Temperature dependence of molecular dynamics and calcium-ATPase activity in sarcoplasmic reticulum

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Introduction

We have studied the role of protein and lipid structure and dynamics in regulating the function of membrane proteins. Our studies have focused on the Ca\textsuperscript{2+}-ATPase enzyme in skeletal muscle sarcoplasmic reticulum (SR), not only because it is one of the key enzymes in muscle, but also because it is one of the best-characterized integral membrane enzymes, serving as a model for the biochemistry and biophysics of active transport systems. The genetic and biochemical characterization of the Ca\textsuperscript{2+}-ATPase enzyme is well underway [1], and the technology has been established for the quantitative analysis of physical parameters such as SR lipid fluidity, Ca\textsuperscript{2+}-ATPase rotational mobility, and Ca\textsuperscript{2+}-ATPase oligomeric state [2-4]. Our goal is to determine the relationships between these physical parameters and Ca\textsuperscript{2+}-ATPase function.

In studying the effects of lipid fluidity, lipid–protein, and protein–protein interactions on Ca\textsuperscript{2+}-ATPase function, we have used spectroscopic probes to monitor the dynamics of lipid and protein components of SR. We have used electron paramagnetic resonance (e.p.r.) of spin-labelled lipids to determine the effective viscosity of the SR lipid environment. By using fatty acid spin labels with nitrooxide groups at different depths in the bilayer, we have been able to measure lipid fluidity near the polar head-group, as well as near the centre of the bilayer [3]. We have also used saturation transfer e.p.r. of spin-labelled Ca\textsuperscript{2+}-ATPase to measure the average rate of rotational diffusion of the Ca\textsuperscript{2+}-ATPase in SR [2, 3, 5].

We have used time-resolved phosphorescence anisotropy (t.r.p.a.) to estimate the rotational rates and mole fractions of different Ca\textsuperscript{2+}-ATPase oligomers. SR is labelled with the phosphorescent dye erythrosin isothiocyanate (ErITC), and the time-dependent decay of the anisotropy of the ErITC emission is measured. Anisotropy, a measure of probe disorder due to protein rotational mobility, is defined as

\[ r = \frac{(I_0 - I_\perp)}{(I_0 - 2I_\perp)} \]  

(1)

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where $I_\parallel$ and $I_\perp$ are the intensities of emitted light measured parallel and perpendicular to the polarity of the exciting beam. The time-dependent decay of anisotropy (Figure 1) is fit to a sum of exponentials plus a residual anisotropy

$$
\frac{r(t)}{r_{\parallel}} = \sum_{j=1}^{n} A_j \exp(-t/\tau_j) + L_{\parallel}
$$

(2)

where each normalized amplitude ($A_j = r_j / r_\parallel$) is related to the mole fraction of species rotating with the corresponding correlation time ($\tau_j$), and $L_{\parallel}$ is the residual anisotropy. In the absence of large aggregates, e.g. in control SR at 25°C, $L_{\parallel} = 0.22$. Any increase in the residual anisotropy $L_{\parallel}$ above $L_{\parallel0}$ is due to the fraction of enzymes that are not mobile (highly aggregated) on the time scale of the phosphorescence lifetime (1 µs–1 ms). In this manner, the number and mole fractions of the different rotating species (oligomeric states) and the mole fraction of ATPase in each oligomeric state can be determined for SR [5]. The rotational correlation times (Equation 2) are inversely proportional to the rotational diffusion coefficients $D_{\parallel}$, which are interpreted according to the equation of Saffman and Delbrück [6]

$$
D_{\parallel} = \frac{kT}{(4\pi\eta^2b\eta)}
$$

(3)

where $b$ is the thickness of the hydrocarbon phase of the lipid bilayer, $\eta$ is the viscosity of the lipid phase and $a$ is the radius of a cylindrical protein rotating about the membrane normal. Thus once $\eta$ has been measured (by e.g. as described by Squier et al. [3]), an observed correlation time can be interpreted in terms of the size of a corresponding oligomeric species. Using these spectroscopic techniques, we have correlated enzymatic activity, SR lipid fluidity, and Ca$^{2+}$-ATPase oligomeric state by selectively perturbing lipid fluidity and/or oligomeric state and measuring the effects on enzyme function.

One of the most useful perturbations of enzymatic activity, lipid fluidity and Ca$^{2+}$-ATPase oligomeric state in SR is temperature variation. With increasing temperature, enzymatic activity and lipid fluidity increase, and Ca$^{2+}$-ATPase oligomeric state shifts toward less aggregated states [5]. Thus, understanding the effect of temperature on SR function is crucial in understanding the roles of lipid fluidity and protein–protein interactions in regulating enzymatic activity. In addition, understanding the ways in which temperature affects activity, fluidity and protein aggregation, as well as how the three parameters are related, may provide insight into the mechanisms of thermal adaptation in biological membranes.

**Temperature dependence of Ca$^{2+}$-ATPase activity**

The first step in investigating temperature effects in SR is to study the temperature dependence of SR function, as measured by Ca$^{2+}$-ATPase activity. Several studies have shown that the Arrhenius plot of Ca$^{2+}$-ATPase activity exhibits a break at approximately 20°C (Figure 2) [2, 7–9]. Once this observation was made, studies of the temperature dependence of SR lipid fluidity, Ca$^{2+}$-ATPase rotational mobility and Ca$^{2+}$-ATPase aggregation state were initiated in an attempt to explain the break in the Arrhenius plot of Ca$^{2+}$-ATPase activity, thus determining the physical parameters which are important in regulating SR function.
Depiction of t.p.a. decays; uniaxial diffusion in the membrane resembles hindered anisotropic diffusion

Arrhenius plot of Ca$^{2+}$-ATPase activity
Reproduced from Bigelow et al. (1986) with permission.

Temperature dependence of SR lipid fluidity and Ca$^{2+}$-ATPase rotational mobility

Changes in SR lipid fluidity were first proposed to explain the break in the Arrhenius plot of Ca$^{2+}$-ATPase activity. It had been observed that Ca$^{2+}$-ATPase
activity and lipid fluidity correlated well above 20°C, and some investigators reported a break in SR lipid fluidity at approximately 20°C [7, 10–12]. However, in these studies a well-defined fluidity parameter was not measured, but rather spectral parameters obtained from e.p.r. spectra of SR membranes were reported. In addition, many of the studies did not consider the effects of the protein component of the membrane on the fluidity of the lipid environment [13].

To determine whether the effects of temperature on lipid fluidity could explain the effects of temperature on Ca²⁺-ATPase activity, Bigelow et al. [2] studied the hydrocarbon mobility of both extracted SR lipid and native SR membrane, using spin labels that detected mobility near the head-group of the lipid (5-SASL (stearic acid spin label)), and near the centre of the bilayer (16-SASL). They found that hydrocarbon mobility correlated well with Ca²⁺-ATPase activity above 20°C, but no break in hydrocarbon mobility was detected, at either spin label position, between 4°C and 40°C [2]. However, it was still not clear how the hydrocarbon mobility measured from e.p.r. spectral parameters related to the true viscosity of the SR lipid environment, and how this fluidity parameter affected SR Ca²⁺-ATPase activity.

To answer this question, Squier et al. [3] used solutions of known viscosity to derive an empirically validated fluidity measurement, based on the hydrodynamic theory of Saffman and Delbrück [6]. The rotational diffusion coefficient of a membrane protein should be proportional to the temperature, and inversely proportional to the viscosity of the membrane and the effective volume of the protein in the membrane (Equation 3). If the membrane viscosity can regulate Ca²⁺-ATPase activity, the mechanism of this action is likely to involve the rate at which a membrane protein can undergo movements or conformational changes in a lipid environment of given viscosity. In this case, the fluidity (defined as T/η) should determine the rate of protein rotational diffusion in SR, which should be related to enzymic activity. Because it is directly related to protein dynamics, the fluidity parameter T/η should be more relevant to Ca²⁺-ATPase activity than other measurements of hydrocarbon mobility.

Squier et al. [3] compared the e.p.r. spectral parameters determined from solutions of known viscosity with spectral parameters obtained from SR lipids at various temperatures, to determine how the SR lipid fluidity varied between 4°C and 40°C. They also used saturation transfer e.p.r. to measure changes in the average rotational mobility of the Ca²⁺-ATPase between 4°C and 40°C. They found that Ca²⁺-ATPase rotational mobility depends directly upon SR lipid fluidity (T/η), and that both rotational mobility and SR lipid fluidity correlate well with Ca²⁺-ATPase activity. In fact, the activation energies for SR lipid fluidity, Ca²⁺-ATPase rotational mobility, and Ca²⁺-ATPase activity are essentially equal above 20°C [3]. However, neither SR lipid fluidity nor Ca²⁺-ATPase rotational mobility showed any break between 4°C and 40°C, suggesting that lipid fluidity (and thus overall rotational mobility of the Ca²⁺-ATPase) alone cannot explain the temperature dependence of Ca²⁺-ATPase activity (Table 1).

Temperature dependence of Ca²⁺-ATPase oligomeric state

The rotational rate determined from saturation transfer e.p.r. is an average, and does not give direct information about multiple oligomeric states of the enzyme that may be present in SR. To resolve different oligomeric species in SR, Birmachau and Thomas [4] performed t.p.a. experiments with ErlTIC-labelled Ca²⁺-ATPase. The t.p.a. decay is sensitive to multiple rotating species, since each
species will contribute a correlation time and amplitude to the decay (Equation 2).

Birmachu and Thomas [4] studied the Ca\textsuperscript{2+}-ATPase in SR using t.p.a. and
resolved at least three species of the Ca\textsuperscript{2+}-ATPase in SR, which probably represent
monomers, dimers and large aggregates of the enzyme.

Now that multiple oligomeric states of the Ca\textsuperscript{2+}-ATPase in SR had been
identified, the question of how lipid fluidity and protein mobility affect SR function
had to be re-examined. Saturation transfer e.p.r. showed that the average
rotational rate of the Ca\textsuperscript{2+}-ATPase is proportional to lipid fluidity, but is this true
for each of the resolved oligomers of the Ca\textsuperscript{2+}-ATPase? Furthermore, what is the
relationship between fluidity and oligomeric state, and between oligomeric state
and enzymic activity? Birmachu and Thomas [4] found that as the temperature
increased from 4°C to 40°C, the rotational rates (reciprocal correlation times,
determined by fitting t.p.a. decays) of all oligomeric states of the enzyme increased
in the same manner as the lipid fluidity. That is, an Arrhenius plot of correlation
times determined from t.p.a. decays, shows a linear increase in all three correlation
times between 4°C and 40°C (Figure 3), as was the case for lipid fluidity, and
average rotational mobility (from saturation transfer e.p.r.). The fluidity story was
now complete. As the temperature is raised from 4°C to 40°C, the fluidity of the
SR lipid increases. This allows all oligomers of the Ca\textsuperscript{2+}-ATPase to rotate at a
faster rate, correlating with increased enzymic activity. However, the effect of fluidity
on rotational rates cannot explain the break in the Arrhenius plot of Ca\textsuperscript{2+}-
ATPase activity at approximately 20°C.

The changes in oligomeric state (as measured by amplitudes determined
by fitting t.p.a. decays) with temperature, however, showed profound differences
from the changes in SR lipid fluidity and Ca\textsuperscript{2+}-ATPase rotational mobility.
Birmachu and Thomas [4] found that as the temperature is raised from 4°C to
20°C, the mole fractions of two populations of smaller aggregates are increased at
the expense of a population of larger aggregates. From 20°C to 40°C, the smallest
oligomeric state (probably monomers) becomes more populated, the number of
medium-sized aggregates (dimers) decreases slightly, while the population of large
aggregates remains essentially unchanged (Figure 4). Thus the aggregation state
changes are significantly different above and below 20°C, suggesting that, in addition
to SR lipid fluidity and Ca\textsuperscript{2+}-ATPase rotational mobility, Ca\textsuperscript{2+}-ATPase
oligomeric state may be important in regulating enzymic activity. In fact, changes
in oligomeric state alone may explain the break in the Arrhenius plot of Ca\textsuperscript{2+}-
ATPase activity at approximately 20°C.

<table>
<thead>
<tr>
<th>Rate Process</th>
<th>(E_a ) (kcal/mol per degree)</th>
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</thead>
<tbody>
<tr>
<td>Lipid fluidity</td>
<td></td>
</tr>
<tr>
<td>5-SASL</td>
<td>11.5 ± 0.3</td>
</tr>
<tr>
<td>12-SASL</td>
<td>11.0 ± 0.4</td>
</tr>
<tr>
<td>Protein mobility</td>
<td>11.2 ± 0.5</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}-ATPase activity</td>
<td></td>
</tr>
<tr>
<td>&gt;20°C</td>
<td>11.8 ± 0.4</td>
</tr>
<tr>
<td>&lt;20°C</td>
<td>23.0 ± 1.0</td>
</tr>
</tbody>
</table>

**Activation energies for SR lipid fluidity, Ca\textsuperscript{2+}-ATPase rotational mobility and Ca\textsuperscript{2+}-ATPase activity**

*Reproduced from Squier et al. (1988) with permission.*
Arrhenius plot of rotational rates of monomers (1), dimers (2) and large aggregates (3) between 4°C and 40°C
Reproduced from Birmachu and Thomas (1990) with permission.

Amplitudes, representing mole fractions of monomers (a), dimers (b) and large aggregates (c) between 4°C and 40°C
Effects of other SR perturbants

In addition to temperature, other perturbants have been used to demonstrate the role that Ca$^{2+}$-ATPase oligomeric state plays in regulating SR function. Squier et al. [14] demonstrated that chemically cross-linking the Ca$^{2+}$-ATPase led to slower average rotational mobility, which led to inhibition of the enzyme. However, because this study employed saturation transfer c.p.r. to detect rotational mobility, it could not directly detect changes between oligomeric states. Voss et al. [15] used t.p.a. to measure changes in oligomeric state due to the addition of the amphiphatic peptide melittin. Melittin, a 26-amino-acid peptide present in bee venom, has been shown to strongly inactivate the Ca$^{2+}$-ATPase in SR (at molar ratios of 10–20:1 melittin:ATPase). This action correlates with a shift in the aggregation state of the Ca$^{2+}$-ATPase towards highly aggregated enzyme complexes (Figure 5). Mahaney and Thomas [16] used c.p.r. to determine that melittin does not affect lipid fluidity to nearly the same extent as it affects protein rotational mobility. Thus, melittin was shown to inhibit the Ca$^{2+}$-ATPase by aggregating the enzyme, not by changing the fluidity of the SR lipid environment.

The identification of an inhibitor that worked by aggregating the Ca$^{2+}$-ATPase supported the notion that oligomeric state was an important regulator of enzymic activity. However, the mechanism of this action remained a mystery. The next step was to identify an enzyme activator that worked by changing the oligomeric state of the enzyme. Bigelow and Thomas [5] showed that the general anaesthetic, diethyl ether, activated the enzyme and increased lipid fluidity. While the maximum activation of the enzyme by ether was 2-fold [at approximately 8% (v/v) ether], the increase in lipid fluidity was substantially less than 2-fold. Bigelow and Thomas [5] then analysed the component of the lipid in close contact with the Ca$^{2+}$-ATPase, i.e. the boundary lipid, and found that it was also mobilized 2-fold. Birmachu and Thomas [4] later used t.p.a. to determine that addition of either results in the formation of smaller Ca$^{2+}$-ATPase oligomers from larger aggregates, which correlates with activation of the enzyme. The Ca$^{2+}$-ATPase can be activated or inhibited by changing the average oligomeric state to favour smaller or larger oligomers.

At this point, it became clear that changes in oligomeric state were central in determining the temperature-dependent activation of the Ca$^{2+}$-ATPase. Between 4°C and 20°C, larger aggregates are broken down into smaller aggregates, and the fluidity of the SR membrane increases, with the net result being a sharp increase in enzymic activity. From 20°C to 40°C, the oligomeric state is shifted slightly to favour monomers as opposed to dimers, and the SR lipid fluidity increases as it does at lower temperatures, with the net result being a more gradual increase in enzymic activity. The activation energies for SR lipid fluidity, Ca$^{2+}$-ATPase average rotational mobility, and Ca$^{2+}$-ATPase enzymatic activity are similar above 20°C, suggesting that lipid fluidity alone dominates the temperature-dependent activation of the enzyme above 20°C. However, the changes in oligomeric state correlate well with the break in the Arrhenius plot, suggesting that changes in oligomeric state dominate Ca$^{2+}$-ATPase activity below 20°C.

To explore further the effects of oligomeric state on Ca$^{2+}$-ATPase activity, we have used halothane, another volatile anaesthetic, to modulate the SR lipid fluidity and Ca$^{2+}$-ATPase aggregation state. The effect of halothane on Ca$^{2+}$-ATPase activity is itself temperature dependent, with addition of halothane at low temperatures resulting in a steep activation, and addition of halothane at higher temperatures resulting in a small activation, followed by an inhibition at higher concentrations of halothane (Figure 6). Halothane was found to increase SR lipid
Time-resolved phosphorescence anisotropy (t.p.a.) decays in the presence of 0, 5, 10, 15 and 20 mol melittin:mol ATPase

The higher \( R_\infty \) represents a greater fraction of highly aggregated enzyme. Reproduced from Voss et al. (1991) with permission.

Fluidity to the same extent at both high and low temperatures. However, there are some differences in the effect of halothane on oligomeric state (detected by t.p.a.) between 4°C and 25°C. At 4°C, addition of approximately 7 mM halothane results in a shift in the aggregation state of the Ca²⁺-ATPase towards the smallest oligomeric state, at the expense of the population of large aggregates. At 25°C, the same concentration of halothane produces the same increase in the population of monomers, but the population of large aggregates is almost depleted at this temperature, so the formation of monomers from dimers is favored [17]. This suggests that interactions between monomers may be important for Ca²⁺-ATPase activity, since the formation of a large number of monomers from dimers results in enzyme inhibition. This is consistent with other studies which have suggested that dynamic transitions between monomers and dimers may be important for enzyme function [13, 18, 19]. Depletion of the dimer state may then be the mechanism of halothane inactivation of the Ca²⁺-ATPase at high temperature.

A model for the temperature-dependent activation of Ca²⁺-ATPase

Using information about the temperature-dependent changes in oligomeric state determined from t.p.a. experiments, and the temperature-dependent changes in SR lipid fluidity determined from e.p.r. experiments, we have now simulated the temperature-dependent activity of the Ca²⁺-ATPase based on the following formula:

\[
\text{Activity} = [\alpha_{\text{Flu}}(T) - B], \text{ above } 20^\circ\text{C}
\]

\[
\text{Activity} = [\alpha_{\text{Flu}}(T) - C] * [X_1(T)I_1 + X_2(T)I_2 + X_3(T)I_3], \text{ below } 20^\circ\text{C}
\]
Ca\textsuperscript{2+}-ATPase activity, normalized to the control activity at each temperature, measured at 4, 7, 10, 15 and 25°C in the presence of halothane

Halothane expressed as membrane concentration (3.15 mM in the membrane = 7 mM solution concentration). Reproduced from Karon and Thomas (1993) with permission.

where \( \alpha \) is the ratio between Ca\textsuperscript{2+}-ATPase activity and SR lipid fluidity, Flu(\( T \)) is the temperature-dependent fluidity of SR lipid determined from c.p.r. experiments [17], and \( B \) and \( C \) are constants. \( \lambda(T) \) are the temperature-dependent mole fractions of Ca\textsuperscript{2+}-ATPase aggregates, determined from amplitudes derived from t.p.a. experiments performed by Birnach and Thomas [4], where \( \lambda_1 = A_1/A_1 + A_2 + A_3 \). \( I_1 \) represents the intrinsic activity of putative monomers (\( I_1 \)), dimers (\( I_2 \)) and larger aggregates (\( I_3 \)) (Figure 7).

To fit the experimental data (determined from ATPase assays between 4°C and 40°C; see Karon and Thomas [17]), Flu(\( T \)) and \( \lambda(T) \) were determined from experimental data as described previously, then \( \alpha \) and \( B \) were varied to find the best fit to the activity data above 20°C, using a least-squares minimization procedure based on the Marquardt algorithm. The value of \( \alpha \) determined from this procedure was then used to find the best fit to the experimental data below 20°C (with \( C, I_1, I_2 \) and \( I_3 \) allowed to vary). The data were best fit using the following parameters: \( \alpha = 14.7, \quad B = 8.35, \quad C = 4.57, \quad I_1 = 0.42, \quad I_2 = 0.56, \quad I_3 = -0.22 \).

The activation energies for the simulated data are 31.7 kcal/mol·K below 20°C, and 16.9 kcal/mol·K above 20°C, in agreement with the experimental data (30.4 and 17.2 kcal/mol·K, below and above 20°C). The model predicts that dimers have the greatest intrinsic activity (\( I_2 = 0.56 \)), with monomers being slightly less active (\( I_1 = 0.42 \)), and large aggregates inhibiting Ca\textsuperscript{2+}-ATPase activity (\( I_3 = -0.22 \)). The model is consistent with all of the observations (about changes in oligomeric state) that have been discussed previously. At low temperatures, a
A significant fraction of the Ca\textsuperscript{2+}-ATPase exists in a highly aggregated form, and shifting the aggregation state towards monomers and dimers activates the enzyme (as occurs with increasing temperature or addition of a volatile anaesthetic). Above 20°C, the Ca\textsuperscript{2+}-ATPase is in the most active aggregation state (an equilibrium between monomers and dimers), and raising the temperature from 20°C to 40°C increases the lipid fluidity, which results in enzyme activation. Adding large amounts of anaesthetic above 20°C results in the formation of monomers from dimers, which inhibits enzymic activity because dimers have greater activity than monomers.

The results of our experiments with melittin, ether and halothane have helped to elucidate the role of the oligomeric state in regulating Ca\textsuperscript{2+}-ATPase enzymic activity. Ca\textsuperscript{2+}-ATPase can be thought of as existing in two basic aggregation states: a sub-optimal aggregation state, either highly aggregated or highly disaggregated (all monomers), in which dynamic transitions between oligomeric states of the Ca\textsuperscript{2+}-ATPase are less likely; or an optimal aggregation state, in which monomers and dimers are the predominant oligomeric states. Melittin, ether and halothane all have the ability to affect aggregation state, and thus affect enzymic activity. Once the Ca\textsuperscript{2+}-ATPase is in an optimal aggregation state, however, SR lipid fluidity is the principal determinant of Ca\textsuperscript{2+}-ATPase activity.
The effect of fatty acid chain length on the Ca\textsuperscript{2+}-ATPase

Evidence against a role for SR lipid fluidity in regulating Ca\textsuperscript{2+}-ATPase enzymic activity has been accumulated by some investigators. One study, in which the Ca\textsuperscript{2+}-ATPase was reconstituted into lipids of different fluidity, found no correlation between lipid fluidity and Ca\textsuperscript{2+}-ATPase activity. The authors used lipids of differing fatty acid chain length and degree of saturation to show that lipid fluidity and Ca\textsuperscript{2+}-ATPase activity are unrelated [20]. In a subsequent study, it was found that fatty acid chain length is an important regulator of Ca\textsuperscript{2+}-ATPase activity. Ca\textsuperscript{2+}-ATPase enzymic activity and Ca\textsuperscript{2+} binding to the Ca\textsuperscript{2+}-ATPase were found to be dependent upon fatty acid chain length, with C\textsubscript{18} lipids supporting the greatest activity, and shorter or longer fatty acids resulting in enzyme inhibition [21]. Together, these results suggest that fatty acid chain length, and not membrane fluidity, is important in regulating Ca\textsuperscript{2+}-ATPase function.

However, a recent study suggests that Ca\textsuperscript{2+}-ATPase aggregation state may be profoundly affected by fatty acid chain length. Cornea and Thomas [22] have shown that the Ca\textsuperscript{2+}-ATPase is least aggregated when reconstituted in C\textsubscript{14} lipids, with shorter or longer fatty acid chains resulting in substantial enzyme aggregation. As with temperature, melittin, ether and halothane, fatty acid chain length profoundly influences the aggregation state of Ca\textsuperscript{2+}-ATPase. When the aggregation state of the enzyme changes to a sub-optimal state due to any of these perturbants, Ca\textsuperscript{2+}-ATPase activity is inhibited. However, these results do not contradict evidence that suggests that the Ca\textsuperscript{2+}-ATPase, in an optimal aggregation state, is regulated by the fluidity of the SR lipid environment. Furthermore, it is important to remember that the Ca\textsuperscript{2+}-ATPase of skeletal SR is in an optimal aggregation state under physiological conditions, and would be expected to be regulated by SR lipid fluidity more than Ca\textsuperscript{2+}-ATPase aggregation state.

The physical mechanism of the aggregation effect

Although we have presented evidence that oligomeric state plays a central role in regulating the enzymic activity of the Ca\textsuperscript{2+}-ATPase, the physical mechanism of the aggregation effect still remains a mystery. Changes in membrane fluidity cannot explain the changes in Ca\textsuperscript{2+}-ATPase oligomeric state observed with increasing temperature. There is no theoretical reason why changing the fluidity of the lipid environment should result in either association or dissociation of proteins. Furthermore, experiments with melittin and halothane have shown that it is possible to change lipid fluidity, and Ca\textsuperscript{2+}-ATPase oligomeric states, to different extents. The finding that ether affects boundary lipid fluidity to a greater extent than bulk lipid fluidity raises the possibility that boundary lipid fluidity may determine Ca\textsuperscript{2+}-ATPase oligomeric state, and thus be responsible for the break in the Arrhenius plot of Ca\textsuperscript{2+}-ATPase activity. This possibility is indeed intriguing since temperature alone also changes boundary lipid properties [23]. Further investigation is needed to determine whether changes in boundary lipid properties correlate with changes in Ca\textsuperscript{2+}-ATPase oligomeric state between 4°C and 40°C.

Conclusion

We have studied the roles of SR lipid fluidity and Ca\textsuperscript{2+}-ATPase oligomeric state in the temperature dependence of Ca\textsuperscript{2+}-ATPase function. We have found that SR
lipid fluidity increases as a function of temperature, and that this effect dominates the temperature-dependent activation of the Ca\(^{2+}\)-ATPase above 20°C. However, an Arrhenius plot of SR lipid fluidity (or Ca\(^{2+}\)-ATPase rotational mobility) shows no break between 4°C and 40°C. The temperature-dependent changes in Ca\(^{2+}\)-ATPase oligomeric state are different above and below 20°C. From 4°C to 20°C, larger (inactive) aggregates of the Ca\(^{2+}\)-ATPase are breaking up into monomers and dimers, resulting in a steep activation of the enzyme. Above 20°C, the equilibrium between monomers and dimers shifts slightly, but this has little effect on enzyme activity. Rather, the temperature-dependent increase in SR lipid fluidity is primarily responsible for enzyme activation. We conclude that the break in the Arrhenius plot of Ca\(^{2+}\)-ATPase activity at approximately 20°C is due to a shift in the aggregation state at approximately this temperature — from a sub-optimal aggregation state in which large aggregates prevent dynamic transitions between smaller oligomers of the enzyme — to an optimal aggregation state in which monomers and dimers of the Ca\(^{2+}\)-ATPase predominate.

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**References**