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Temperature dependence of kinetic parameters of $(Ca^{2+} + Mg^{2+})$-ATPase in rabbit and winter flounder sarcoplasmic reticulum

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The effect of temperature on the activity of $(Ca^{2+} + Mg^{2+})$-ATPase in rabbit (R) and winter flounder (F) sarcoplasmic reticulum (SR) has been investigated. The enzymes from the two sources appear to be differently adapted to temperature. $(Ca^{2+} + Mg^{2+})$-ATPase activity of FSR was high at 35 °C, and declined to zero at 40 °C, whereas RSR was still active above 45 °C. K_m for ATP increased with temperature in a biphasic fashion for both enzymes. The K_m values for FSR were 69–75 μM at lower temperatures (9–18 °C) and 80–187 μM at higher temperatures (18–35 °C). Values for RSR were 6.5–13 μM below 32 °C and 37–186 μM above this temperature. At their respective physiological temperatures the enzyme from both rabbit and flounder exhibited similar K_m (70–80 μM). Effective ATP binding enthalpies were 3–5-times lower for FSR than for RSR in both temperature regions. Binding energies increased with temperature 3–4-fold for enzyme in both SR. The enzyme in FSR is suggested to be a more effective catalyst than the one in RSR in the sense that its activation energy for ATP hydrolysis is lower. These variations may arise from dissimilarities in either the protein, or in its surrounding lipid, or both. RSR and FSR are significantly different in the nature of the unsaturated chains in their constituent lipids. The difference in lipid composition might account for some of the deversity in the kinetic parameters.

Introduction

In order to determine the potential influence of the lipid millieu on membrane enzymes, it has been common to manipulate the lipids in the membrane by actions such as treatment with phospholipases or to reconstitute purified enzymes in different pure lipid environments. In these sorts of studies the manipulations themselves place the enzyme in an environment which is non-physiological. If one assumes that there is normally a close matching between an enzyme and its lipid environment in order to achieve optimal function, then it would be worthwhile to also attempt studies where the normal millieu is maintained but there were differences in the millieu of the samples studied.

To attempt this we have chosen to investigate the properties of one enzyme, $(Ca^{2+} + Mg^{2+})$-ATPase, in two different natural environments. We have investigated the $(Ca^{2+} + Mg^{2+})$-ATPase from rabbit (R) and winter flounder (F) sarcoplasmic reticulum. It is known that the lipid environments in those membranes are different from one another. At this point it is not possible to determine how much, if any, structural difference there is between the enzyme molecules from the two sources. The ultimate objective is to study the matching of protein and lipid in both membranes to see how they optimize function.

It is first necessary to determine if there are differences in the physiological properties of the enzyme from the two environments. Here we report on the influence of temperature on the kinetic properties of the $(Ca^{2+} + Mg^{2+})$-ATPase from the two sources. Studies

Abbreviations: $(Ca^{2+} + Mg^{2+})$-ATPase, Ca^{2+}-stimulated, Mg^{2+}-dependent adenosinetriphosphase; SR, sarcoplasmic reticulum; RSR, rabbit sarcoplasmic reticulum; FSR, flounder sarcoplasmic reticulum; K_m, Michaelis-Menten constant; V_m, maximum initial velocity; V_i, initial velocity; E_a, activation energy.

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of the temperature dependence of ATP-hydrolysis by $(Ca^{2+} + Mg^{2+})$-ATPase isolated from rabbit white skeletal muscle resulted in non-linear Arrhenius plots [1,2]. In contrast to rabbit, the $(Ca^{2+} + Mg^{2+})$-ATPase of fish SR showed a linear Arrhenius plot, a disparity which is believed to reflect the differences in the lipid composition of the membranes [3]. Analysis of fatty acids in rabbit SR showed that palmitate, oleate and linoleate represented more than 85% of fatty acid content of membrane lipids [4,5]. In flounder SR, approx. 20% of the fatty acids was palmitate, and high amounts of eicosapentaenoate and docosahexaenoate (more than 55% in total) were observed [6]. The nature of fatty acyl chains in the microenvironment of the protein affected the thermostability of the Ca-ATPase from crayfish abdominal muscle and locust flight muscle [7].

The variation with temperature of the substrate-binding affinity of the membrane Mg^{2+}-ATPase of *Acholeplasma laidlawii* B can strongly influence the behavior of Arrhenius plots. This can lead to altered values of E_a and break temperatures, and even create artifactual breaks in some cases if enzyme activity is assayed at a single fixed substrate concentration [8]. Therefore the present study deals with detailed investigation of temperature dependence of the main kinetic parameters, the maximum initial velocity (V_m) and the Michaelis-Menten constant (K_m), and the Van't Hoff enthalpies for substrate binding for $(Ca^{2+} + Mg^{2+})$-ATPase in SR isolated from fish and rabbit. The activation energy of the reaction at various concentrations of substrate has been evaluated. The findings are discussed in the light of data on overall fatty acid composition, and on the thermal properties of the membranes as seen by different scanning calorimetry.

Material and Methods

Sarcoplasmic reticulum from the white skeletal muscle of rabbit (RSR) and from winter flounder (FSR) were isolated essentially by the procedure of East and Lee [9]. Protein concentration was determined by the method of Lowry et al. [10]. $(Ca^{2+} + Mg^{2+})$-ATPase activities in the presence of increasing concentrations of ATP in the range of 0.002–2.0 mM were estimated by incubation of 25 μg of FSR or 10 μg of RSR membrane protein in the presence of 50 mM (pH 7.0) 3-morpholinopropanesulfonic acid, 1.1 mM $CaCl_2$, 1 mM EGTA and 80 mM KCl. All activity measurements were repeated at various temperatures within the range of 9–46 °C. Activity was monitored spectrophotometrically using the coupled assay method of Anderson and Murphy [11].

Total lipid extracts were obtained from suspensions of membrane preparations by the method of Bligh and Dyer [12]. Following 4.5 h of transmethylation of the lipid in a mixture of absolute methanol and concentrated sulfuric acid (47 : 3, v/v) at 65°C, the methyl esters were extracted [13] and the fatty acid composition was determined by gas chromatography using a Perkin-Elmer 8310 apparatus (Norwalk, CN) equipped with 30 m × 0.25 mm capillary column coated with SP2330 (Supelco Inc., Bellefonte, PA). Methyl heptadecanoate was used as an internal standard.

Differential scanning calorimetry was performed using a Microcal MC-2 Calorimeter (Amherst, MA). Membrane suspensions in assay buffer (containing calcium but not ATP) at 6.95 mg protein per ml for FSR and 6.95 mg protein for RSR were subjected to successive heating scans form approx. 5 °C up to 40 °C twice, followed by two successive scans from 5 °C to 80 °C. The assay buffer containing calcium was used as a reference.

Results

Investigation of isolated SR membrane fractions by electrophoresis according to Laemli [14] showed a major band with molecular mass 100–110 kDa, consistent with that of $(Ca^{2+} + Mg^{2+})$ATPase. Visual inspections suggested that about 70% of RSR and FSR proteins were $(Ca^{2+} + Mg^{2+})$-ATPase. Possible contamination by sarcolemmal $(Na^+ + K^+)$-ATPase (its α-subunit is also approx. 100 kDa) was tested by measuring its activity in the presence of 5 mM $MgCl_2$, 10 mM KCl and 100 mM NaCl. In RSR the $(Na^+ + K^+)$-ATPase activity was not detected, and in FSR it was less than 1% of the activity of $(Ca^{2+} + Mg^{2+})$-ATPase.

The activity of $(Ca^{2+} + Mg^{2+})$-ATPase in the presence of a constant amount of Ca^{2+} was determined at ten to twelve concentrations of ATP over the range of 0.002 to 2.0 mM (examples are given in Fig. 1). Kinetic data were fit to the Michaelis-Menten equation using the Graphpad© program from ISI Software (3501 Market Street, Philadelphia, PA 19104, U.S.A.). At all temperatures, the K_m for ATP was lower for the rabbit

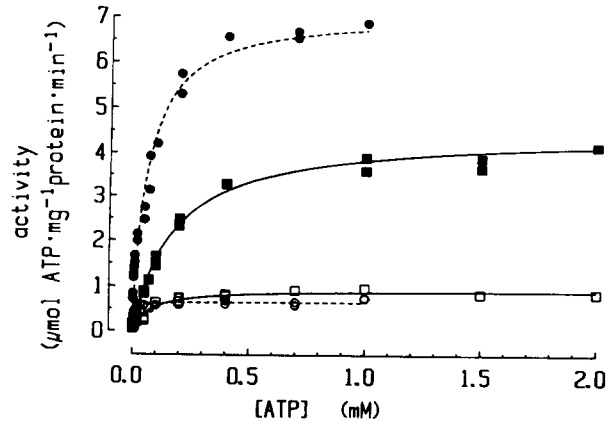

Fig. 1. [ATP] and the activity of $(Ca^{2+} + Mg^{2+})$-ATPase in SR at various temperatures. ●-----●, RSR, $t = 35.2$ °C; ○-------○, RSR, $t = 16.8$ °C; ■———■, FSR, $t = 35.2$ °C; □———□, FSR, $t = 18.1$ °C.

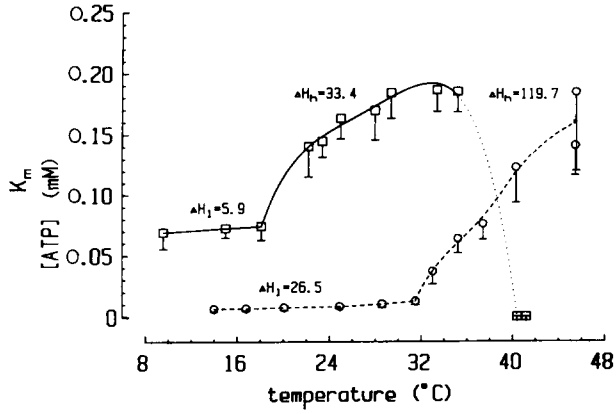

Fig. 2. Temperature dependence of K_m of $(Ca^{2+} + Mg^{2+})$-ATPase in SR. The numerical values give effective Van't Hoff binding enthalpies ($kJ \cdot mol^{-1}$) in the low-temperature and high-temperature regions. The plot for FSR shows a 'reduction' in K_m above 35.6 °C, with it having a value of zero at 40.5 °C and above. It should be noted that this is an artificial effect occurring as a consequence of the thermal denaturation of the enzyme. Error bars represent one S.D. as determined from the numerical analysis, by non-linear curve fitting. o- - - - -o, RSR; □———□, FSR.

SR in comparison to the flounder SR, but the K_m for both RSR and FSR were similar (70–80 μM) in the regions of their respective physiological temperatures (Fig. 2). K_m increased with temperature in a biphasic fashion for the enzyme from both sources, slowly below 18 °C for FSR or 32 °C for RSR, and more rapidly above these 'transition' temperatures. Effective average ATP binding enthalpies were calculated from Van't Hoff plots of K_m values. In the low temperature region, ΔH for ATP binding was determined to be 5.9 $kJ \cdot mol^{-1}$ for FSR and 27 $kJ \cdot mol^{-1}$ for RSR. Above the 'transition' temperatures binding enthalpies increased 4–5-fold for the enzyme from both sources to values of 33 $kJ \cdot mol^{-1}$ for FSR and 120 $kJ \cdot mol^{-1}$ for RSR. The 'reduction' of the K_m of the enzyme from FSR above

TABLE I

Loss of activity with time of incubation at 36.5 °C in FSR

An estimate of initial rate was made over the first 12 s. Rates were estimated at various periods after start of the reaction. Initial rates were 30–55% of the rates measured at 29 °C. Values are mean ± range of two assays.

Time period of measurement of reaction rate (s)	% of initial reaction rate during time period
0– 12	100
60– 72	64± 8
120–132	55±11
180–192	39± 5
240–252	21± 7

32 °C is used as an artificial device to show the thermal denaturation of the enzyme.

Maximum velocities (V_m) of ATP hydrolysis increased with temperature for ATPase in both membranes (Fig. 3). Below 29 °C, values of V_m were slightly higher for enzyme in FSR than in RSR (e.g., 2.25 μmol ATP hydrolysed/min per mg protein for FSR and 1.33 μmol/min per mg for RSR at 25 °C). Near 29 °C the V_m were similar for the enzyme from both sources (2.05 μmol ATP hydrolysed/min per mg for FSR at 29.4 °C and 2.38 μmol/min per mg for RSR at 28.6 °C). Above 30 °C, V_m increased more profoundly with temperature for RSR than for FSR. Activity of ATPase declined to zero between 35.2 and 40.4 °C in FSR whereas in RSR the enzyme was still active at 45.6 °C (13.67 μmol/min per mg). Linear progress curves for the enzyme in FSR were obtained at 35.2 °C, but at 36.5 °C the enzyme rapidly lost activity during incubation. Table I shows specific information about the thermal denaturation of the FSR enzyme at 36.5 °C. The initial reaction rates (over 0–12 s) were about 30–55% of rates measured at 29 °C, but the rate declined rapidly with time as a result of thermal denaturation.

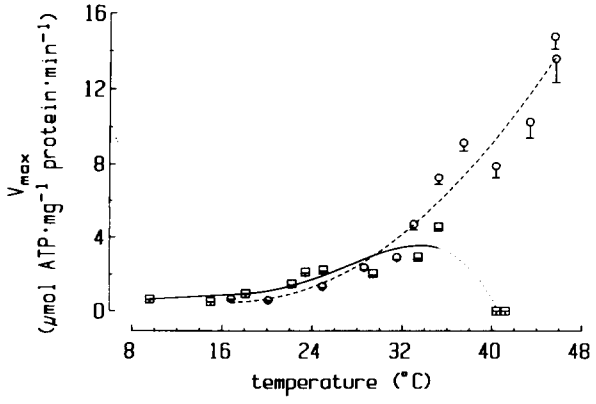

Fig. 3. Temperature dependence of V_m of $(Ca^{2+} + Mg^{2+})$-ATPase in SR. Error bars represent one S.D. as determined from the numerical analysis by non-linear curve fitting. o- - - - -o, RSR; □———□, FSR.

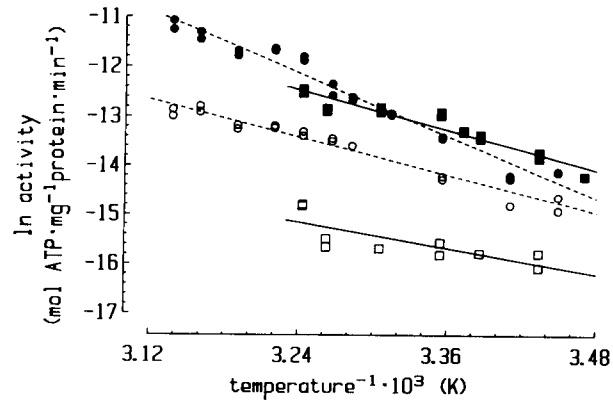

Fig. 4. Temperature and the activity of $(Ca^{2+} + Mg^{2+})$-ATPase in SR; Arrhenius plot. ●- - - - -●, RSR, [ATP] = 1 mM; o- - - - -o, RSR, [ATP] = 0.01 mM; ■———■, FSR, [ATP] = 1 mM; □———□, FSR, [ATP] = 0.01 mM.

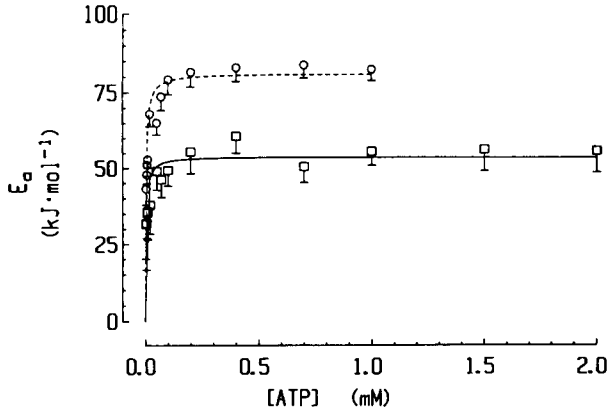

Fig. 5. [ATP] and the activation energy of ATP hydrolysis by (Ca^{2+} + Mg^{2+})-ATPase in SR. Error bars represent one S.D. as determined from the numerical analysis by non-linear curve fitting. o------o, RSR; □———□, FSR.

Evaluation of the temperature dependence of actual ATPase activity at various concentrations of ATP by Arrhenius plots resulted in straight lines for enzyme in both membranes, FSR and RSR (Fig. 4). Up to a limiting value of substrate concentration, the activation energy (E_a) of the reaction was dependent on substrate concentration (Fig. 5). Al all concentrations of ATP investigated the E_a was significantly higher for RSR than for FSR (Fig. 5). The values for E_a at high substrate concentrations were 50–55 and 80–83 kJ · mol^{-1} for the enzyme in FSR and RSR, respectively.

TABLE II

Fatty acid composition of FSR and RSR membranes

n.d., not detected.

Fatty acid	(weight %)	
	FSR	RSR
14:0	3.0	1.2
16:0a *	0.9	3.5
16:0	24.6	31.0
16:1	3.2	2.2
18:0	3.5	7.5
18:1	11.8	15.0
18:2	0.9	17.1
18:3	0.2	1.4
18:4	0.1	n.d.
20:0	0.2	n.d.
20:1	3.6	0.2
20:2	0.2	0.3
20:3	0.1	1.3
20:4	3.0	8.1
20:5	16.5	0.8
22:0	n.d.	0.1
22:1	0.4	n.d.
22:4	0.2	1.6
22:5	2.2	2.5
22:6	23.2	1.0
24:0	0.1	0.2
24:1	0.7	0.5
Other	1.4	4.5

* Tentative identification as the hemiacetal of 16:0 aldehyde

Table II gives the composition of fatty acids found in the preparations of the sarcoplasmic reticulum from the skeletal muscle of rabbit and flounder. In keeping with previous observations [4] the RSR had high proportions of palmitate, oleate and linoleate. The FSR had relatively large amounts of eicosapentaenoate (16.5%) and docosahexaenoate (23.2%), and it contained 24.6% palmitate, 11.8% oleate with only a small content of linoleate (0.9%). The high contents of $(n-3)$ polyunsaturated acids are consistent with, although somewhat lower than, the values found by Luo and Hultin [6].

Fig. 6 shows differential scanning thermograms of FSR and RSR. FSR showed a transition extending from about 32°C to 38°C with a maximum excess specific

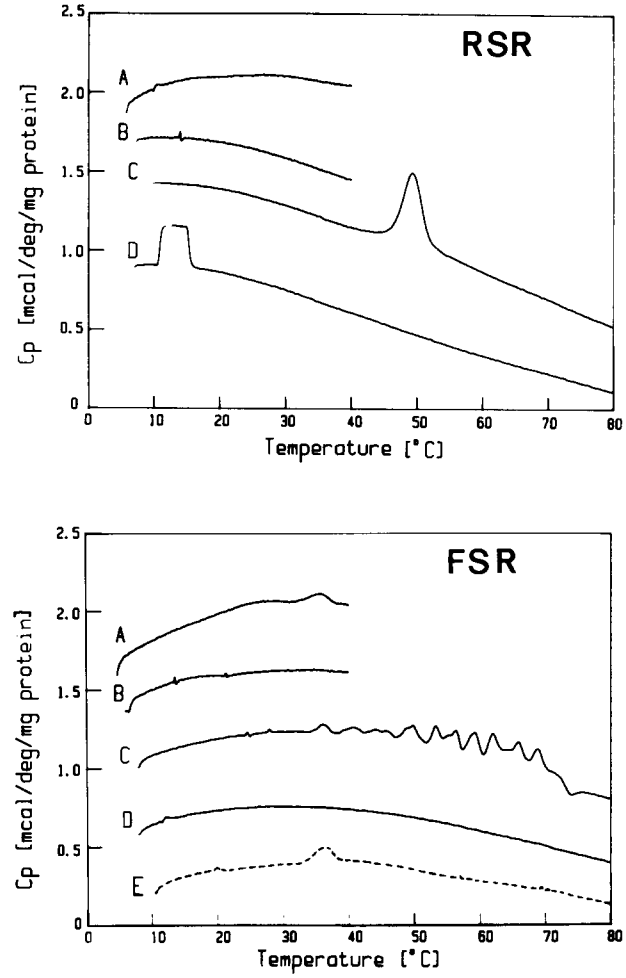

Fig. 6. Differential scanning thermograms of RSR (A) and FSR (B). (A) First scan, to 40°C; (B) second scan, to 40°C; (C) third scan, to 80°C; (D) fourth scan, to 80°C. (E) A different sample of FSR scanned from 4°C to 90°C to show extremes of observed transition profiles in FSR samples. For RSR, the enthalpy change of the endotherm in scan C cup was 1.7 mcal/mg membrane protein. For FSR the enthalpy change for the endotherm in scan A was 0.2 mcal/mg protein. For scan C of FSR the total enthalpy change for all the endotherms was 1.9 mcal/mg protein.

heat at 35.6 °C. This transition was not apparent on a second heating scan to 40 °C. However, a small transition from about 34–38 °C was apparent on a third heating scan followed by a series of endotherms up to about 70 °C. The multiple endotherms were not apparent on a second heating scan to 80 °C. Subsequent investigation of three other preparations of FSR produced a variety of profiles in the calorimeter ranging from two up to the multiple transitions shown in Fig. 6. Trace E in the lower panel of Fig. 6 shows a sample of FSR scanned from 4 °C to 90 °C in order to show the extremes of patterns seen in the calorimetric scans. Some samples showed a simple pattern such as in E whereas others showed more complex patterns such as in D. The one constant factor was that all showed a transition centered near 36 °C. We have not been able to correlate the appearance of the transition profiles with either the presence of calcium or ATP, the pattern of Coomassie blue staining bands on sodium dodecyl sulphate-polyacrylamide gel electrophoresis, or the sample history. RSR showed only one endotherm between 42 and 54 °C with a maximum at 49.3 °C during a scan from 5 °C to 80 °C. This endotherm was not seen on a repeat scan to 80 °C. We note that additional smaller endotherms have been seen in samples of RSR by others [15,16] although we did not observe them in our preparation. The enthalpy change associated with the single endotherm in RSR was 1.7 mcal/mg (7.1 J/g) membrane protein. The enthalpy change associated with the endotherm of FSR at 32 to 38 °C was 0.2 mcal/mg (0.8 J/g) protein. The total enthalpy change for all the endotherms in the scan of FSR to 80 °C was 1.9 mcal/mg (7.9 J/g) membrane protein.

Discussion

In a number of previous papers it has been reported that the $(Ca^{2+} + Mg^{2+})$-ATPase of sarcoplasmic reticulum exhibits complex kinetics of activation with respect to ATP [1,17–19]. The K_m is a function of several rate constants as suggested by the kinetic model for the $(Ca^{2+} + Mg^{2+})$-ATPase of SR proposed by Gould et al. [19]. Over the total temperature range studied, values of K_m for ATP changed more for enzyme in SR from rabbit than in SR from flounder. The lowest and highest values of K_m in the RSR were 6.5 and 186 μM, measured at 14 and 45.6 °C. This 29-fold change in K_m from RSR is compared to a 5–6-fold change in the K_m of the FSR enzyme over the range of temperatures investigated. This indicates that the binding site of the rabbit enzyme is more sensitive to temperature than is the site in the FSR enzyme. The reduced sensitivity to temperature of the FSR might be a consequence of an evolution to a more 'adaptable' enzyme because the fish has to live at a variety of temperatures, while the enzyme of RSR only encounters one physiological temperature. On the other hand, the enzyme in RSR is more heat-stable, that is, the enzyme retains activity at higher temperature than does the enzyme from FSR.

A stimulating observation is that the K_m for enzymes from the two sources are similar at their respective physiological temperatures. While the physiological temperature of the rabbit is at or near 37 °C, the flounder can live in waters with a range of temperatures from about −1.7 °C to 25 °C. Local waters in which the fish are found vary in temperature from −1.7 °C to about 16 °C. The water temperature of the fish used to obtain the FSR in these experiments was approx. 8 °C. Perhaps this observation of nearly equal K_m for enzymes from both sources is purely coincidental. However, there might be an optimal K_m that is related to other cellular factors which themselves are also temperature sensitive. For example, it might be that the concentrations of the substrate in the skeletal muscles of the fish and rabbit are similar at their respective physiological temperatures.

Van't Hoff plots of K_m values yielded the average effective binding enthalpies for ATP which have significantly different values for the enzyme in flounder and in rabbit SR membranes. Over the temperature ranges investigated the ATPase of the FSR membranes exhibited lower values of ATP binding enthalpies than those for ATPase of RSR. The ratio of the binding energies for the enzyme from both sources is similar in the low-temperature and high-temperature regions. This might suggest that the discontinuities which occur near 18 °C and 32 °C in the plots of K_m vs. temperature for FSR and RSR, respectively, represent transitions of a similar nature in the enzyme in both membranes. The suggestion is that the binding sites are changing in a similar fashion, but the changes occur at different temperatures.

Contrary to the increase of K_m for ATP with temperature (Fig. 2) the K_{Ca}, the Ca^{2+} concentration which gives half-maximal activation of the ATPase, was observed to decline with increasing assay temperature for the $(Ca^{2+} + Mg^{2+})$-ATPase of SR from plaice [3]. This difference in the behaviour of binding sites for ATP and for Ca^{2+} suggests that variation of temperature induces different conformational changes in the polypeptide chain in areas of the respective binding sites for Ca^{2+} and ATP.

Earlier studies indicated that Arrhenius plots of ATPase activity of rabbit SR could be resolved into two, or more, straight lines with different slopes [1,2]. The corresponding break temperatures were 18 °C, or 15 °C and 21 °C [1,2]. Our investigation on the ATPase in rabbit SR in the temperature range of 15–46 °C produced linear Arrhenius plots without discontinuities. We have insufficient data at low temperatures to indicate if there was a discontinuity in the region of 15–21 °C. Interpretation of Arrhenius plots has to be done

with caution because of the change in error distributions when activity and temperature are transformed to ln(activity) and T^{-1}. Others have commented on the difficulties associated with construction of Arrhenius plots and the complicated relationship between K_m, velocities and temperatures [8]. The Arrhenius plots for ATPase in winter flounder SR (measured over the range 9–35.2 °C) also fit straight lines without breaks, in agreement with the observation on the enzyme from plaice SR made by McArdle and Johnston [3].

The activation energy for ATP hydrolysis (Fig. 5) suggests that the ATPase in FSR is a more effective catalyst than ATPase in RSR in the sense that its activation energy is lower. This might represent chemical or conformational differences at the active sites. Conformations might in turn be influenced by the lipid environments in the two membranes.

The dependence of the activation energy on substrate concentration is not unexpected (see also Ref. 3). A combination of the Arrhenius and Michaelis-Menten equations predicts the influence of substrate concentrations on E_a:

$$V_i = A \cdot e^{-E_a/R \cdot T} = V_m \cdot [S]/(K_m + [S]) \quad (1)$$

$$E_a = R \cdot T \cdot \{\ln A - \ln V_m - \ln[S] + \ln(K_m + [S])\} \quad (2)$$

When $K_m \gg [S]$,

$$E_a = R \cdot T \cdot \{\ln A - \ln V_m - \ln[S] + \ln K_m\} \quad (3)$$

and E_a is dependent on [S].

When $K_m \ll [S]$,

$$E_a = R \cdot T \cdot \{\ln A - \ln V_m\} \quad (4)$$

and E_a is independent of [S].

When $K_m = [S]$, substitution in Eqn. 2 of $K_m = \{(V_m \cdot [S])/V_i\} - [S])$ yields

$$E_a = R \cdot T \cdot \{\ln A - \ln V_m - \ln[S] + \ln(V_m \cdot [S]/V_i)\}$$

Since $V_m/V_i = 2$,

$$E_a = R \cdot T \cdot \{\ln A - \ln V_m + \ln 2\} \quad (5)$$

and E_a is independent of [S].

At a given temperature when K_m and V_m are constant, high substrate concentrations do not influence the activation energy (E_a) (Eqn. 4) but at low concentrations of substrate, E_a depends on [S] (Eqn. 3). These relationships between the concentration of substrate and E_a were also observed experimentally for the influence of $[Ca^{2+}]$ on ATP hydrolysis by $(Ca^{2+} + Mg^{2+})$-ATPase in plaice SR [3].

The $(Ca^{2+} + Mg^{2+})$-ATPases from RSR and FSR display differences in temperature optima, effective ATP binding enthalpies, activation energies, and temperature dependences of K_m and V_m. These variations may arise from dissimilarities in either the protein, or in its surrounding lipid, or both. Volmer and Veltel [7] found that the surrounding lipid had an influence on the thermal stability of $(Ca^{2+} + Mg^{2+})$-ATPase from SR of crayfish and locust membranes. Our data on RSR and FSR indicate a significant difference in the nature of the unsaturated chains in their constituent lipids. The difference in lipid composition possibly accounts for some of the diversity in the kinetic parameters. Further work on the surrounding lipid, and on the influence of temperature on the protein structure will help to establish more precisely the contributions of lipid and protein to the observed kinetic differences.

The differential scanning thermograms of the membranes from the two sources provide some insight into the contributions of structural changes in the membranes to the observed variations in kinetic parameters with temperature. First, there were no detectable calorimetric events near 18 °C in FSR or 32 °C in RSR, those temperatures representing the points of change in the temperature dependence of K_m for the respective membranes (Fig. 2). This suggests that structural rearrangements in either the protein or lipid or both which accompany these changes in K_m are fairly either small or noncooperative so that they are not detected calorimetrically. Changes in the temperature dependence of K_m might involve small, but critical rearrangements in enzyme, say at the active site. Or they might involve redistribution of lipid and protein contacts in the membrane.

No transitions unequivocally attributable to lipids in the membrane were observed in either case. Given the relatively unsaturated lipid present in each membrane (Table II), this finding is not surprising. The lipid transitions of these membranes would be expected at temperatures below the scan ranges accessible in the calorimeter unless an additive were used to depress the freezing-point (see, for example, Ref. 20). Martonosi [21] observed a lipid-derived transition at about 15 °C in freeze-dried SR membranes. Upon hydration of the membranes the lipid transition seen by Martonosi [21] moved to lower temperatures, a finding consistent with the current observations, and with the influence of hydration on phospholipid transitions [22].

All the calorimetric transitions in both membranes were attributable to protein unfolding by virtue of their irreversible character. The irreversible transition seen in RSR at 49.3 °C is undoubtedly due to the thermal denaturation of the $(Ca^{2+} + Mg^{2+})$-ATPase. It occurs at a temperature consistent with ATPase denaturation seen in other studies using differential scanning calorimetry [15,16] and Fourier transform infrared spectroscopy [23]. The enthalpy change associated with this denaturation (1.7 cal/g) was somewhat lower than those reported for the unfolding of soluble erythrocyte spec-

trin (2.0 cal/g) [24], or for cytochrome oxidase reconstituted in dimyrisytoylphosphatidylcholine (approx. 3.3 cal/g) [25]. Martonosi [21] observed a transition near 52° C in dehydrated membranes which might represent a similar structural change. Other work in this laboratory (unpublished observations) suggests that the enzyme in RSR begins to lose activity above 51° C. The loss of activity in RSR is thus associated with a fairly major change in protein structure. It is interesting that the calorimetric transition at 35.6° C in the FSR, which correlated with loss of activity of the ATPase, is only about one tenth the magnitude of the transition in the RSR. In FSR there were also multiple transitions over a wide range of temperatures whose total enthalpy change (1.9 cal/g) was similar to that of the single RSR transition. One potential interpretation of those findings is that in the FSR the transitions arise from various domains of the ATPase (and other proteins) unfolding at different temperatures. The transition at 35.6° C might then be associated with unfolding in the region of the active site. Some FSR preparations showed less complex patterns on scanning to high temperatures (see Fig. 6, FSR, E). We have no simple explanation for this varied behavior as yet. Further detailed analysis of these multiple transitions will be necessary to elucidate their specific origins. Multiple transitions have been seen in RSR preparations by other workers [15,16]. While one can postulate that these multiple transitions arise from the melting of different domains it is not possible to determine as yet which ones they are, and why the profile is variable. It is fair to say, however, that the domain which unfolds near 36° C in FSR includes the active site for ATP hydrolysis.

These membrane enzymes are examples of the remarkable adaptations which occur in nature. Such adaptations are expressed in both lipid and protein structures. The enzyme from FSR, which operates at lower temperatures than the one from RSR, has lower binding enthalpies for ATP and lower activation energy. Both these factors would contribute to a higher hydrolytic activity for the FSR enzyme at lower temperatures. The K_m for both enzymes is similar in the regions of their respective physiological temperatures. This presumably represents an adaptation to allow for efficient utilization of the substrate pools which in themselves are presumably adapted to physiological needs at the respective temperatures.

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