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Activation Energies of Mitochondrial Adenosine Triphosphatase under Different Conditions

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Abstract

Arrhenius plots of mitochondrial ATPase show a two- to three-fold increase in the activation energy below 17°. The breaks in the Arrhenius plots are not profoundly modified by the uncoupling agent, DNP, and by the inhibitor oligomycin. Detergents however, which disaggregate the membrane and make the ATPase oligomycin insensitive, induce a decrease of the differences in slope above and below the transition temperatures. The results are discussed in relation to the physical changes of the membrane phospholipids.

The involvement of phospholipids in the function of membrane enzymes is well recognized and a phospholipid requirement has been demonstrated for a number of membrane enzymes [1, 2]. The lipid specificity in restoration of enzymic activities has been studied mainly for the polar part of the lipids. However different experimental findings have suggested that membrane proteins may interact hydrophobically with the nonpolar portion of the phospholipids [3-5]; moreover it is recognized that lipids in membranes are in a liquid-crystalline state where the nonpolar fatty acyl chains constitute a mobile phase [6].

If such mobility of the core of the lipid bilayer is required for membrane functions, we should expect the membrane activities to be affected by the physical state of the phospholipids. Since such a state depends on the temperature, a correlation between the temperature effect on the activity of membrane enzymes and physical transitions in the phospholipids may cast some light on the role of phospholipids in membrane functions.

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Breaks in Arrhenius plots of membrane-linked enzymic systems have been observed in the past, as being related to the concomitant presence of processes having different activation energies [7]; this interpretation has been questioned by Kumamoto *et al.* [8] who have suggested that in this case a catenary curve should result rather than a broken line. Raison and his coworkers are of the opinion that breaks in Arrhenius plots reflect physical transitions of the micro-environment in which the enzymes are situated [9].

The mitochondrial Mg^{++}-dependent ATPase is a typical lipid-requiring enzyme [10-13]; such a property is characteristic of the enzyme *in situ,* while the isolated enzyme F_1 [14], once detached from the membrane, does not contain lipids and does not require lipids for activity. This "allotopy" [15] of the ATPase is also expressed by the cold lability of F_1 and its insensitivity to the inhibitor oligomycin. In our opinion these properties make it worth while to investigate the mitochondrial ATPase in connection with its lipid environment. This study concerns the effect of temperature on the activity of the mitochondrial ATPase in intact mitochondria and after disruption of the membrane with detergent.

Methods

Beef heart mitochondria (BHM) were prepared with a small scale procedure [16]. The protein content of mitochondria was determined according to Gornall *et al.* [17]. ATPase activity was assayed as described by Margolis *et al.* [18]. When detergents were used, they were added directly to the incubation medium and activity was measured immediately, after the usual preincubation at the desired temperature.

Results

Figure 1 shows an Arrhenius plot of ATPase activity of frozen BHM under standard conditions, in the presence of the uncoupler, 2,4-dinitrophenol (DNP), and finally in the presence of the inhibitor, oligomycin. The slopes, which express the activation energies of the enzymic reactions, sharply increase below about 17° under all three experimental conditions. This pattern is anomalous in comparison with soluble enzymes, which usually show a linear temperature dependence. Since these results were obtained using frozen mitochondria we have tested also fresh BHM where the ATPase is in a more controlled state, and the membrane has not undergone possible structural changes due to the freezing or thawing processes. Figure 2 shows that the results do not differ appreciably from those obtained with frozen mitochondria. Table I reports the calculated activation energies for the various

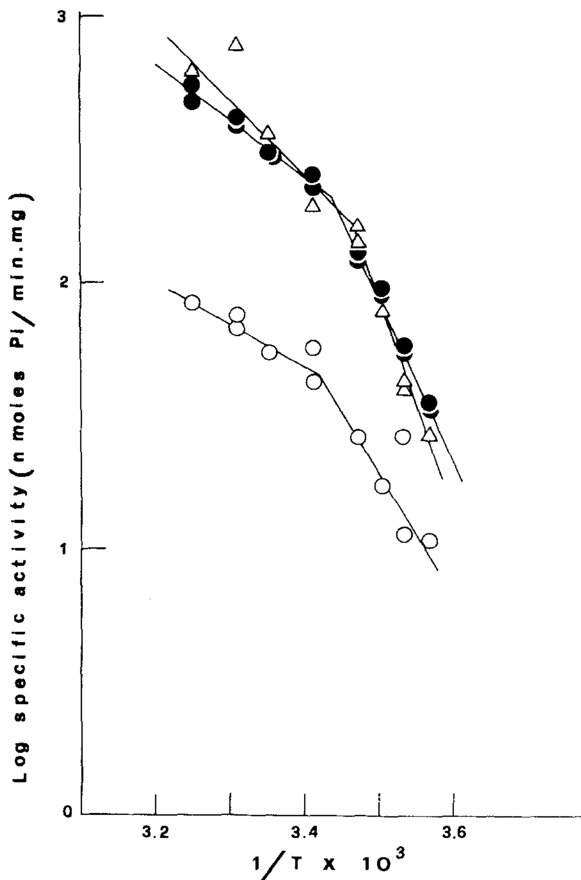

Figure 1. Arrehenius plot of ATPase in frozen BHM. ●——●, no addition, △——△, +DNP (5×10^{-5} M); ○——○, +oligomycin (1 μg/ml).

TABLE 1. Break temperature in Arrhenius plots and activation energies of mitochondrial ATPase

Condition	Break temperature (°C)	Activation energy (kcal/mole) Above transition temperature	Activation energy (kcal/mole) Below transition temperature
BHM, frozen	17.7	9.8	27.1
BHM, frozen + DNP (5×10^{-5} M)	15.0	13.3	37.3
BHM, frozen + oligomycin (1 μg/ml)	19.4	6.9	20.6
BHM, fresh	16.8	12.8	23.4
BHM, fresh + DNP (5×10^{-5} M)	15.2	13.1	38.2
BHM, fresh + oligomycin (1 μg/ml)	17.7	6.4	18.9

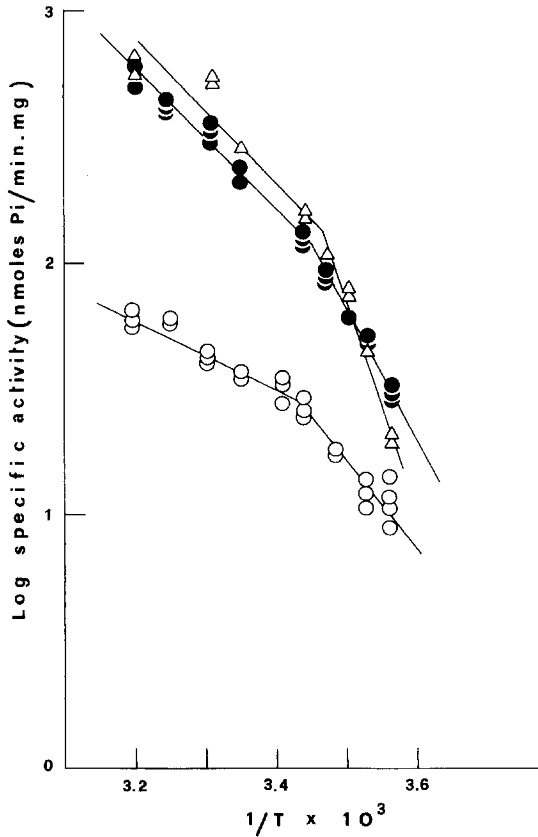

Figure 2. Arrhenius plot of ATPase in fresh BHM. ●———●, no addition; △———△, +DNP (5×10^{-5} M); ○———○, +oligomycin (1 μg/ml).

reactions. As an average, the activation energies increase up to three-fold below the break point. The residual oligomycin insensitive ATPase of the mitochondrial preparation, although maintaining the break, has an activation energy close to half that of the ATPase in absence of the inhibitor, thus raising the doubt that a contaminant activity from a different membrane was assayed.

Figure 3 shows that the nonionic detergent, Triton-X-100, at the concentration of 2.5 mg/ml, which solubilizes the mitochondrial membrane, almost abolishes the break in the Arrhenius plot of ATPase, characterized, in this case, by a much smaller slope variation. Deoxycholate (DOC) has a similar but weaker effect (Table II). Triton-X-100 makes the ATPase insensitive to oligomycin (Table III), which indicates

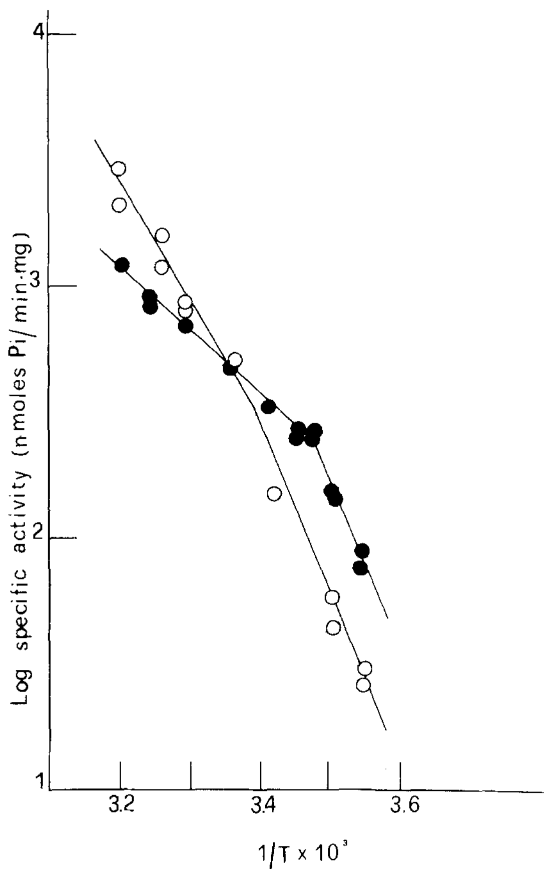

Figure 3. Effect of Triton-X-100 on the temperature dependence of ATPase in frozen BHM. ●——●, no addition; ○——○, +Triton-X-100, 2.5 mg/ml.

TABLE II. Effect of detergents on activation energies of mitochondrial ATPase

Exp.	Addition	Break temperature (°C)	Activation energy (kcal/mole) Above transition temperature	Below transition temperature
1	–	16.0	11.6	36.3
	Triton (1 mg/ml)	21.0	19.3	36.3
	Triton (2.5 mg/ml)	22.0	22.6	30.9
2	–	16.8	9.0	30.5
	DOC (0.5 mg/ml)	16.3	17.2	35.2

TABLE III. Effect of Triton-X-100 on oligomycin sensitivity of mitochondrial ATPase at different temperatures

Temperature (°C)	– Triton –oligomycin	+ oligomycin	% (°°)	+ Triton (2 mg/ml) –oligomycin	+ oligomycin	% (°°)
	Specific activity (°)			Specific activity (°)		
9°	55	14	25	26	25	96
12°	90	17	19	46	40	87
15°5	126	26	21	119	90	76
21°	251	48	19	345	336	97
25°	301	55	18	467	450	96
30°	398	71	18	722	733	101
36°	545	83	15	1145	1145	101

(°) nmoles P_i produced/min mg protein.

(°°) Activity in presence of oligomycin (1 μg/ml) expressed as % of the uninhibited ATPase activity.

that the enzyme becomes detached from the membrane and lipid-independent [15]. The oligomycin insensitive ATPase is cold-labile [19], and this fact could explain a lower activity at low temperatures in the presence of Triton. The existence of a break in the residual oligomycin insensitive ATPase in intact BHM would be only apparently in contrast with the Triton effect, if we assume the occurrence of contamination with nonmitochondrial membranes having oligomycin insensitive ATPase activity.

Discussion

The possibility exists that the break points in the Arrhenius plots of ATPase depend upon changes in the physical state of the lipids associated with the membranous enzyme. The transition temperatures of membrane phospholipids from a crystalline to a liquid-crystalline state vary with the lipid composition [6], and are apparently not modified significantly by extraction of the lipids from the membrane, as shown for *Mycoplasma laidlawii* membranes [20] and rat liver mitochondria [21]. A break temperature at 16°-17° was observed by others for some activities connected with energy transduction either in mitochondria [22] or in microorganisms [23, 24], while higher temperature values were reported for the same activities by other authors [9, 25, 26]. None of these values however correspond to the transition temperatures of mitochondrial lipids as studied by differential calorimetry [21], which are centered at 0° C. However since the endothermic transitions begin at temperatures far below and end at temperatures quite above 0° C, the breaks could be related only to the upper portion of this temperature range. The significance of the breaks in the Arrhenius plots of ATPase is, therefore, difficult to interpret, and cannot be directly ascribed to the

transition temperatures where the nonpolar chains of membrane phospholipids "freeze". To this purpose it is significant to consider here that the membrane is not necessarily homogeneous in fluidity. In fact mixed vesicles of dioleyl and dibehenoyl lecithin at 26°C have been described as patches of crystalline dibehenoyl lecithin in a semifluid dioleyl lecithin bilayer [27]. Moreover, the phospholipid environment of the (Na^+ + K^+) activated ATPase experiences a higher fluidity in comparison with the average state of the bilayer as shown by spin labelling studies [28]. Although Kline *et al.* [29] found no break in Arrhenius plots of either membrane-bound or isolated (Na^+ + K^+)-ATPase, in another study of the same enzyme a distinct break, dependent on Na^+ concentration, was observed at 20°C; on the other hand Kimelberg and Papahadjopoulos [30, 31] found that reconstitution of ATPase with dipalmitoyl phosphatidyl glycerol resulted in a biphasic Arrhenius plot with an inflection at 36°, while reconstitution with unsaturated phosphatidylglycerol resulted in no break in the temperature range studied.

The breaks might be correlated with the temperature at which the chains begin to be less mobile: since there is an indication that the fatty acyl chains are less mobile in correspondence of the polar part of the phospholipids [32], "freezing" of the membrane could be a process initiating from the polar part and extending towards the core of the bilayer. The effect on an enzymic activity could be evident much before the transition to the crystalline state is completed.

Due to the above mentioned complications, there is not yet sufficient evidence for the interpretation of the break points in the Arrhenius plots based on changes in the physical state of the phospholipids alone. The membrane proteins are expected to change their conformation while changes in phospholipids are occurring; to this purpose we have found interesting reversible changes in the circular dichroism spectra of mitochondria due to lipid removal [33]. Breaks in Arrhenius plots might be due to temperature-dependent changes in protein conformation, probably as the result of changes in phospholipid physical state.

The accompanying communication [34] extends the results of this investigation to respiratory activities both in intact and lipid-depleted mitochondria.

Acknowledgements

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