-ATPase activity was measured by an enzyme-linked assay performed in microtiter plates (200 μl total volume in each well) as described previously (15, 29). Each well contained 0.2–0.6 μg of Ca-ATPase (1–3 μl of vesicles) and was added to a buffer containing 50 mM imidazole, pH 7.0, 0.1 M KCl, 5 mM MgCl_2, 0.5 mM EGTA, 0.5 mM phosphonoxyglycine, 2.5 mM ATP, 0.2 mM NADH, 2 IU of pyruvate kinase, 2 IU of lactate dehydrogenase, and 1–2 μg of calcium ionophore (A23187). Each assay was done in triplicate at each of 12 different free calcium concentrations. The absorbance of NADH was monitored at 340 nm to determine the rate of ATP hydrolysis. The assays were performed at 15 and 25 °C in a Thermomax microplate reader (Molecular Devices). Each data point represents average ± S.E. (n ≥ 6). A t test was used to determine the statistical significance of the differences between peptides and the effects of temperature.

Computational Modeling—We started with our previously constructed, experimentally verified model for the PLB transmembrane domain (residues 35–52) (5). This model was derived from that of Adams et al. (30) (PDB entry 1PSL) by capping the N and C termini with N-acetyl and N′-methylamide groups, respectively, and then rotating each helix counter-clockwise around its axis by about 50° to produce a structure conforming to a leucine-zipper motif (12), as supported by cysteine reactivity and site-directed spin labeling (5). This structure was then used as the starting point for simulations carried out using AMBER 5.0 (31, 32), as recently updated for the peptide backbone parameters (parm96.dat). Before energy minimization, side chain rotamers were generated using the program SQWRL (33) to relieve unfavorable interactions between side chains. Side chains were found to be in the most favorable conformation for an α-helix (35).

Results

The template was energy-minimized using a 12Å nonbonded cutoff, a distance-dependent dielectric constant of 4 r, and a converge criterion of $5 \times 10^{-6}$ kcal/mol Å in the root mean square of the Cartesian elements of the energy gradient. Positional constraints were imposed on the backbone heavy atoms using a harmonic potential with a force constant of 5 kcal/mol Å². Minimum energy conformations of the mutants were generated by replacing the side chains while maintaining the same backbone templates as in the wild-type protein.

Interhelical energy differences were obtained as $\Delta E_{ij} = E_{ij} - E_{ii}^0$, where $E_{ij}$ is the nonbonded interchain energy per helix (34), and $E_{ii}^0$ is the value for WT-PLB.

The buried surface area was defined as $\Delta A = (A_i + A_j) - A_{ij}$, where $A_i$ and $A_j$ are the surface areas of the individual helices i and j, and $A_{ij}$ is the surface area of the dimer complex. Values were averaged over the five helix interfaces of the pentamer. Surface areas were generated using GRASP (35) with a probe size of 1.4 Å. Free energies of dissociation were estimated from the buried surface area by considering a penalty of 20 cal mol⁻¹ Å⁻² upon loss of interfacing surface (36).

Replacement of Cys with Abu in Phospholamban

FIG. 1. Amino acid sequence of PLB (residues 1–52). X is Cys in WT-PLB, Abu in Abu-PLB, and Ala in Ala-PLB.

FIG. 2. Matrix-assisted laser desorption/ionization time of flight mass spectrum of Ac-Abu-PLB. The spectrum shows the [M + H] peak of Ac-Abu-PLB, m/z 6066.

FIG. 3. SDS-PAGE of WT-PLB, Abu-PLB, and nonacylated Abu-PLB at 25 °C.
a peak at 6025 m/z, [M + H], which corresponded to the calculated molecular mass of 6023.40 Da. Amino acid analysis was also consistent with the expected composition (data not shown).

**Oligomeric Distribution of WT-PLB and Abu-PLB on SDS-PAGE**—We compared expressed WT-PLB (Fig. 3, lane 1) with synthetic acetylated (lane 2) and nonacetylated Abu-PLB (lane 3) on SDS-PAGE. WT-PLB and Abu-PLB each appear primarily as a 30-kDa pentamer, with a faint band at 6 kDa (monomer). This shows that in SDS solution at 25 °C, the pentamer is just as stable for Abu-PLB as for WT-PLB.

**Inhibitory Function of WT-PLB and Abu-PLB**—Fig. 4 shows the effects of synthetic WT-PLB and Abu-PLB on Ca-ATPase activity as a function of Ca2+/H1001 concentration, measured in reconstituted membranes. Both WT-PLB and Abu-PLB decrease the activity of the Ca-ATPase at pCa below 5.5, resulting in an increase in pKCa (the calcium concentration, in pCa units, required for 50% calcium activation). Abu-PLB shifted pKCa by 0.27 (control, 6.23 ± 0.02; Abu-PLB, 5.96 ± 0.02), whereas the shift by WT-PLB was 0.20 (6.03 ± 0.03) for this co-reconstitution system. There is a significant difference between the control and Abu-PLB (p = 2.5 E-06) but not between Abu-PLB and WT-PLB (p = 0.2).

Thus, Abu-PLB shows similar inhibitory activity to WT-PLB, and Cys residues are not required for inhibition of the Ca-ATPase.

**Inhibitory Function of Acetylated and Nonacetylated Abu-PLB**—To study the function of the acetyl group capping the N terminus, acetylated and nonacetylated Abu-PLB were reconstituted in membranes. Both peptides showed the same increase in pKCa (Fig. 5). There is no significant difference between acetylated and nonacetylated Abu-PLB (p = 0.60). This result shows that the acetyl group plays no role in the inhibition of the Ca-ATPase.

**Inhibitory Function of WT-PLB and Ala-PLB**—Fig. 6 shows the inhibitory effects of Ala-PLB and WT-PLB on Ca-ATPase activity. Ala-PLB decreases pKCa by 0.29 (control, 6.31 ± 0.02; Ala-PLB, 6.02 ± 0.02), whereas the decrease from WT-PLB is 0.20 %

**TABLE I**  
**Inhibitory effects and monomeric fractions of WT-PLB, Abu-PLB, and Ala-PLB, at 12, 25, and 37°C**

|        | 12°C |        | 25°C |        | 37°C |
|--------|------|--------|------|--------|------|
|        | pKCa shift | % | pKCa shift | % | pKCa shift | % |
| WT-PLB | -0.23 ± 0.07 | 12 ± 7 | -0.25 ± 0.03 | 18 ± 5 | -0.25 ± 0.05 | 22 ± 5 |
| Abu-PLB | -0.34 ± 0.08 | 8 ± 5 | -0.27 ± 0.02 | 32 ± 4* | -0.30 ± 0.04 | 78 ± 4* |
| Ala-PLB | -0.25 ± 0.07 | 100 ± 2* | -0.29 ± 0.02 | 100 ± 2* | -0.27 ± 0.04 | 100 ± 2* |

* Significantly different from WT-PLB (p < 0.01).

FIG. 4. Regulation of purified skeletal SR Ca-ATPase at 25 °C, after reconstitution in DOPC bilayers. □, control (Ca-ATPase only); ▼, Ca-ATPase plus Abu-PLB; □, Ca-ATPase plus WT-PLB. The curves are fits to experimental data (symbols), means ± S.E. (n ≥ 6).

FIG. 5. Regulation of Ca-ATPase activity at 25 °C by acetylated (●) and (○) nonacetylated (non-Ac) Abu-PLB after reconstitution in DOPC bilayers. The curves are fits to experimental data (symbols), means ± S.E. (n ≥ 6).

FIG. 6. Regulation of purified skeletal SR Ca-ATPase at 25 °C after reconstitution in DOPC bilayers. □, control (Ca-ATPase only); ▼, Ca-ATPase plus Ala-PLB; □, Ca-ATPase plus WT-PLB. The curves are fits to experimental data (symbols), means ± S.E. (n = 9).
(6.03 ± 0.03). There is a significant difference between the control and Ala-PLB ($p = 1.7 \times 10^{-09}$) but not between Ala-PLB and WT-PLB ($p = 0.6$).

Table I summarizes the inhibitory potencies, measured as in Figs. 4 and 6, and oligomeric stabilities, measured from densitometry of SDS-PAGE, of the three PLB derivatives at three different temperatures. There was no significant difference in inhibitory potency among the three peptides, nor was there a significant temperature dependence. In contrast, there were substantial differences in oligomeric stability. At all three temperatures, WT-PLB was predominantly pentameric, and Ala-PLB was completely monomeric. At low temperature, Abu-PLB exhibited high pentameric stability, comparable to that of WT-PLB, but the Abu-PLB pentamer was much less stable at 37 °C. Fig. 7 shows a comparison of the mobilities of the WT-PLB, Abu-PLB, and Ala-PLB on SDS-PAGE at room temperature. WT-PLB and Abu-PLB showed mobility characteristic of pentamers, whereas Ala-PLB was completely monomeric.

**DISCUSSION**

Previous studies have shown that mutations of the three cysteines in PLB to serine, alanine, or phenylalanine disrupts the pentameric structure of PLB, suggesting that the cysteine side chains are crucial for the oligomeric stability of PLB (12, 13). The principal goal of the present study was to determine whether the steric properties of Cys are sufficient for pentameric stability or whether the specific chemical properties of the thiol group are important. Another goal was to clarify the role of the N-terminal acetyl group for pentameric stability and interaction with the Ca-ATPase. We synthesized acetylated and nonacetylated Abu-PLB, an analogue of PLB in which the three Cys residues were replaced with Abu (Fig. 1), an amino acid analogue that is known to be isosteric with cysteine. SDS-PAGE of Abu-PLB indicated that it is primarily pentameric, as is WT-PLB (Fig. 3), indicating clearly that the apparent requirement of the three Cys residues for pentameric stability of PLB is based primarily on steric packing, not on the chemical properties of the thiol groups.

N-terminal acetylation of Abu-PLB indicated no difference in the oligomeric stability and inhibitory function.

**Fig. 7.** SDS-PAGE of WT-PLB, Abu-PLB, and Ala-PLB at room temperature.

**Fig. 8.** Theoretical model of WT-PLB and comparison with Abu-PLB, and Ala-PLB. Models show residues 35–43 as viewed from the outside of the pentameric bundle. Residues at positions 36 and 41 are shown in orange using a ball-and-stick representation. The solvent-accessible surfaces for one helix and for position 36 of the second helix are shown using their corresponding atom color. Surfaces were generated using a probe radius of 1.4 Å and displayed using WebLab ViewerPro 3.7.
between Cys at position i and the carbonyl of residue i-4 (37). Although such interactions can also occur in the current model, Cys-36 and Cys-41 may also be involved in interhelical hydrogen bonds.

The structural model of PLB, based on a “leucine zipper” template, places Cys-36 and Cys-41 at positions g and e, respectively, of the helical heptad repeat, whereas Cys-46, at position c, faces the lipid environment (Fig. 9A) (3). According to this model, side chain-side chain interactions can occur between Cys-36 and Cys-41 and between these two residues and the backbone atoms of Leu-37 (Fig. 9B). The lower oligomeric stability of Abu-PLB and Ala-PLB compared with WT-PLB, could, therefore, depend on differences between interhelical interactions at positions 36 and 41 of these peptides.

A close inspection of the interfacial region between Cys-36 and Cys-41 shows that additional types of interactions are also possible. Whereas the \( \chi_1 = \text{gauche}^+ \) conformer of Cys-36 is preferred in \( \alpha \)-helices (33), wild-type PLB has an additional state (\( \chi_1 = \text{trans} \)) available for interaction. In this orientation, the interhelical energies are similar to those with \( \chi_1 = \text{gauche}^+ \) (Table II), whereas Cys-36 can form a hydrogen bond with the carbonyl oxygen of Leu-37 in the neighboring helix. The S . . O distance (3.2 ± 0.7 Å) and the \( C_p - S - O \) angle (127 ± 17°) are in agreement with those observed for hydrogen bonded Cys in proteins (S . . O = 3.5 ± 0.1 Å and \( C_p - S - O \) = 104 ± 27°) (38). Furthermore, side chain rotation of Cys-36 results in a S . . S distance of 4.0 ± 1.0 Å, which is similar to that observed for hydrogen-bonded S . . S atoms in cysteine crystals (S . . S = 3.854 Å) (39).

**Inhibitory Function**—Abu-PLB and Ala-PLB have the same inhibitory function as WT-PLB (Table I), showing clearly that neither the steric properties of the Cys residues nor their chemical and polar properties are important for their inhibition of the Ca-ATPase. The similar potency of these two PLB analogues does not correlate with their significant differences in oligomeric stability (Table I). At first glance, this appears to contradict the proposal that only the monomeric form of PLB binds to the Ca-ATPase and inhibits it (3, 4), and it raises the possibility that the SDS-PAGE assay used in the present study does not accurately reflect PLB oligomeric stability in the membrane. However, previous analyses of PLB oligomeric stability in lipid bilayers, using spectroscopic probes, have shown that SDS-PAGE is remarkably accurate in determining the distribution of oligomers for PLB and its mutants in lipid bilayers (3, 4, 6). Spectroscopic analysis also shows that the Ca-ATPase depolymerizes PLB, so that even WT-PLB, which is only 10–20% monomer in SDS or in lipid bilayers (6), is 40% monomeric in the presence of Ca-ATPase (7). Thus, it is quite likely that each of the PLB variants in the present study is sufficiently monomeric in the presence of the Ca-ATPase to exert full inhibitory function.

![Fig. 9. Structural model for the PLB transmembrane domain (residues 35–47). A, helical wheel representation (12). B, theoretical model of PLB, viewed as in Fig. 8. The figure was generated using WebLab ViewerPro 3.7 (Molecular Simulations Inc.).](image)

These results are consistent with previous findings that there is sometimes a lack of quantitative correlation between oligomeric stability and inhibitory potency, suggesting that other structural factors are important (27). Although some PLB mutants that have greatly decreased pentameric stability, as shown by SDS-PAGE, have greater inhibitory activity than WT-PLB, others have negligible inhibitory activity (9). A few mutants have been shown to be more potent inhibitors than WT-PLB, despite retaining full pentameric stability on SDS-PAGE (25). Of course, one possible explanation is that the oligomeric state of a PLB mutant in SDS-PAGE does not correspond to its state in the lipid bilayer and does not reflect the effects of the Ca-ATPase on this oligomeric state. This possibility has been addressed by spectroscopic probes, which have been used to measure oligomeric interactions of PLB in lipid bilayers (4). These studies showed that there is a dynamic equilibrium between monomeric and pentameric PLB in a lipid bilayer and that this equilibrium agrees with SDS-PAGE in the absence of Ca-ATPase (3, 6), but the equilibrium shifts in favor of the monomer in the presence of the Ca-ATPase (4, 7). It has been suggested that inhibitory activity of PLB or its mutants...
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depends not only on the oligomeric state but also on the affinity for the Ca-ATPase (7, 9, 27). The present study underscores the complexity of this functional interaction.

We found that the N-terminal acetyl group of Abu-PLB has no effect on oligomeric stability or functional significance on Ca-ATPase inhibition (18). N-terminal acetylation, in addition to its role in targeting proteins for distribution within the cell, has been shown to be important in the function of actin (40, 41), and the proteolytic stability of peptides (42). In addition to an increase in steric bulk of the N-terminal end of PLB, the acetyl capping reduces the net positive charge, which could be critical for the interaction with the Ca-ATPase if electrostatic interactions were vitally important. It can be argued that the addition of extra charge would disrupt any interaction surface that existed, even if the forces involved in the interaction were largely hydrophobic. Because no change in PLB inhibitory function occurs when the acetyl cap is omitted, we speculate that the extreme N-terminal end of PLB does not interact at all with the Ca-ATPase. This result is in agreement with the literature (15, 43), and it suggests that the N terminus of PLB can be labeled without affecting the interaction with the Ca-ATPase (44). With the recent publication of a high resolution x-ray crystal structure of SERCA1 (19), modeling of the PLB/SERCA interaction surface should be possible in the near future.

In summary, substitution of the three cysteine residues in PLB with alanine (Ala-PLB) completely destabilizes the PLB pentamer, but substitution by α-amino-γ-butyric acid (Abu-PLB) causes only slight destabilization. Thus, the thiol groups of the Cys residues of PLB are not required for pentamer formation. In particular, intermolecular disulfide bonds are not necessary. Because Abu is isosteric to Cys, these results indicate that steric packing is the principal factor determining pentameric stability. However, the Abu-PLB pentamer is significantly less stable than WT-PLB at temperatures above 25 °C, indicating that specific chemical properties of the thiol groups do play a significant role. Examination of the structure reveals a possible hydrogen bond between Cys-36 and between Cys-36 and Cys-41. WT-PLB, Abu-PLB, and Ala-PLB have no significant differences in bone oxygen of Leu-37 and between Cys-36 and Cys-41. WT-PLB) causes only slight destabilization. Thus, the thiol groups existed, even if the forces involved in the interaction were vitally important. It can be argued that the addition of extra charge would disrupt any interaction surface that existed, even if the forces involved in the interaction were largely hydrophobic. Because no change in PLB inhibitory function occurs when the acetyl cap is omitted, we speculate that the extreme N-terminal end of PLB does not interact at all with the Ca-ATPase. This result is in agreement with the literature (15, 43), and it suggests that the N terminus of PLB can be labeled without affecting the interaction with the Ca-ATPase (44). With the recent publication of a high resolution x-ray crystal structure of SERCA1 (19), modeling of the PLB/SERCA interaction surface should be possible in the near future.

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