Temperature-dependent conformational changes in isolated oligomycin-sensitive ATPase

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Isolated oligomycin-sensitive ATPase undergoes a kinetic change at 20–25°C with a higher activation energy and a lower K_m for ATP below this temperature range. This observation has been correlated with temperature-dependent structural changes detected by circular dichroism in the UV region in the isolated enzyme. The negative ellipticities in the 208–225 nm region, which are proportional to the α-helix content, increase with rise in temperature to a maximum above 25°C.

ATPase *Mitochondria* *Conformational change* *Kinetics* *Temperature effect* *Lipid role* *Membrane circular dichroism*

1. INTRODUCTION

The temperature dependence of the activity of membrane-bound enzymes often shows an anomalous behaviour, with discontinuities or breaks in the Arrhenius plots and higher activation energies calculated below the discontinuities [1]. The break has been observed in mitochondrial oligomycin-sensitive ATPase, but not in the isolated oligomycin-insensitive moiety of the enzyme, denominated F_1 [2]. The break appears related to the lipids, since it occurs at a temperature varying with lipid composition [3], although there is no quantitative correlation between lipid phase transition and the temperature at which the break occurs [1]. Detergents and organic solvents, which have marked effects on lipid–protein interactions [4], abolish the break while increasing the activation energy over a wide temperature range [5], suggesting that alteration of lipid–protein interactions decreases the catalytic power of the enzyme. The question whether such a decrease of catalytic power be linked to a structural change of the enzymic protein is thus of interest.

Although theoretical studies have predicted structural modifications of membrane-bound enzymes as a result of lipid fluidity changes, on the basis of observed changes of activation energy and cooperativity [6,7], only one study showing a conformational change accompanying a discontinuity in the Arrhenius plot of a non-membrane-bound enzyme has appeared so far [8]. Conformational changes in a plasma lipoprotein have also been shown as a consequence of lipid and temperature changes [9]. However, no temperature-dependent changes were observed in the circular dichroic spectra of synaptic membranes [10].

2. METHODS

Oligomycin-sensitive ATPase was purified from bovine heart mitochondria as in [11]. ATPase activity was assayed with an ATP-regenerating system [11]. Protein and phospholipid were determined as in [12] and [13], respectively.

The circular dichroism (CD) spectra were recorded in the range 180–300 nm in a Jasco

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J-500A spectropolarimeter equipped with a thermostatic jacket in the cuvette holder. Spectra were recorded at various intervals between 10–37°C; for each temperature the scan was repeated 3 times. The time constant was 16 s and the scan rate 5 nm/min. All the measurements were made in the same cell with an optical path of 0.2 mm at 64 μg protein/ml in 0.01 M Tris–HCl (pH 7.5). The UV absorption spectra were recorded in a Perkin Elmer 554 spectrophotometer; the readings were always done against a reference cuvette containing the same additions except the membrane. The CD-values were reported as molar ellipticities, calculated assuming a mean residue weight of 115. The percentage of α-helix was calculated from the ellipticity at 208 nm as in [14].

3. RESULTS AND DISCUSSION

The isolated oligomycin-sensitive ATPase had 0.32 mg phospholipid/mg protein and displayed an activity which was only slightly stimulated by exogenous phospholipids. The activity exhibits a sharp break near 20°C, which is evident in an Arrhenius plot, and the activation energy below the break increases ~2-fold (cf. fig.1 in [2] for an assay in a similar preparation). The K_m for ATP increases with increasing temperature, but the increase is much greater over 20–30°C (table 1).

Fig.1 shows the CD and absorption spectra of the ATPase in the UV region at 10°C and 25°C. The red shift, currently seen with membrane preparations [15], shown by crossing at wavelengths >201 nm and the absorption flattening [16] do not appear to be prominent in this preparation. The absorption values in fig.1B are identical at all temperatures, indicating that if spectral distortions due to the particulate state of the membrane suspension [15] occur, they are not of major importance. For this reason we have not attempted to correct the spectra, as recommended in [17] for turbid suspensions. An examination of the CD spectra in fig.1A shows a lower negative ellipticity in the 210–225 nm region at 10°C than at 25°C; the difference is accompanied by a crossover slightly shifted to higher wavelength in the 10°C scan.

Table 2 shows the molar ellipticities at different wavelengths and temperatures, calculated from the CD spectra. Analysis of the values shows a general increase of the amplitude of the negative bands of the spectrum over 10–25°C. The changes were found reversible; i.e., they were observed by either raising or lowering the temperature. An attempt to calculate the percentage of α-helix according to [14] showed a progressive increase of helical content from 23.1–34.5% over 10–25°C, and then a steady level (fig.2). The decreased amplitude of the spectrum at 10°C, particularly in the 210 nm region, might be caused in part by a decreased length of helical segments [18].

In [19], a structural change between 25–30°C was postulated in oligomycin-sensitive ATPase and in beef heart submitochondrial particles on the basis of a plateau in the temperature dependence of intrinsic tryptophan fluorescence. In [20], a break was observed at a somewhat lower temperature in the temperature dependence of the rotational correlation time of a fatty acid spin label in bovine heart mitochondria but not in

Table 2

Ellipticity values of oligomycin-sensitive ATPase at different temperatures

T (°C)	192 nm	196 nm	208 nm	221 nm	224 nm	Crossover
		($[\theta] \times 10^{-3}$, deg.cm^2.dmol^{-1})				(λ, nm)
10	29.1	19.5	−10.7	−12.3	−10.4	201.5
13	28.0	18.5	−12.8	−13.5	−11.7	201.1
17	27.6	18.6	−12.6	−13.1	−11.6	201.1
22	30.1	20.0	−13.2	−14.6	−12.2	201.3
25	29.5	20.0	−14.0	−14.8	−12.8	201.1
28.5	29.9	19.4	−14.1	−13.9	−11.9	201.2
37	26.7	19.3	−14.0	−13.8	−11.7	201.2

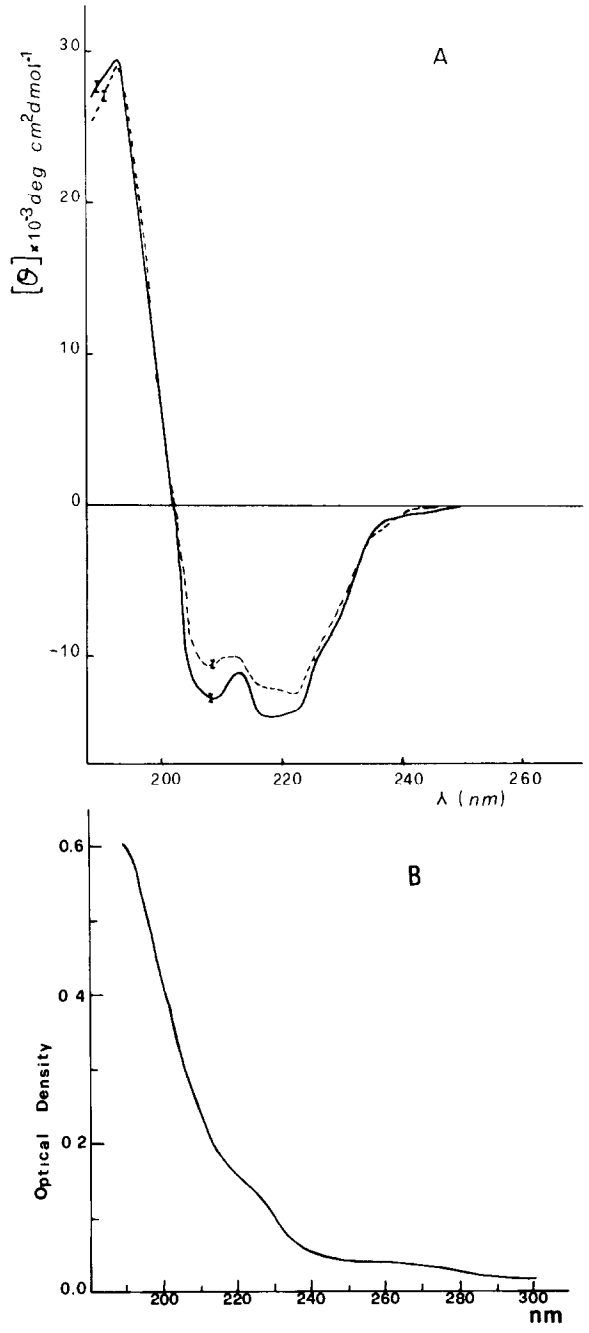

Fig.1. (A) Typical CD spectra of oligomycin-sensitive ATPase at two different temperatures: (——) 25°C; (– – –) 10°C. The bars in the experimental spectra represent the statistical range within one spectrum (square root of the noise amplitude). (B) UV absorption spectra of oligomycin-sensitive ATPase. The spectra are indistinguishable at all temperatures tested (from 10–37°C).

Table 1

Effect of temperature on the V_m and the K_m for ATP of oligomycin-sensitive ATPase

T (°C)	K_m (mM)	V_m (μmol.min^{-1}.mg^{-1})
14	0.098	0.144
17	0.123	0.210
20	0.151	0.356
26	0.312	1.048
30	0.385	1.820
35	0.400	2.659

unilamellar vesicles obtained from phospholipids extracted from the same mitochondria.

Although the latter studies have indicated the existence of temperature-dependent structural changes either in mitochondrial membranes or in the isolated ATPase, this investigation appears to demonstrate that a change occurs at the level of secondary structure. The results of this study cannot demonstrate whether the change is an intrinsic property of the enzyme or is related to the lipid environment. Studies are in progress with ATPase reconstituted with different lipids.

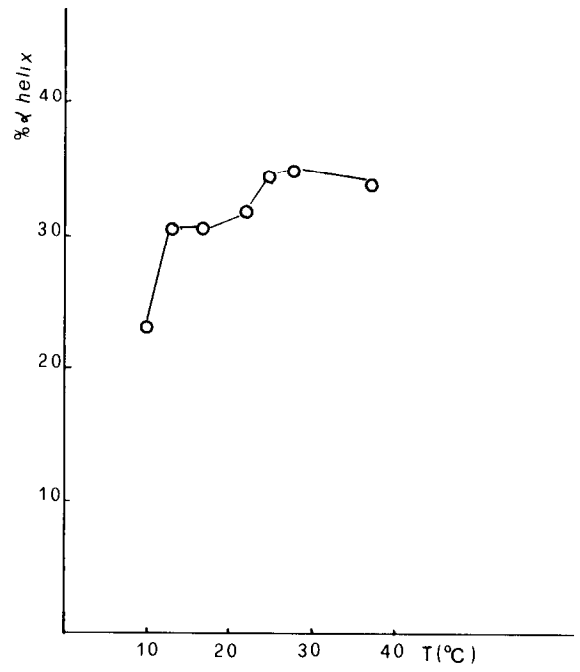

Fig.2. Computed amounts of α-helix in oligomycin-sensitive ATPase at different temperatures.

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