Relationship between Protein Rotational Dynamics and Phosphoenzyme Decomposition in the Sarcoplasmic Reticulum Ca-ATPase*

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We have investigated the role of large-scale protein rotational mobility in the reaction mechanism of the Ca-ATPase in sarcoplasmic reticulum using conditions that have previously been found to inhibit selectively phosphoenzyme decomposition, i.e. 1) partial delipidation (by detergent extraction or phospholipase treatment) and 2) the addition of nonaqueous solvents (dimethyl sulfoxide, glycerol, and sucrose). Using saturation-transfer electron paramagnetic resonance to probe the microsecond rotational motion of the spin-labeled Ca-ATPase, we find that both calcium-dependent ATPase activity and protein rotational mobility decrease in parallel, suggesting that protein mobility is important to the enzymatic step(s) involving phosphoenzyme decomposition. Using conventional EPR to measure the nanosecond rotational dynamics of spin-labeled lipid hydrocarbon chains, we find that neither the removal of lipid nor the addition of nonaqueous solvents significantly affects the lipid dynamics. We propose that the physical mode of inactivation under these conditions is the reduction in protein mobility through enforced protein-protein interactions, the result of which is a reduction in a motion essential for Ca-ATPase activity.

Previous spectroscopic studies of sarcoplasmic reticulum (SR)† have provided evidence for conformational changes within the Ca-ATPase upon calcium binding and phosphorylation (reviewed by Martonosi and Beeler, 1983). These studies have usually relied upon changes in spectral properties of probes, the nature of which is not well defined with respect to physical events that may be coupled to calcium transport. For this reason, we have focused our attention on the microsecond rotational mobility of covalent probes bound to the Ca-ATPase polypeptide chain, a well-defined measurement that is sensitive to the degree of protein-protein interaction between Ca-ATPase polypeptide chains and to the fluidity of the lipid hydrocarbon environment (Saffman and Delbrück, 1975; Thomas, 1985, 1986; Squier et al., 1988a, 1988b). Independent spectroscopic measurements of lipid hydrocarbon chain dynamics allow us to determine whether a change in the microsecond rotational mobility of the Ca-ATPase is due to a change in lipid mobility or protein-protein association.

There is considerable evidence that interactions among Ca-ATPase polypeptide chains occur (Vanderkooi et al., 1977; Watanabe et al., 1981; Papp et al., 1987) and that these interactions may change during enzymatic activity (Watanabe and Inesi, 1982; Yantorno et al., 1983; Yamamoto et al., 1984; Silva and Verjovski-Almeida, 1985; Dux et al., 1985; Verjovski-Almeida et al., 1986) in a way that may be critical to the rate-limiting step in the Ca-ATPase reaction mechanism (i.e. phosphoenzyme decomposition) (Yantorno et al., 1983). Therefore, in this study, we have used conditions that selectively inhibit phosphoenzyme decomposition and measured their effect on both protein and lipid mobility. These perturbations involve partial delipidation and the addition of nonaqueous solvents. The latter conditions also stabilize the formation of phosphoenzyme from inorganic phosphate (de Meis et al., 1980). The inhibition of the calcium-dependent ATPase activity by nonaqueous solvents has been interpreted as evidence for a thermodynamic role for water, i.e. solvation of inorganic phosphate at the active site (de Meis et al., 1980). However, both the electrostatic interactions (Tanford, 1961) and the hydration repulsive force (Rau et al., 1984) among Ca-ATPase polypeptide chains are presumably also affected by the addition of nonaqueous solvents, which alter the structure of water (Tanford, 1980). Therefore, in this study, we have related the enzymatic inactivation, by both partial delipidation and the addition of nonaqueous solvents, to the protein rotational mobility in order to explore an alternative hypothesis: that inhibition is due primarily to a reduction in protein mobility, presumably as a result of changing the degree of interaction among Ca-ATPase polypeptide chains.

In previous studies using saturation-transfer EPR (ST-EPR), a quantitative correlation was found between protein rotational mobility and enzymatic activity as temperature, lipid type, detergent concentration, and diethyl ether concentration were varied (Thomas and Hidalgo, 1978; Hidalgo et al., 1978; Bigelow et al., 1986; Bigelow and Thomas, 1987; reviewed by Thomas, 1985; and by Hidalgo, 1985, 1987), suggesting that protein mobility is a fundamental determinant of ATPase activity. However, these perturbions alter both the lipid and protein mobility, thus obscuring the underlying physical requirements for enzyme activity. In this study, which is related to a previous study in which the functional effects of delipidation on the partial reactions of the Ca-

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| The abbreviations used are: SR, sarcoplasmic reticulum; ST-EPR, saturation-transfer EPR; L/P, lipid/protein ratio; L'/L, ST-EPR lowfield line height ratio; MOPS, 3-(N-morpholino)propanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylenedinitrilotetraacetic acid; SASL, stearic acid spin label(s).
ATPase reaction mechanism were studied (Hidalgo et al., 1986), we make a quantitative analysis of the relationship between the functional and dynamic effects of selectively inhibiting phosphoenzyme decomposition using methods of inhibition that do not significantly alter the lipid fluidity. This study is complementary to one involving protein-protein cross-linking (Squier et al., 1988a), which also inhibits protein mobility without affecting lipid fluidity.

**EXPERIMENTAL PROCEDURES**

**Fragmented SR**

Vesicles were prepared from rabbit skeletal white (fast twitch) muscle essentially as described previously (Fernandez et al., 1980). The fraction that sedimented between 25 and 50% (w/w) sucrose was collected and used for all experiments. All preparation was done at 4 °C. The membrane vesicles were suspended in 0.3 M sucrose, 1 mM NaNO3, 20 mM MOPS (pH 7.0) and stored in liquid nitrogen.

**Dilipidation**

The lipid/protein (L/P) ratio was varied using two different procedures. The first procedure involved incubating SR with varying amounts of deoxycholate (recrystallized twice from ethanol) for 30 min in the presence of 1 M KCl, 50 mM sodium phosphate (pH 8.0) at 0 °C at a protein concentration of 10 mg/ml (Hidalgo et al., 1976, 1986; Thomas et al., 1982). The incubation solution was then collected on a discontinuous sucrose gradient and washed twice in 10% sucrose, 20 mM MOPS (pH 7.0) so as to remove any remaining deoxycholate (radioactive [3H]deoxycholate indicated less than one deoxycholate molecule remained per Ca-ATPase). Control samples subjected to the same procedure in the absence of deoxycholate retained full Ca-ATPase activity. Alternatively, native SR was partially delipidated using phospholipase A2 (Sigma; stock was 50 IU/ml) essentially as described by Nakamura and Ohnishi (1979). SR was suspended at a concentration of 2 mg/ml in a medium containing 100 mM KCl, 10 mM CaCl2, 1% (w/v) bovine serum albumin, and 20 mM HEPES (pH 8.0) at 25 °C. Between 0.1 and 10 IU of phospholipase A2 was added to 25 mg of SR (2 mg/ml) and incubated at 25 °C for 1 h. The SR was then washed four times with a buffer containing 1% (w/v) bovine serum albumin, 100 mM KCl, and 10 mM MOPS (pH 7.0) to remove free fatty acids.

**Lipid/Protein Molar Ratio**

The molar ratio of phospholipids to Ca-ATPase was determined by comparing the molar concentration of total phospholipids, determined from phosphorus assays, to that of the Ca-ATPase, determined by dividing the protein concentration by a molecular weight of 115,000 and multiplying by the fraction of the total protein that had this molecular weight, as determined from densitometer scans of polyacrylamide gels (see below). This SR preparation typically has about 80 ± 6 mol of phospholipid/mol of Ca-ATPase. The partially delipidated samples varied from L/P = 9 to that of native SR. No significant decrease in Ca-ATPase activity was observed due to either the spin labeling or the delipidation procedures, as determined by a control (no deoxycholate or phosphorylase added).

**Enzymatic Assays**

Calcium-dependent ATPase activity was measured in a solution containing 0.05 mg of protein/ml, 60 mM KCl, 6 mM MgCl2, 2 mM ATP, 5 mM MOPS (pH 7.0), and either 0.1 mM CaCl2 (free calcium is 40 μM) or 2 mM EGTA (free calcium is 2 μM) at both 4 and 25 °C. The reaction was started by the addition of 5 mM ATP, and the initial rate of release of inorganic phosphate was measured by the method of Lanzetta et al. (1979). Activity assayed in the presence of EGTA (basal activity) was subtracted from that assayed in the presence of CaCl2 (total Ca-ATPase activity) in order to obtain calcium-dependent ATPase activity. Protein concentrations were determined by the biuret method using bovine serum albumin as a standard.

**Gel Electrophoresis**

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by the method of Laemmli (1970) using a 7.5% acrylamide gel with a 3% stacking gel. The Ca-ATPase was resolved from a 58-kDa protein, presumably phosphorylase b (Pickart and Jencks, 1984), which we found to be less than 4% of the protein in this preparation (Bigelow et al., 1986). Before electrophoresis, samples (3 mg of protein/ml) were denatured with 1% (w/v) sodium dodecyl sulfate. Gels were stained for protein with Coomassie Blue. Such gels indicated that 80 ± 5% of the proteins in our SR preparation migrated as a 100-kDa band, indicating that our preparation contains about 7 nmol of Ca-ATPase/mg of SR.

**RESULTS**

**Dependence of Calcium-dependent ATPase Activity on Lipid/Protein Ratio**

The data in Fig. 1 represent samples whose extent of delipidation is varied from a L/P ratio characteristic of native SR, i.e. L/P = ~80 ± 8, to L/P = 9. As the L/P ratio is decreased, there is a constant (maximal) calcium-dependent ATPase activity above L/P = 48 ± 4; below this critical lipid number, there is a linear decrease in ATPase activity.
activity, with total inactivation occurring at about L/P = 12. This inactivation pattern is the same regardless of the delipidation procedure used (see "Experimental Procedures"), although membrane asymmetry is altered by deoxycholate solubilization (Herbette et al., 1981), but not by phospholipase digestion (Nakamura and Ohnishi, 1975).

Dependence of Protein Rotational Mobility on Lipid/Protein Ratio—In this study, we define the protein mobility to be the rotational mobility of the spin label covalently bound to the Ca-ATPase, as measured by ST-EPR (see legend to Fig. 2). Previous studies have provided considerable evidence that the covalently bound spin label undergoes rotational motion primarily in the microsecond time range and that this slow motion probably corresponds to overall rotation of the Ca-ATPase about the membrane normal (Thomas and Hidalgo, 1978; Squier and Thomas, 1986b; Squier et al., 1988a, 1988b). Large-scale intramolecular (segmental) protein rotational motions also contribute to the observed motion, although to a much lesser extent (Lewis and Thomas, 1986; Squier et al., 1988a).

ST-EPR spectra are shown for samples with different L/P ratios in Fig. 2. The effective rotational correlation times (τr) obtained from the experimental ST-EPR spectra were determined by comparing these spectra with reference spectra obtained from isotropically tumbling spin-labeled hemoglobin in solutions of known viscosity (η) and temperature (Thomas et al., 1976; Squier and Thomas, 1986a). As seen from both the spectra (Fig. 2) and the related graph depicting the dependence of protein rotational mobility on the L/P ratio (Fig. 3), there is initially no change in the spectral shape as lipid is removed; however, below the critical lipid number necessary for maximal ATPase activity (L/P = 48 ± 5), there is a progressive increase in the total spectral intensity as well as diagnostic line height ratios (i.e. L'/L; see Fig. 2), indicating that the microsecond rotational mobility of the Ca-ATPase decreases only as the L/P ratio is decreased below the critical lipid number necessary for enzymatic activity.

The quantitative relationship between protein rotational mobility, defined in the legend to Fig. 2, and the L/P ratio, shown in Fig. 3, mirrors the loss in calcium-dependent ATPase activity shown in Fig. 1. A linear correlation between the calcium-dependent ATPase activity and protein mobility is obtained when the enzymatic activity is plotted directly against protein mobility (Fig. 4; data taken from Figs. 1 and 3). A similar correlation between enzymatic activity and protein rotational mobility is observed independent of the...
method of delipidation. Phosphoenzyme decomposition is selectively inhibited under these conditions (Hidalgo et al., 1986), suggesting that protein mobility is critical to the partial reactions involving phosphoenzyme hydrolysis.

Conventional EPR measurements using spin-labeled lipid analogs resolve two motional populations of lipid in native SR. As the L/P ratio is reduced, an increasing fraction of lipid spin labels have restricted motion on the conventional (nanosecond) EPR time scale (Thomas et al., 1982). However, the number of lipids in this restricted fraction (i.e. 20–32 lipids/Ca-ATPase) (Nakashima and Ohnishi, 1975; Jost and Griffith, 1978; Thomas et al., 1982; Silvius et al., 1984; East et al., 1985; Bigelow and Thomas, 1987) as well as the motional properties of both the mobile and restricted lipids are virtually unchanged upon decreasing the L/P ratio (Thomas et al., 1982; East et al., 1985). This “boundary lipid” has been proposed to be critical to function for many membrane-bound enzymes (reviewed by Marsh and Watts, 1982; and Marsh, 1985). We find that significantly more lipid (i.e. L/P = 45–50) is necessary for maximal enzymatic activity than this restricted fraction, indicating that the presence of the motionally restricted lipid alone is not sufficient to maintain optimal enzymatic activity.

**Dependence of Calcium-dependent ATPase Activity on Water Concentration—**In order to further investigate the role of protein mobility in the process involving phosphoenzyme decomposition, we have made use of other conditions (i.e. nonaqueous solvents) that stabilize the phosphoenzyme intermediate (de Meis et al., 1980). When varying amounts of dimethyl sulfoxide, sucrose, or glycerol are substituted for water, the Ca-ATPase activity is inhibited, showing a linear relationship with the water content in all three cases (Fig. 5). Although the relationship between ATPase activity and water concentration is similar, suggesting that water may play a direct role in the enzymatic reaction mechanism (e.g. the solvation of inorganic phosphate) (de Meis et al., 1980), dimethyl sulfoxide causes a much larger inhibition, at the same water content, than either glycerol or sucrose (Fig. 5). All three solvents inactivate the enzyme in a manner that is reversible upon dilution.

**Relationship between Protein Rotational Mobility and Water Concentration—**In Fig. 6, ST-EPR spectra are shown in the presence of increasing amounts of dimethyl sulfoxide; similar results are observed with sucrose and glycerol. As the concentration of water is decreased through the addition of nonaqueous solvents, there is a corresponding increase in both spectral intensity and line height ratio parameters (e.g. $L^*/L$), indicating a reduction in protein rotational mobility. The quantitative dependence of protein mobility on water concentration is shown in Fig. 7. As in the case of Ca-ATPase inhibition, dimethyl sulfoxide causes a larger effect than the other two solvents. A plot of the calcium-dependent ATPase activity (from Fig. 5) against protein rotational mobility (from

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**Fig. 5. Effects of nonaqueous solvents on Ca-ATPase activity.** The calcium-dependent ATPase activity (relative to the control) was assayed in the presence of dimethyl sulfoxide ($\Delta$), glycerol ($\bigcirc$), or sucrose ($\square$) and plotted against the water content. The control activity (normalized to 1.0) was typically 2.6 μmol of P, mg $^{-1}$ min $^{-1}$ at 25 °C. Similar inactivation profiles are observed at 4 and 37 °C.

**Fig. 6. Effect of dimethyl sulfoxide on ST-EPR spectra of spin-labeled Ca-ATPase.** Dimethyl sulfoxide was used to reduce the concentration of water in the medium. The concentration of dimethyl sulfoxide (w/w) was as follows: 0% (a), 10% (b), 20% (c), and 40% (d). These spectra were recorded at 4°C and have been normalized to a constant number of spins. The base line is 110-G wide.

**Fig. 7. Effect of nonaqueous solvents on protein rotational mobility.** Protein mobility ($\rho_{21}/\rho_0$) was measured from ST-EPR spectra (see Fig. 6) as a function of the water content for dimethyl sulfoxide ($\Delta$), glycerol ($\bigcirc$), and sucrose ($\square$). The integrated intensity of the ST-EPR spectrum was used to calculate correlation times, in this case, due to variations in signals due to weakly immobilized probes (Squier and Thomas, 1986a). Indistinguishable results were obtained at 4, 25, and 37 °C; the data represent the average of these measurements.
Conventional EPR spectra of stearic acid spin labels indicates that the addition of nonaqueous solvents (i.e. dimethyl sulfoxide, glycerol, and sucrose) has only a slight effect on lipid hydrocarbon chain dynamics (Table I). The order parameters of 5-SASL and 16-SASL, which probe hydrocarbon chain dynamics near the polar head group region and bilayer center, respectively, are increased only slightly by the addition of these nonaqueous solvents. These increases in order parameter correspond to increases in lipid viscosity with less than 20% for 5-SASL and less than 8% for 16-SASL using the model-independent calibration plots of Squier et al. (1988b).

The protein rotational mobility of the Ca-ATPase in SR, as measured by ST-EPR, has been shown to be inversely proportional to $N \pi/T$, where $\pi$ is the membrane viscosity and $N$ is the mean number of associated proteins in a rotating unit (Squier et al., 1988a), as predicted by a general theory for the rotational motion of membrane proteins (Saffman and Delbrück, 1975). Since the viscosity of the aqueous medium varies only slightly over this range of experimental conditions (1-6 centipoise) (Mellan, 1970) and is in all cases much smaller than that of the bilayer (Squier et al., 1988b), its effect on the overall rotational mobility is negligible (Saffman and Delbrück, 1975; Hughes et al., 1982; Peters and Cherry, 1982).

Furthermore, since the increases in lipid viscosity upon the addition of these nonaqueous solvents are also small (see above), the large decreases in protein mobility must be due to increases in $N$, i.e. to protein aggregation.

![Fig. 8. Solvent-independent correlation between ATPase activity and protein mobility. The data obtained from the ordiates of Figs. 5 and 7 are directly compared for the solvents dimethyl sulfoxide (D), glycerol (C), and sucrose (L).](image)

**TABLE I**

| Solvent                | 5-SASL Order Parameter | 16-SASL Order Parameter |
|------------------------|------------------------|-------------------------|
| Control                | 0.74 ± 0.01            | 0.065 ± 0.002           |
| 40% (w/w) dimethyl sulfoxide | 0.75 ± 0.01          | 0.070 ± 0.002           |
| 40% (w/w) glycerol      | 0.77 ± 0.01            | 0.059 ± 0.002           |
| 40% (w/w) sucrose       | 0.73 ± 0.01            | 0.059 ± 0.002           |

*S is the measured order parameter obtained from the anisotropic motion of the stearic acid spin label in SR at 25°C (see Squier et al., 1988b); the nitroxide was positioned at the indicated position from the carbonyl group. Control indicates a solution containing only 20 mM MOPS (pH 7.0). The other solutions contained the same buffer plus the indicated nonaqueous solvent. Uncertainties represent the standard errors of the mean.

**DISCUSSION**

**Summary of Results**—We observe, under conditions previously shown to selectively inhibit phosphoenzyme decomposition (de Meis et al., 1980; Hidalgo et al., 1986), that enzymatic inactivation correlates with protein immobilization (probably due to protein association), suggesting that enforced interactions among Ca-ATPase polypeptide chains inhibit enzymatic activity.

Other studies have provided evidence that ST-EPR primarily measures the overall rotational mobility of the Ca-ATPase (Thomas and Hidalgo, 1978; Squier et al., 1988a), that stearic acid spin labels provide a valid measure of lipid fluidity (inverse of viscosity) in SR (Squier et al., 1988b), that lipid fluidity modulates Ca-ATPase rotational mobility (Hidalgo et al., 1978; Bigelow et al., 1986; Bigelow and Thomas, 1987; Squier et al., 1988a, 1988b), that both the calcium-dependent ATPase activity and associated transport activity correlate strongly with protein rotational mobility (Hidalgo et al., 1978; Thomas et al., 1982; Bigelow et al., 1986; Bigelow and Thomas, 1987; Squier et al., 1988a), and that interactions between Ca-ATPase polypeptide chains are important to the enzymatic reaction mechanism (Hill and Inesi, 1982; Kurobe et al., 1983; Yantorno et al., 1983; Squier et al., 1988a). The question remained, however, whether the dependence of enzymatic activity on protein mobility was primary or was a secondary consequence of the dependence on lipid fluidity. This study, using conditions that selectively inhibit protein mobility without significantly affecting lipid hydrocarbon chain dynamics, suggests that protein mobility is a primary determinant of enzymatic activity in SR.

**Partial Delipidation**—If protein-protein interactions are important to the Ca-ATPase reaction, then it might be predicted that the activity would increase over some range of L/P ratio, but both enzymatic activity and protein rotational mobility are constant from L/P = 48 to 90. The simplest explanation is that the interactions among proteins are constant over this range due to attractive interactions that maintain optimal protein-protein contacts as long as a minimum lipid concentration (L/P ≥ 48) is present. This model is supported by the virtually identical patterns of inactivation observed independent of the method of delipidation, which results in Ca-ATPase polypeptides whose mass is symmetrically or asymmetrically distributed across the bilayer (Herbette et al., 1981; Nakamura and Ohnishi, 1975), thereby altering the relative spacing of the large aqueous portion of the Ca-ATPase. Furthermore, when the Ca-ATPase is reconstituted in the presence of a large excess of lipid, large protein-free lipid domains are observed (Lenz et al., 1985), suggesting the presence of an attractive force between Ca-ATPase molecules that maintains optimal interactions between Ca-ATPase monomers (reviewed by Abney and Owicki, 1985).

The motional properties of stearic acid spin labels in either the mobile or restricted population of lipids surrounding the Ca-ATPase are virtually unchanged upon decreasing L/P ratio (Thomas et al., 1982; East et al., 1985), indicating that the decrease in protein mobility upon decreasing the L/P ratio is not the result of changing the fluidity of the membrane lipids associated with the Ca-ATPase, as had previously been suggested (Napier et al., 1987). Our data indicate that the requirement of a minimal L/P ratio for maximal calcium-dependent ATPase activity is due to a requirement for enough solvating lipid to mobilize the Ca-ATPase (Figs. 1 and 3). The hydrophilic portion of the Ca-ATPase protrudes into the cytoplasm and has a significantly larger diameter than the membrane-bound hydrophobic portion of the molecule (Le Maire et al., 1981; Blasie et al., 1985; Taylor et al., 1984, 1986).
and therefore defines the minimal space necessary for enzymatic function \((L/P = 45-50)\). This is considerably greater than the lipid number in the first annulus around the protein, i.e. \(L/P = 20-32\) (Nakamura and Ohnishi, 1975; Jost and Griffith, 1978; Thomas et al., 1982; Silviu et al., 1984; East et al., 1985; Bigelow and Thomas, 1987). Therefore, we propose that inactivation at lipid/protein ratios less than 48 ± 5 is not due to depletion of the boundary layer, but is due to enforced protein-protein contacts (aggregation) among hydrophilic portions of the Ca-ATPase. Thus, the effects considered in this paper should be distinguished from, but do not rule out, a proposed specific role for annular lipids in the enzyme mechanism (Bigelow and Thomas, 1987).

**Nonaqueous Solvents**—Although the addition of nonaqueous solvents exerts numerous nonspecific effects, their ability to promote the formation of the phosphoenzyme intermediate from inorganic phosphate (de Meis et al., 1986) suggests that they may promote and/or stabilize a conformation of the Ca-ATPase associated with this intermediate of the catalytic cycle. In order to better understand the conformational changes associated with phosphoenzyme stabilization, we have examined the relationship among the calcium-dependent ATPase activity, lipid fluidity, and protein rotational mobility. The calcium-dependent ATPase activity correlates better with protein mobility (Fig. 8) than with water content (Fig. 6) or lipid fluidity (Table I), suggesting that protein mobility is a primary determinant of enzymatic function. In principle, a possible explanation for the solvent effects on protein mobility could be changes in the aqueous viscosity; but these effects are predicted to be much smaller than observed (Saffman and Delbrück, 1975), and the solvent having the smallest effect on aqueous viscosity (dimethyl sulfoxide) has the largest effect on protein mobility and Ca-ATPase activity. Therefore, we propose that the effects of the nonaqueous solvents are due to their promotion of interactions among hydrophilic portions of the Ca-ATPase polypeptide chains, presumably due to the disruption of the water structure.

**Role of Protein Mobility in Ca-ATPase Mechanism**—In this study, we have found that, in general, protein aggregation inhibits the calcium-dependent ATPase activity; and we have found a direct correlation between the ATPase activity and overall protein rotational mobility (Figs. 4 and 8), suggesting a functional role for protein mobility. The experiments involving nonaqueous solvents provide evidence that phosphoenzyme stabilization need not be limited to hydrophobic interactions within the active site, but may be a result of global interactions between polypeptide chains. In fact, protein mobility may be critical to the process involving phosphoenzyme hydrolysis. Overall rotational motions of the Ca-ATPase could be directly involved in the enzyme mechanism (e.g. due to a requirement for rotational rearrangement within an oligomer). Alternatively, the essential motions may be intramolecular conformational changes that are inhibited by enforced protein aggregation. Future studies on the internal structure of the Ca-ATPase will be necessary in deciding among these and other alternatives.

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