THE ROLE OF LIPID IN REGULATION OF MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE

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1. Introduction

Interactions between lipid and protein within the mitochondrial membrane are important in the regulation of many membrane-bound functions [1–3]. Such membrane activities show characteristic discontinuities in the Arrhenius kinetics, which probably reflect the influence on the enzymes of lipid phase transitions [4–6]. However, in some cases discrepancies have been observed between activity transition temperatures and the membrane lipid phase transition temperatures. As shown by Lenaz et al. [7], several mitochondrial enzymes exhibit breaks in Arrhenius plots at temperatures which are well above the observed lipid phase transition temperature of the whole membrane [8]. Disparities between the transition temperature of membrane lipids and the breaks in Arrhenius plots of proline uptake and succinate-dehydrogenase were also observed by Esfahani et al. [9] in membranes of *Escherichia coli*, and recently in our laboratory for the membrane-bound mitochondrial ATPase [10]. These observations might indicate that the distribution of the lipids within the membrane is heterogeneous.

One direct approach to resolving this problem is to study the physical and functional properties of the lipid micro environment surrounding the enzyme. In this paper we provide direct evidence that the change in activation energy of oligomycin sensitive adenosine triphosphatase is a consequence of a liquid-crystalline to a gel-phase transition in the lipid molecules which form the immediate environment of the enzyme.

Abbreviations: OS-ATPase-oligomycin sensitive adenosine triphosphatase. EPR-electron paramagnetic resonance.

2. Materials and methods

2.1. *Preparation of oligomycin-sensitive ATPase*

Yeast mitochondrial particles were extracted with 0.25% (w/v) Triton X-100. The solubilised OS-ATPase was purified on a linear glycerol gradient as described by Tzagoloff et al. [11]. ATPase activity was measured as previously described [12]. Protein was estimated by the method of Lowry et al. [13].

2.2. *Electron paramagnetic resonance spectra*

The spin label *N*-oxyl-4′4′-dimethyl-oxazolidine derivative of 5-ketostearic acid (5NS) (Synvar Associates, Calif., USA) was dissolved in ethanol at 10^{-2} M and added to mitochondria particles and OS-ATPase suspensions to a final concentration of 2×10^{-4} M spin label. The spectra were recorded using a Decca Radar Spectrometer with a Newport Instrument 7 inch magnet.

2.3. *X-ray diffraction*

Samples for diffraction were taken from pellets centrifuged at 500 000 *g* for 1 h and accommodated into an evacuated diffraction camera in a sealed chamber maintained at ~ 100% humidity and at temperatures within the range – 25 to + 50°C with variations of up to ± 1°C. The diffraction camera had Elliott Toroid optics and was used in conjunction with an Elliott GX 6 rotating anode X-ray generator. Exposure times were 30 to 60 min. using packs of 2 or 3 films (Ilford Industrial G). Patterns were recorded at temperature intervals of 3 or 5°C over the transition range.

3. Results and discussion

The activity of the isolated OS-ATPase was studied as a function of temperature and the break (at 15 to 19°C) in the Arrhenius plot proved to be identical with that of the membrane bound activity (fig.1). This observation suggests that the change in the activation energy of the membrane-bound OS-ATPase activity is a reflection of the phase transition of phospholipid microenvironment surrounding the enzyme. The relation between the discontinuity in the Arrhenius plots of the enzyme activity and the lipid phase transitions has been demonstrated in $(Na^+ + K^+)$ adenosine triphosphatase, indicating that the enzyme activity is very sensitive to the fluidity of the phospholipid membrane [14,15].

Separation of membrane lipids into different domains either on the basis of their different melting points or their specific interaction with protein has been proposed [5,7,9]. The physical state of a lipid microenvironment associated with the enzyme molecule can be detected by X-ray diffraction studies, the rigid-crystalline state giving a sharp reflection at 4.2 Å, and the liquid-crystalline state giving a diffuse reflection at about 4.6 Å [9,16]. The order–disorder lipid phase transitions, in mitochondrial particles and isolated OS-ATPase, were compared. Both systems showed a change from a broad diffraction band centred at about 4.6 Å and representing the packing of disordered hydrocarbon chains to a sharp reflection at 4.2 Å representing close-packing of rigid hydrocarbon chains. For the isolated OS-ATPase (table 1), this change spanned a temperature range (+ 10 to + 15°C) which was both significantly narrower than that for the intact membrane (−15 to 5°C) and much closer to the activity transitions (+ 15 to + 19°C).

Table 1
Summary of EPR and X-ray diffraction data from isolated OS-ATPase and native membrane

Preparation	EPR[a] (gauss)	X-Ray[b] Range of transition temperature
Mitochondria	56.2	− 15 to − 5°C
OS-ATPase	60.7	+ 10 to + 15°C

[a]Estimation of hyperfine splitting ($2T_{11}$) was as described in [17].
[b]Disappearance of 4.2 Å sharp ring.

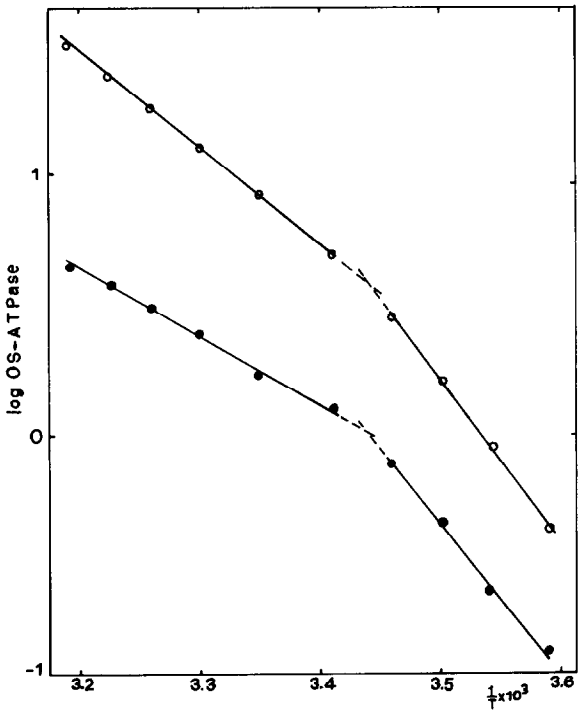

Fig.1. Temperature dependence of (•) membrane-bound OS-ATPase activity and (○) isolated OS-ATPase activity. The activity is expressed as μmoles of P_i liberated/min per mg of protein.

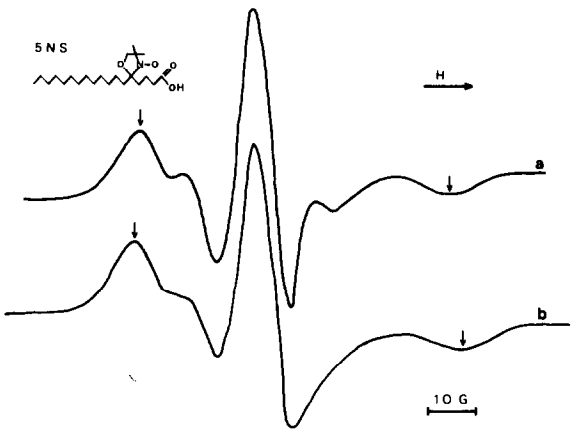

Fig.2. Electron paramagnetic resonance spectrum of 5NS spin label in buffered aqueous dispersions of (a) mitochondrial particles and (b) isolated OS-ATPase. The spin label was introduced at room temperature and the arrows indicate the positions of the outer hyperfine extrema.

Further evidence of these differences arises from EPR studies. Comparison of the EPR spectra of the spin label 5 NS in the isolated OS-ATPase and in the intact membrane showed a substantially higher value for the magnitude of the splitting between the high and low magnetic field peaks (fig.2). The hyperfine splitting ($2T_{11}$) is related to the rotational mobility of the spin label and reflects the local viscosity of the lipid environment [17]. However, the 5NS spin label apparently experiences in whole membrane an environment with greater fluidity than that of isolated OS-ATPase (table 1). This would indicate that the lipid retained in the isolated enzyme provided a much more viscous environment for the spin label.

These data provide strong evidence that the lipid isolated along with the OS-ATPase protein is substantially that which influences the activity in the intact membrane, and that this lipid exhibits a greater rigidity than the bulk of the membrane lipid.

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