ACTIVATION ENERGIES OF THE ATPase ACTIVITY OF SARCOPLASMIC RETICULUM

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Received April 12,1974

SUMMARY

Arrhenius plots of Ca^{++}-stimulated ATP hydrolysis by sarcoplasmic reticulum (SR) show breaks (T_t) at 16.7°C and 11.5°C for rabbit and lobster preparations, respectively. The energies of activation (Eact) are about 10 and 19.5 Kcal/mole above and below T_t, respectively, and are similar for both lobster and rabbit SR. The antibiotic filipin increases T_t by about 7°C for both preparations, but the Eact above and below the new T_t values remain similar to those of the controls. Desintegrated membranes do not show breaks in Arrhenius plots and the Eact assume relatively high values. The Ca^{++} ionophore X-537A does not affect either the T_t values nor the Eact.

INTRODUCTION

Sarcoplasmic reticulum membranes (SR) function as a transducer system which interconverts chemical and osmotic energies (1-3). Recent evidence suggests that the functional system in SR is composed only by a major protein (ATPase) and lipids (4). The simplicity of this system makes it particularly attractive for studying the regulation of the activity of membrane bound enzymes.

Using a continuous recording system, we measured the initial rates of the Ca^{++}-stimulated ATP hydrolysis by SR of skeletal muscles of rabbit and lobster. We then estimated the energies of activation and the temperature of phase transition of the ATPase of the two types of SR which differ in their cholesterol content. Filipin, which complexes cholesterol, increases the temperatures at which a break is observed in the Arrhenius plots of the ATPase activity of the SR membranes, whereas the ionophore X-537A, which increases the steady state ATPase of SR (19), does not seem to interact directly with

the enzyme since the energy of activation is unaffected by the ionophore.

MATERIALS AND METHODS

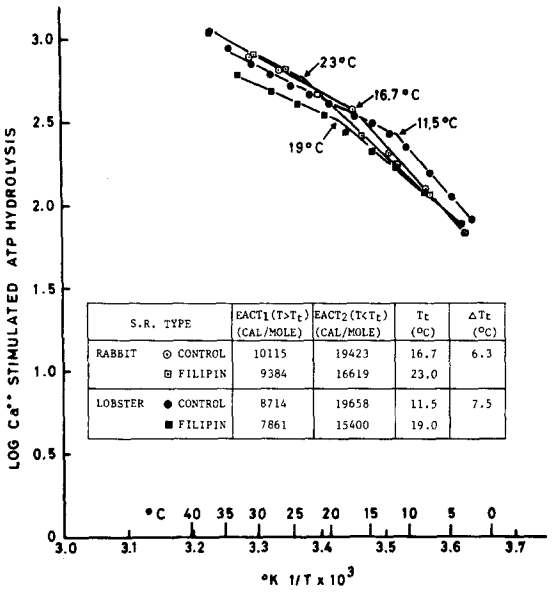
Fragmented SR membranes were isolated from rabbit and lobster muscles as described elsewhere (5). The amount of Ca^{++}-pump protein (ATPase) in the preparations was estimated by SDS polycrylamide gel electrophoresis (6) to be about 50% and 65% of the total protein for rabbit and lobster preparations, respectively.

The ATP hydrolysis was followed by recording continuously the production of H^{+}. The reactions were conduced in a thermostated vessel containing reaction media (50 mM KCl, 5 mM $MgCl_2$, 1 mM ATP, 5 mM Tris, pH 7.2 and about 2.5 mg of SR protein).

Alterations in pH (no more than 0.1 unit for each complete experiment) were followed by a system composed of a Radiometer pH meter, Model 26, and a Perkin Elmer recorder, Model 56, at 1 mV sensitivity. The system was calibrated at the end of each experiment by adding known amounts of titrated NaOH. The Ca^{++}-stimulated ATP hydrolysis was initiated by adding 200 nmoles $CaCl_2$ (40 μM final concentration) to the medium (5 ml) containing the SR. This method permitted us to measure initial rates of ATP hydrolysis.

Lipid fractions were extracted from SR suspensions in chloroform-methanol mixtures (7) which were evaporated under low pressure in a rotatory evaporator and the fractions were freed of H_2O by dissolving in absolute ethanol and evaporating. The protein residues were removed by filtration and centrifugation. The final residues in chloroform were analysed for cholesterol (8) and phospholipids. The phospholipids were separated in TLC plates (0.5 mm silica gel G Merck) run with $CHCl_3$: CH_3OH: 25% NH_4OH: H_2O (70: 30: 4: 1) (9). The amount of phospholipids was determined by measuring the amount of Pi in the scraped spots previously digested in 70% $HClO_4$ (10).

The ionophore X-537A was supplied by Dr. Julius Berger, Hoffmann - - La Roche Inc., Nutley, New Jersey, 07110, U.S.A., and filipin was supplied by Dr. George B. Whitfield, The Upjohn Company, Kalamazoo, Michigan, U.S.A.



S.R. TYPE		EACT1 (T>Tt) (CAL/MOLE)	EACT2 (T<Tt) (CAL/MOLE)	Tt (°C)	ΔTt (°C)
RABBIT	⊙ CONTROL	10115	19423	16.7	6.3
	⊞ FILIPIN	9384	16619	23.0	
LOBSTER	● CONTROL	8714	19658	11.5	7.5
	■ FILIPIN	7861	15400	19.0	

Fig. 1 - Arrhenius plots for the Ca^{++}-ATPase activity of SR isolated from rabbit or lobster skeletal muscle. The experiments were performed in media containing 2.5 mg of SR protein, and the reactions were initiated by adding 200 nmoles $CaCl_2$ (40 μM final concentration). The filipin treatment was made by incubating buffered suspensions of SR (10 mM Tris-maleate, pH 7.2, in 100 mM KCl) with filipin in dimethyl formamide (0.2 μmoles filipin/mg SR) for 1 hr at 0°C. About 2 μl of 100 mM solution of filipin was added per mg protein and the same volume of dimethyl formamide was without effect on the ATPase activity.

RESULTS AND DISCUSSION

Arrhenius plots for the Ca^{++}-stimulated ATP hydrolysis by SR were resolved into two straight lines with transition points at about 16.5°C for rabbit and 11.5°C for lobster SR preparations (Fig.1). Our results for the energy of activation recorded in Fig.1 are essentialy in accord with those obtained by Deamer (4) for lobster SR. However, the value for T_t which we report for lobster SR (11.5°C) is much lower than the value of 17°C reported by Deamer (4). The discrepancy may arise because the value reported by Deamer was obtained by extrapolation in plots with rather scattered experimental points. Other workers (11,12) have failed to find a break in the Arrhenius

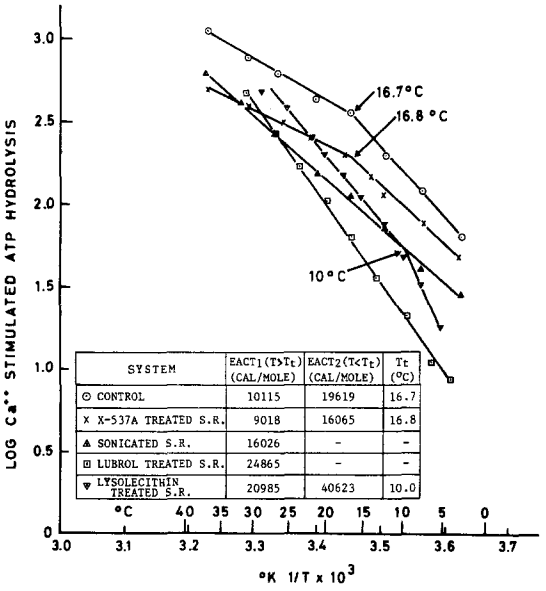
plot for the ATPase activity of SR of rabbit skeletal muscle, probably because they did not examine the initial rates as we have done. It should be noted that the rate of the ATPase reaction during Ca^{++} uptake changes continuously after the first few seconds of the reaction until it stabilizes at a low steady state value.

The activation energies are similar for rabbit and lobster SR, but the breaking points (T_t) differ appreciably. Therefore, we investigated whether the two membrane systems vary in their phospholipid and cholesterol content which in other membrane systems has been shown to be fundamental in determining membrane fluidity (13-16).

The membrane cholesterol of SR was complexed by using the antibiotic filipin (15) in concentrations which did not affect the rate of ATP hydrolysis or the Ca^{++} accumulation by SR (17). Treatment of SR with filipin resulted in an increase of T_t for both rabbit (16.7°C to 23°C) and lobster (11.5°C to 19°C) preparations (Fig.2), while the energies of activation remained similar for both preparations before and after filipin treatment. These results agree well with those found for other types of membrane bound enzymes (16).

The amount of cholesterol in lobster and rabbit SR is summarized in Table I. It is evident that the ratio of cholesterol to other lipids is nearly twice as high in lobster as in rabbit SR. It can also be calculated that the amount of cholesterol per unit of Ca^{++}-pump is 2.1 times higher in lobster SR as in the rabbit preparations. These difference in cholesterol content may account for the different T_t values observed in the two types of SR. It has been shown that cholesterol acts as a regulator of the fluidity of the membrane lipid core by inducing condensation of the hydrocarbon chains at high temperatures and by preventing the crystallization of the chains at low temperatures (13,14).

The content in total lipid on a protein basis is higher in lobster than in rabbit SR (Table I). Since the content in Ca^{++}-pump of SR of rabbit and lobster is 50 and 65%, respectively, of the total protein, the total lipid



SYSTEM	EACT1 (T>Tt) (CAL/MOLE)	EACT2 (T<Tt) (CAL/MOLE)	Tt (°C)
⊙ CONTROL	10115	19619	16.7
x X-537A TREATED S.R.	9018	16065	16.8
▲ SONICATED S.R.	16026	-	-
⊡ LUBROL TREATED S.R.	24865	-	-
▼ LYSOLECITHIN TREATED S.R.	20985	40623	10.0

Fig. 2 - Arrhenius plots for the Ca^{++}-ATPase activity of modified SR isolated from rabbit skeletal muscle. Experimental conditions were similar to those of Fig.1. Sonicated SR was obtained by treatment of suspensions (4 ml of 10 mg/ml) for 5 periods of 10 sec each in a Branson sonifier, Model W 140, at power 8, equipped with standard tip. Lubrol and lysolecithin treated membranes were obtained by incubating buffered suspensions (10 mM Tris-maleate, pH 7.2, in 100 mM KCl) with 0.33 or 1.0 mg of lubrol or lysolecithin per mg of SR protein, for 1 hr at 0°C.

expressed in mg per mg Ca^{++}-pump can be calculated to be 1.36 for rabbit and 1.52 for lobster preparations. Therefore, the ratio of lipid to protein in the two types of SR is not very different if we assume that proteins other than Ca^{++}-pump are not associated with lipid, and are merely proteins trapped in the vesicles during the isolation procedure (4). Furthermore, the relative amount of the various phospholipids is similar in both preparations (Table I), but we did not determine whether the fatty acid compositions of rabbit and lobster are different. Differences in saturation of the fatty acids could also determine different values of T_t which may reflect the temperature for the phase transition from a liquid crystalline to a crystalline state of the

TABLE I

Lipid composition of rabbit and lobster sarcoplasmic reticulum. Values are the average of two experiments

Component	Rabbit SR	Lobster SR
Total lipid (mg/mg protein)	0.68	0.99
Phospholipids		
µg Pi/mg protein	12.3	20.5
µg Pi/mg total lipid	18.3	20.7
Phospholipid classes (% of total Pi)		
Phosphatidyl Choline	68.9	69.4
Phosphatidyl Ethanolamine	17.9	16.5
Phosphatidyl Serine	6.5	6.5
Sphingomyelin	3.5	3.7
Lysophosphatidyl Choline	2.4	2.3
Phosphatidyl Inositol + Origin on chromatogram	1.5	2.2
Cholesterol		
µg/mg protein	19.6	54.3
µg/mg lipid	29.0	54.5
mg/mg lipid Pi	1.6	2.6

phospholipid hydrocarbon chains which interact with the enzyme molecules (14). The breaks in the Arrhenius plots depicted in Figs. 1 and 2 probably are related to the transition temperatures of those lipids which actually interact with the Ca^{++}-pump and not to the bulk of the alkyl groups which apparently have a transition at about 22°C (18).

Arrhenius plots for SR membranes desintegrated either by sonic oscillation or by solubilization do not show a breaking point, and the energy of activation assumes the higher of the two values reported here and which, in the case of intact membranes, is obtained only at temperatures below the T_t. It appears that the substitution of the natural lipid by the lubrol detergent (or lysolecithin) results in a lower catalytic efficiency for the enzyme. Sonic oscillation may affect the native interaction between the lipids and

the enzyme, or it may cause modification of the membrane lipids.

The ionophore X-537A releases Ca^{++} previously accumulated by SR or impedes its accumulation (19). It has been assumed that the antibiotic increases the permeability of the membrane to Ca^{++} and does not affect the Ca^{++}-pump, although there is not deffinite evidence for the latter. Recent investigations in our laboratory revealed that X-537A stimulates the steady state rate of ATP hydrolysis, but does not affect the Ca^{++}-stimulated ATP hydrolysis by solubilized or sonicated membranes. Furthermore, the antibiotic does not affect either the energies of activation or the T_t for ATPase activity in intact membranes (Fig.2). Therefore, it seems that X-537A does not in fact interact with the enzyme itself. Work presently in course indicates that the effect of X-537A on the steady state ATPase activity is mediated by the dissipation of the Ca^{++} gradient which regulates the ATP hydrolysis by the Ca^{++}-pump.

The results reported show that the energies of activation for ATP hydrolysis are similar for rabbit and lobster SR, but the breaking point which occurs in the Arrhenius plots of the ATPase activity differs for the two SR preparations (16.7°C for rabbit SR and 11.5°C for lobster SR). Filipin, which combines with cholesterol, increases the value of the breaking points by 6-7°C without affecting the energies of activation, whereas X-537A has no significant effect on the Arrhenius plot of the ATPase activity.

ACKNOWLEDGEMENTS

This work was supported by Grant CB/2 from I.A.C. (Portuguese Ministry of Education) and by the Gulbenkian Foundation.

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