LIPID DYNAMICS AND LIPID–PROTEIN INTERACTION IN ISOLATED BEEF-HEART MITOCHONDRIAL ATPase COMPLEX

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1. Introduction

Phospholipid requirement for the activity of several membrane-bound enzymes has been well established [1–3]. The activity of these enzymes exhibits a break in the Arrhenius plot at certain specified temperatures. The existence of such a break is attributed to temperature-induced phase transitions and/or separations of the membrane lipids interacting with the enzyme proteins [4,5]. One of the main problems regarding membrane-bound enzymes is how their function is affected by the physical state of the phospholipid molecules close to the enzyme protein. Studies on the lipid dependency of enzymes, involving lipid removal and replacement, have to be interpreted with caution because it is difficult to correlate directly the properties of the original enzyme with its lipid-depleted form.

Therefore, we have undertaken a study of the relation between the enzymatic activity and lipid–protein organization in beef heart mitochondrial ATPase, isolated as a particulate membrane preparation without separating the protein from adjacent lipids. This provides a useful approach to questions concerning the nature and importance of the interactions between ATPase hydrophobic subunits and lipid molecules with regard to ATP hydrolysis.

2. Materials and methods

N-Oxyl-4′,4′dimethyl-oxazolidine derivative of 5-ketostearic acid (5-NS) was purchased from Syva assoc. Other chemicals were of analytical grade. Beef heart mitochrondrial ATPase was purified according to [6]; 0.8% (instead of 1.5%) cholic acid neutralized with NaOH was used. ATPase preparations contained 0.12 μmol phospholipids/mg protein and ATPase activity was stimulated by addition of asolectin. F_1-ATPase was prepared by the chloroform method [7].

2.1. *ATPase assay*

ATPase activity was measured by determining P_i released. The reaction mixture contained 2–20 μg protein fraction in 50 mM Tris-HCl, 2 mM $MgCl_2$, 20 μg pyruvate kinase and 2 mM phosphoenolpyruvate (pH 7.5) in 1 ml final vol. The reaction was started by addition of 5 μmol sodium ATP (pH 7.5) and stopped after 5 min by addition of 0.5 ml 20% trichloroacetic acid. P_i released was determined as in [8].

2.2. *Protein determination*

Protein was determined by the method in [9], using bovine serum albumin as standard.

2.3. *Electron paramagnetic resonance measurements*

Electron paramagnetic resonance (EPR) spectra were recorded on a Varian E-3 EPR spectrometer modified for a variable temperature. The mitochondrial membrane and the purified ATPase were spin labeled with 5-NS. EPR spectra were evaluated in terms of the order parameter S, calculated according to the equation in [10].

3. Results and discussion

To gain information on lipid organization in the domain of ATPase complex, temperature-dependence of ATPase activity was measured at 9–37°C.

The Arrhenius plot for ATP hydrolysis by isolated mitochondrial ATPase shows a discontinuity at ~16°C

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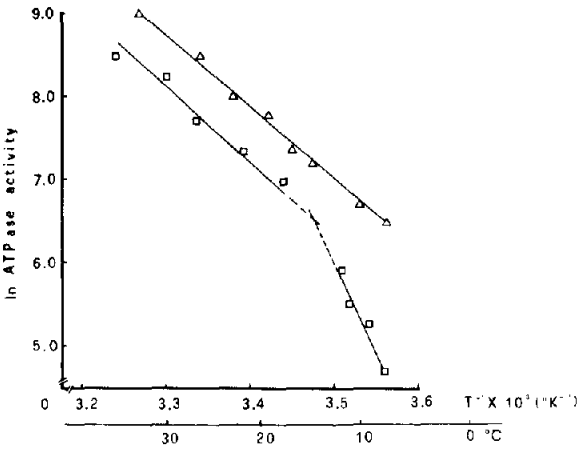

Fig.1. Arrhenius plot of mitochondrial ATPase activity: abscissa, $1/T$; ordinate, ln of ATP hydrolysis rate; (△) soluble ATPase; (□) F_oF_1–ATPase. The hydrolysis rate is expressed as nmol P_i liberated . min^{-1} . mg protein^{-1}.

(fig.1). Above the break point ATPase activity increases only very slightly when the temperature is increased (E_a low, ~7.6 kJ/mol), whilst below this temperature, the activity increases very sharply (E_a high, ~19.3 kJ/mol). This is consistent with previous results for OS-ATPase from yeast mitochondria [11] and from the membrane-bound enzyme [12]. In contrast no break was observed in the Arrhenius plot when the ATPase complex was depleted of the hydrophobic proteins (F_o), indicating that F_1 behaves as a soluble enzyme with an activation energy of ~6.9 kJ/mol.

These functional changes may be associated with the changes in the state of order of the lipid molecules around the ATPase complex.

The relationship between the discontinuity in the Arrhenius plot of ATPase activity, and membrane

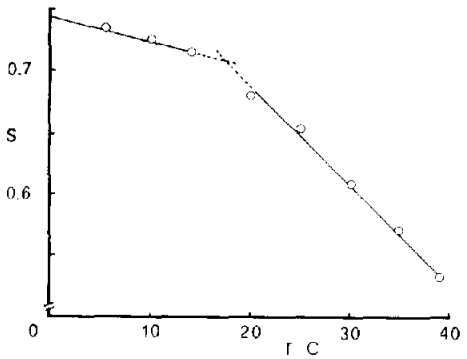

Fig.2. The order parameter S of 5-nitroxide stearate label in isolated mitochondrial ATPase preparation as a function of the temperature.

lipid phase transition were then studied. The physical state of the lipid microenvironment of ATPase was detected by EPR spectroscopy of spin-labelled fatty acid (5-NS) incorporated into the purified F_oF_1–ATPase complex. Throughout the temperature range tested (0–39°C) the order parameter S was markedly dependent on temperature (fig.2) and the order–disorder lipid-phase transitions were found close to the inflection point observed in the Arrhenius plot for mitochondrial ATPase activity.

These data provide evidence that the lipids, isolated along with the ATPase complex, play an important role on the functional arrangement of the enzyme. The change in the activation energy of ATPase might be a consequence of a thermotropic phase transition of the lipid molecules, which form the microenvironment of the enzyme or might arise from some specific protein–lipid interactions. Such interactions involving the hydrophobic sector (F_o) of the enzyme with the surrounding phospholipid molecules, seem also to influence the conformation and/or the catalytic activity of the hydrophylic sector (F_1) of the enzyme, resulting in an increase in E_a, when F_1 is associated with the membrane sector F_o.

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