THERMOTROPIC TRANSITIONS IN SARCOPLASMIC RETICULUM

V.M.C. Madeira and M.C. Antunes-Madeira

Departamento de Zoologia, Coimbra, Portugal

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SUMMARY

Functional transitions of nucleoside triphosphatase activity (NTPase) of sarcoplasmic reticulum (SR) occur at about 17 °C independently of the substrate hydrolysed. Changes in the enthalpies (ΔH^*) and entropies (ΔS^*) of activation occur at the transition temperature (T_t), but the free energies of activation (ΔG^*) remain constant over the range of temperatures studied. Phospholipase-A_2 from pig pancreas attacks phospholipids of intact SR only at temperatures above 18 °C, but the enzyme is ineffective at all temperatures in liposomes prepared with isolated SR lipids. The rotational parameter of the fluorescent probe, perylene, imbeded in SR membranes showed a transition at about 18 °C not observed in liposomes of SR lipids.

INTRODUCTION

Sarcoplasmic reticulum is one of the simplest systems which interconvert chemical and osmotic energies (1-3), since it is formed primarily by an ATPase functionally associated to the membrane lipids (4).

The physical state of membrane lipids regulates the membrane activities which often show functional temperature transitions closely related to changes in lipid fluidity (5-10). In the present study we show that the functional transitions of NTPase activity of SR are independent of the substrate hydrolysed. Good correlation was obtained between the values of T_t and the temperature above which Phospholipase-A_2 penetrates the SR membranes. Furthermore, the rotational parameter of perylene imbeded in the membrane exibits a transition temperature similar to the value of T_t obtained for the NTPase activity. The results also indicate that the membrane proteins of SR modify the behaviour of membrane lipids with respect to their thermotropic transitions.

MATERIALS AND METHODS

Membranes of SR were isolated from rabbit skeletal muscles

by standard centrifugation procedures (11). The protein contents of suspensions were determined by means of the biuret reaction (12).

The liposomes for fluorescence studies were prepared by vigorous mixing (4 min. in Vortex) 2 mg of SR phospholipids with 3 ml of a standard medium containing 50 mM KCl, 5 mM $MgCl_2$ and 5 mM Tris (pH 7.0). The suspension was then diluted with 3 ml of medium and mixed again. Finally, $CaCl_2$ was added to a concentration of 40 μM. The liposomes for phospholipase-A_2 treatment were prepared in a similar way except that 9 mg of lipid were mixed with 13.5 ml of standard medium.

The hydrolysis of the nucleoside triphosphates (NTP) was followed by recording continuously the production of H^+ in standard medium, at pH 6.9, containing 1 mM NTP and 2.0 mg of SR as described previously (13). The Ca^{++}-stimulated NTP hydrolysis was initiated by adding 200 nmoles $CaCl_2$ (40 μM final concentration).

Digestion experiments were performed by incubation SR (10 mg of protein) or liposomes (1 mg of lipid) in standard medium containing 40 μM $CaCl_2$ and 10 μg pig pancreas phospholipase-A_2 (total volume 1.6 ml). The reactions were terminated at the end of 2 hrs. by adding 50 mM EGTA. Lipid extractions and analyses were performed as described previously (13, 14).

Fluorescence polarization measurements were made in a Perkin Elmer, Model MPF-3, spectrofluorimeter equiped with polarization filters. Corrections were made for the light scattering of suspensions and instrumental polarization. The excitation monocromator was set at 410 nm (6 nm band pass) and the emission was measured at 472 nm (6 nm band pass). The cuvettes contained SR (2.0 mg of protein) or liposomes (1.0 mg phospholipid) in 3.0 ml of standard medium plus 40 μM $CaCl_2$ and 0.4 μM perylene. The molar ratio of perylene to lipid was about 1:1000.

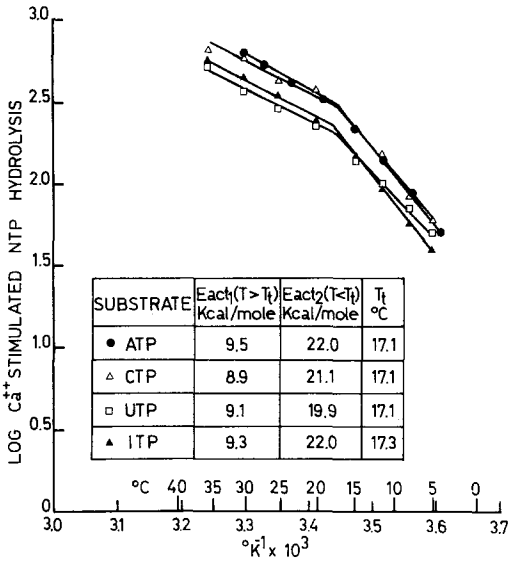
Polarizations were calculated according to Shinitzky et. al. (15). The rate constants of rotation ($\bar{R}$) were calculated as described elsewhere (15-17). Excited state lifetime for perylene was assumed to be 5.4 nsec at 25 °C (18).

Phospholipase-A_2 from pig pancreas was a generous gift of Dr. J. Op den Kamp.

RESULTS AND DISCUSSION

Arrhenius plots for the Ca^{++}-stimulated NTP hydrolysis by SR were resolved into two straight lines with breaks at about 17 °C (Fig. 1). These results agree with those reported before for the Ca^{++}-stimulated ATP hydrolysis by SR (13, 19). The thermodynamic parameters of activation stated in Table I were estimated in accordance with the theory of rate processes (20).

These parameters and the transition temperatures (T_t) are very similar for all the nucleotides utilized as substrates (Table I). Therefore, they are characteristic of the enzyme system and do not depend on the substrate hydrolysed. The sharp change in Δ H*



SUBSTRATE	E_{act_1} (T > T_t) Kcal/mole	E_{act_2} (T < T_t) Kcal/mole	T_t °C
• ATP	9.5	22.0	17.1
△ CTP	8.9	21.1	17.1
□ UTP	9.1	19.9	17.1
▲ ITP	9.3	22.0	17.3

Fig. 1. Arrhenius plots of the Ca^{++}-stimulated NTP hydrolysis by SR. The experiments were performed in standard media containing 2.0 mg of SR protein and 1 mM NTP and the reactions were initiated by adding 200 nmoles $CaCl_2$ (40 µM final concentration). The vesicles used exibited very low basal NTPase activity even at high temperatures.

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at T_t is compensated by a change in Δ S*, since Δ G* remains constant over the range of temperatures studied.

Although some controversy exists about the interpretation of the breaks in Arrhenius plots, there are evidences that the breaks occur often as a consequence of phase transitions of membrane lipids on which enzymic activities depend (5-10, 19). Thus, the manipulation of lipid composition of most membranes in which the degree of fluidity was varied led to shifts in T_t of membrane activities (6, 7, 10, 21). We have also shown previously that T_t for ATP hydrolysis by rabbit or lobster SR occur at about 17 °C and 11.5 °C, respectively (13). Recent studies in lipid composition revealed that lobster SR phospholipids have chains more unsaturated than those of rabbit SR (22). The unsaturated fatty acids of lecithins make up about 73% and 53% of the total for lobster and rabbit SR, respectively, and the relative contents

TABLE I

THERMODYNAMIC PARAMETERS OF ACTIVATION OF THE NTPase ACTIVITY OF SR.

The first number of each pair is the mean value for temperatures above T_t, while the second number is the mean for temperatures below T_t.

Substrate	T_t °C	ΔH* Kcal/mole	ΔG* Kcal/mole	ΔS* cal/°K.mole
ATP	17.1	8.9 21.5	21.3 21.3	-41.7 + 1.3
CTP	17.1	8.3 20.5	21.3 21.0	-43.8 - 1.7
UTP	17.1	8.5 19.3	21.5 21.2	-44.0 - 6.8
ITP	17.3	8.7 21.4	21.5 21.3	-43.1 + 0.7

in polyunsaturated fatty acids are about 9% for rabbit and 39% for lobster SR. These differences may, thus, influence the behaviour of the ATPase enzyme relatively to the T_t.

Calorimetric studies in SR membranes (23) led to the conclusion that the discontinuities in Arrhenius plots of SR ATPase probably do not reflect the thermotropic transition of membrane lipids. However, this type of studies may only yield information about the macrolipid enviroment rather than about the microlipid environment of the enzyme which seems to be relatively imobilized as compared with the rest of the lipid (24).Moreover, studies

using spin labels (19, 25) and nuclear magnetic resonance of protons (26) indicate that transitions of membrane SR lipids occur at temperature values comparable with T_t. On the other hand, the results obtained here for the changes in ΔS^* at T_t (about 40 cal/^{o}K.mole) may mean that at temperatures below T_t there is an increase of order in the enzyme environment which impedes its mobility freedom.

Phospholipase-A_2 from pig pancreas can only attack efficiently the SR lipids at temperatures above 18 ^{o}C (Fig.2), a value comparable with T_t for the NTPase activity. This finding suggests that some change occurs in the membrane at 18 ^{o}C which favours the penetration of phospholipase-A_2 into the bilayer, but the same change does not occur when liposomes of SR lipids are submitted to digestion (Fig. 2). Thus, the membrane protein seems to favour the occurence of a thermotropic transition at about 18 ^{o}C.

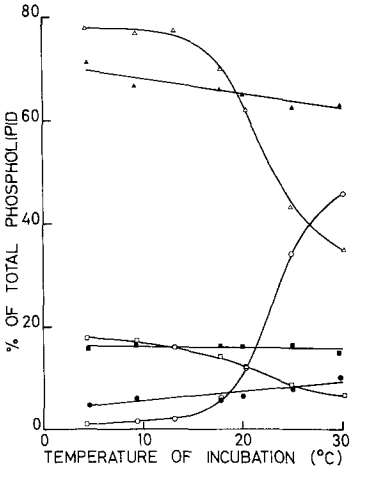

Fig. 2. Lipid digestion of SR membranes (open symbols) or liposomes of SR lipids (full symbols) by pig pancreas phospholipase-A_2. Digestions were performed in standard media containing 40 µM $CaCl_2$, liposomes or SR membranes and 10 µg phospholipase-A_2 (see text). Digestion is greatly enhanced above 18 ^{o}C only in intact SR. Symbols: triangles, phosphatidylcholines; circles, lysophosphatidylcholines; squares, phosphatidylethanolamines.

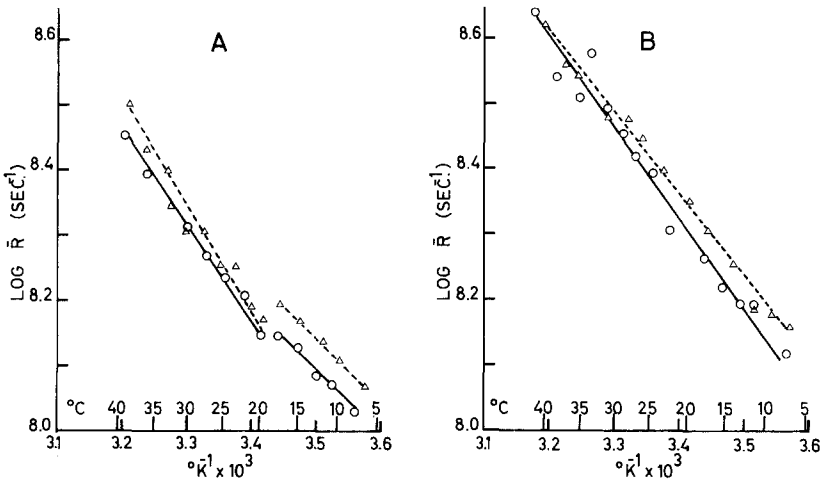

Fig. 3. Arrhenius plots of perylene rotation rate ($\bar{R}$) in SR membranes (A) and in liposomes of SR lipids (B). The results of two experiments with different preparations are depicted. It was not possible to define a break in B as it was in A. The temperature of the break is at about 18.5 °C.

Other approach to the problem was achieved by studying the temperature dependence of the rotational rate of perylene included in the membranes. This parameter (Fig. 3A) suffers a sharp transition at about 18.5 °C which indicates that a change in membrane viscosity occurs at this temperature. A similar effect was not observed in liposomes of SR lipids which suggests again that the protein moiety of the membrane modifies the behaviour of the lipid part relatively to thermotropic phase changes.

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