Effects of cholesterol on the kinetics of mitochondrial ATPase

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Enrichment of the inner mitochondrial membrane with cholesterol induces an increase in ATPase activity with a decrease in the K_m for ATP. Cholesterol also abolishes the discontinuity normally found in the Arrhenius plot of ATPase activity. Since no change is detected in the rate of proton translocation through the ATPase membrane sector, it is concluded that cholesterol incorporation induces changes in the hydrolytic step of ATPase via a conformational change transmitted from the membrane sector to the catalytic sector F_1.

ATP hydrolysis *Enzyme kinetics* *Proton translocation* *Cholesterol* *Temperature effect* *Mitochondria*

1. INTRODUCTION

The activity of several membrane-bound enzymes has an anomalous temperature dependence, showing breaks or discontinuities in the Arrhenius plots with strong increase of activation energy below the discontinuity [1]. Although such a behaviour is not unique for membrane enzymes, there is some consensus that phase changes in the lipid microenvironment in many cases are responsible for the nonlinear temperature dependence [2].

Mitochondrial ATPase is a lipid-dependent membrane enzyme, composed of a water-soluble moiety (F_1) containing the site for ATP hydrolysis, and a membrane sector (F_0) involved in H^+ translocation across the mitochondrial inner membrane [3]. Previous studies from our laboratory have demonstrated that the kinetics of mitochondrial ATPase is affected by perturbation of the membrane with organic solvents [4] or by incorporation of the isolated enzyme into phospholipid vesicles having different lipid composition [5].

Cholesterol is a major component of biological membranes [6] and is responsible for some of their physico-chemical features; changes in cholesterol content are associated with alterations of membrane-associated functions [7,8]. In the inner mitochondrial membrane, under normal conditions, cholesterol is practically absent; nevertheless, a strong increase of its content has been found in tumor mitochondria [9,10]. It has been shown that cholesterol enrichment of the inner mitochondrial membrane leads to alterations of the activity of transport systems and of membrane-bound enzymes [10,11], and these alterations have been postulated to be somehow involved in the malignancy state [10].

In view of these considerations, and of the fundamental role of ATPase in mitochondrial energy conservation, we have found it of interest to investigate the effect of cholesterol enrichment of bovine heart and rat liver mitochondria in vitro on the kinetics of ATPase. Here, we show that cholesterol enrichment to levels corresponding to those found in tumor mitochondria induces an increase in ATPase activity with decrease in the K_m for ATP; moreover, cholesterol abolishes the discontinuity normally found [5] in the Arrhenius plot of ATPase activity.

2. MATERIALS AND METHODS

Bovine heart mitochondria were prepared according to [12] and submitochondrial particles by

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sonication [13]. Rat liver mitochondria were prepared as in [14]. The outer membrane was ruptured by osmotic shock of the freshly prepared mitochondria [15].

Cholesterol was incorporated into mitochondria or submitochondrial particles by incubation with beads of Sephadex G-10 essentially by the method of Coleman et al. [16]. Control mitochondria were incubated with Sephadex beads by the same procedure except that cholesterol was omitted. ATPase activity was measured by an ATP-regenerating method as described [5]. Proton translocation through the ATPase in submitochondrial particles was measured by following the kinetics of oligomycin-sensitive 9-aminoacridine extrusion [17] at the onset of anaerobiosis in the succinate-energised membrane [18]. Controls in the presence of uncouplers showed that 9-aminoacridine penetration was not rate-limiting.

Cholesterol was assayed according to [19], lipid phosphorus as in [20] and proton by a biuret method [21].

3. RESULTS AND DISCUSSION

Preliminary investigations demonstrated that reproducible results could be obtained only performing cholesterol incorporation on freshly prepared non-frozen mitochondria. A likely explanation for the irreproducibility of cholesterol incorporation in frozen material is in the formation of inner membrane vesicles in the matrix space by pinching off of the cristae [22]. Such vesicles, hidden in the matrix, are likely to be excluded from interaction with exogenous cholesterol.

Table 1 lists the cholesterol contents of the different preparations used here; the levels of incorporation with both bovine heart and rat liver mitochondria are similar to those found by Coleman et al. [16]. The effect of cholesterol incorporation on the double-reciprocal plots of ATPase activity in bovine heart mitoplasts is shown in fig.1, at two different temperatures (14 and 33°C); cholesterol induces a slight increase in V_m and a substantial decrease in the apparent K_m for ATP.

Similar results were obtained in rat liver mitochondria and in bovine heart submitochondrial particles. The kinetic constants are also summarised in table 1.

The presence of cholesterol strongly affects the temperature dependence of ATPase; an Arrhenius

Table 1

Cholesterol content of the inner mitochondrial membrane and kinetic constants of ATPase activity in cholesterol-enriched mitochondria

Membrane	Cholesterol content (nmol/mg protein)	Temperature (°C)	ATPase activity	
			V_m (μmol/min per mg)	K_m (mM)
Bovine heart mitochondria	30 (control)	14	0.14	0.25
		33	0.67	0.36
	150 (enriched)	14	0.15	0.12
		33	0.84	0.20
Rat liver mitochondria	12 (control)	32	0.49	0.23
	207 (enriched)		0.55	0.13
Bovine heart submitochondrial particles	50 (control)	14	1.08	0.20
		33	6.45	0.36
	150 (enriched)	14	1.17	0.13
		33	5.11	0.20

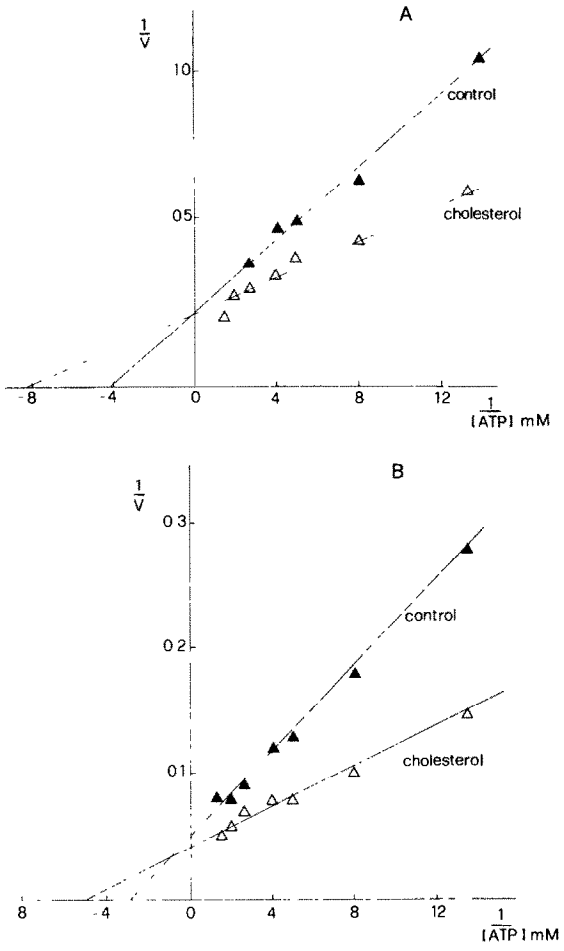

Fig.1. Double-reciprocal plots of ATPase activity in bovine heart mitochondria. Effect of cholesterol. (A) at 14°C, (B) at 33°C.

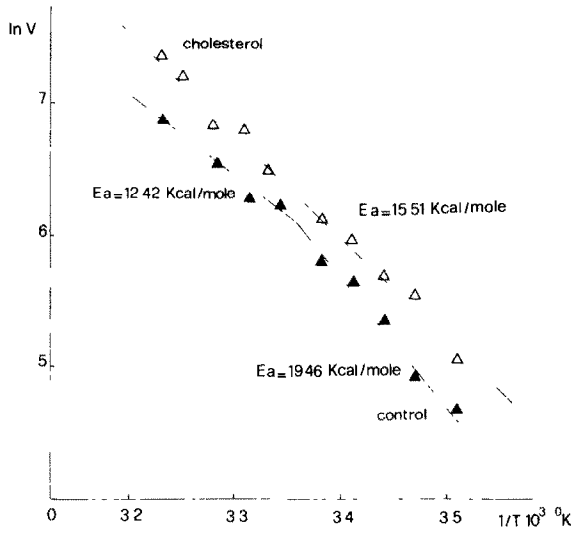

Fig.2. Arrhenius plot of ATPase activity in bovine heart mitochondria. Effect of cholesterol.

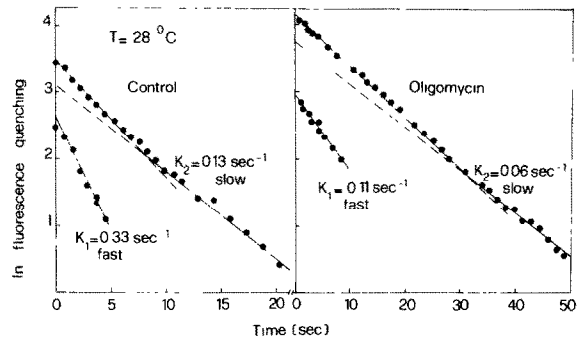

Fig.3. Proton release from succinate-energised submitochondrial particles.

plot of ATPase activity in bovine heart mitochondria (fig.2) shows that cholesterol abolishes the discontinuity found at 18°C in control mitochondria, giving rise to a linear plot with an intermediate activation energy. Similar results have been obtained in rat liver mitochondria and bovine heart submitochondrial particles.

A break in an Arrhenius plot may be the consequence of either a change in the rate-limiting step, or a phase change of the solvent, or finally a conformational change of the enzymic protein, as a function of temperature [23]. Whatever the reason, a change in the lipid composition of the membrane is able to affect the properties of the catalytic site, which is present in the hydrophilic sector of the enzyme. This finding suggests that cholesterol, by altering the physical state of the membrane or by direct interaction with the membrane sector F_0 of the enzyme, induces a conformational change in the enzymic protein capable of modifying the catalytic activity.

Experiments on the proton release by submitochondrial particles enriched with cholesterol have failed to show any effect on the kinetics of proton translocation, thereby excluding the possibility that the effects on ATP hydrolytic activity are due to mass law effects of changed H^+ translocating activity on ATP hydrolysis. The results are shown in fig.3 and table 2.

Table 2

Proton release from bovine heart submitochondrial particles (28°C)

Membrane (submitochondrial particles)	First order rate constant (fast) (s^{-1})		
	− oligomycin	+ oligomycin	Oligomycin-sensitive
Control	0.33	0.11	0.22
Cholesterol-enriched	0.33	0.11	0.22

It is therefore postulated that cholesterol incorporation induces a change in the hydrolytic step of ATPase via a conformational change transmitted from the membrane sector to F_1. The transfer of a conformational change from F_0 to F_1 has been also postulated in the action of energy transfer inhibitors, such as oligomycin and dicyclohexylcarbodiimide, on ATPase activity [24]. Evidence for a temperature-dependent conformational change of isolated mitochondrial ATPase has been obtained in our laboratory [25].

The mechanism by which cholesterol affects ATPase is not clear; fluorescence polarization studies using the probe diphenylhexatriene have shown no significant effect of the relatively low cholesterol levels on membrane viscosity (not shown); it seems therefore likely that cholesterol acts by direct interaction with the protein. Similar results were obtained by Krämer [11] on the adenine nucleotide carrier of the inner mitochondrial membrane.

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