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Temperature Dependence of Mitochondrial Oligomycin-Sensitive Proton Transport ATPase

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Abstract

The temperature dependence of the oligomycin-sensitive ATPase (complex V) kinetic parameters has been investigated in enzyme preparations of different phospholipid composition. In submitochondrial particles, isolated complex V, and complex V reconstituted in dimirystoyl lecithin vesicles, the Arrhenius plots show discontinuities in the range 18–28°C, while no discontinuity is detected with dioleoyl lecithin recombinant. Van't Hoff plots of K_m also show breaks in the same temperature interval, with the exception of the dioleoyl-enzyme vesicles, where K_m is unchanged. Thermodynamic analysis of the ATPase reaction shows that DMPC–complex V has rather larger values of activation enthalpy and activation entropy below the transition temperature (24°C) than those of the other preparations, while all enzyme preparations show similar free energies of activation (14.3–18.5 kcal/mol). The results indicate that temperature and lipid composition influence to a different extent both kinetic and thermodynamic parameters of ATP hydrolysis catalyzed by the mitochondrial ATPase.

Key Words: OS-ATPase; temperature effect; kinetics; lipid role; membrane enzyme; protein–lipid interaction (bovine mitochondria).

Introduction

Study of the temperature dependence of an enzyme reaction can provide important mechanistic information (Dixon and Webb, 1964). Nonlinear Arrhenius plots ($\ln K_c$ vs. $1/T$) and Van't Hoff plots ($\ln K_m$ vs. $1/T$) have been occasionally reported for soluble enzymes, but breaks or discontinuities in the temperature dependence have been considered as a special marker of membrane-bound enzymes (Raison and McMurchie, 1974).

The proton transport ATPases (Amzel and Pedersen, 1983) are a special

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class of membrane-bound enzymes where a proton gradient established across the membrane (usually by electron transport) is used to drive the synthesis of ATP and ADP and P_i. The ATP synthesizing site is located in a peripheral moiety of the enzyme complex named F_1, while the protons are conveyed through an integral membrane sector called F_0, from the opposite side of the membrane (the process being inhibited by oligomycin and DCCD), to the catalytic site. Under nonenergized conditions the enzyme catalyzes ATP hydrolysis, and this activity has been shown to require phospholipids in the intact membranes or in isolated F_0–F_1 complexes, whereas isolated watersoluble F_1 does not have any lipid requirement (Kagawa and Racker, 1966; Racker, 1970).

The temperature dependence of the oligomycin-sensitive ATPase from mitochondria has been previously investigated in our laboratory (Lenaz *et al.*, 1975, Parenti Castelli *et al.*, 1979): a break in the Arrhenius plot of ATPase activity in mitochondrial membranes was found at temperatures ranging between 18 and 23°C depending on the systems and the experimental conditions used. A break was also found when the isolated oligomycin-sensitive ATPase was investigated (Solaini and Bertoli, 1981). Treatment of submitochondrial particles or mitochondria with Triton X-100 (Parenti Castelli *et al.*, 1979), organic solvents, and anesthetics (Lenaz *et al.*, 1978) abolished the break, without physical detachment of the F_1 moiety (Lenaz *et al.*, 1971), with an increased activation energy on the whole temperature range investigated.

In the present study we report further kinetic data on the temperature dependence on the mitochondrial oligomycin-sensitive ATPase, also in relation to modifications of the lipid environment of the enzyme.

Materials and Methods

Dimyristoyl phosphatidylcholine, dioleoyl phosphatidylcholine, and cholic acid were purchased from Sigma Chemical Co. Cholic acid was recrystallized before use. Pyruvate kinase and lactate dehydrogenase were from Boerhinger, Mannheim; all other reagents were of analytical grade.

Submitochondrial particles (SMP) derived from bovine heart mitochondria were prepared essentially according to Beyer (1967). Oligomycin-sensitive ATPase (complex V) was purified according to Stiggall *et al.* (1978) using 0.35 mg deoxycholate/mg mitochondrial protein for solubilization.

The purified OS-ATPase was reconstituted with pure dimyristoyl phosphatidylcholine (DMPC) or dioleoyl phosphatidylcholine (DOPC) following a scheme similar to that of Hesketh *et al.* (1976). Complex V was suspended with sonicated synthetic phospholipids (2 mg/mg protein) and sodium cholate

(0.8 mg/mg protein) in a buffer containing 2.5% methanol, 50 mM Tris/SO_4, pH 7.5, 0.25 M sucrose, 0.2 mM EDTA, 0.8 mM $MgSO_4$, and 0.1 mM ATP. Protein concentration was kept at about 10 mg/ml; the mixture was incubated for 30 min at 25°C. Afterwards it was layered on top of a discontinuous sucrose density gradient formed by 10 ml of 60% and 20 ml of 15% sucrose in the same buffer as above. These gradients were centrifuged at 150.000 × *g* for 90 min at 25°C. A band of protein–lipid vesicles was recovered, washed in the sucrose buffer, and subjected to a second substitution as follows: protein (approximately 10 mg/ml) was incubated with lipids sonicated to clarity in sucrose buffer (3 mg/mg protein) and sonicated in a water soni-cleaner bath for 20 min at 25°C. Cholate (0.2 mg/mg protein) was then added and the mixture incubated for 30 min at the same temperature. The protein/lipid/cholate mixture was centrifuged in a 15–16% linear sucrose gradient (30 ml) at 150.000 × *g* for 2 h at 25°C. The recombinants were recovered in bands and washed in sucrose buffer. The gas–liquid chromatography of the recombinant fatty acids showed that at least 95% of the lipids were of exogenous origin.

Although we did not measure the residual detergent in the recombinant, less than 0.2 mol cholate/mol protein was left in preparations of Ca^{2+}-ATPase similar to ours (Gomez-Fernandez *et al.*, 1980).

Protein was determined by the biuret method (Gornall et al., 1949) in the presence of 1% deoxycholate or/and according to Lowry *et al.* (1951).

Quantitation of lipid phosphorus was performed according to Marinetti (1962).

The ATPase activity was measured at pH 7.5 using an ATP-regenerating system as follows: the reaction mixture (1 ml) contained 25 μmol of Tris/acetate, 25 μmol of KOH, 0.3 mmol sucrose, 5 μmol $MgCl_2$, 160 nmol NADH, 1.5 μmol phosphoenolpyruvate, 2.5 units of lactate dehydrogenase, and 2.5 units of pyruvate kinase. Rotenone (1 μg) was present when SMP's activity was assayed. The enzyme preparation and ATP from a 0.025 M stock solution were finally added. The decrease of NADH, which is a measure of ADP formation from ATP, was followed at 340 nm on a Zeiss PMQ III spectrophotometer equipped with a Servogor recorder and a thermostating system.

The kinetic parameters K_m and V were evaluated from Lineweaver–Burk plots and the data were fitted using linear regression equations. The errors associated with the calculated values of V and K_m were less than 15% in all cases described. The catalytic constant K_c was obtained by dividing the maximal rate of ATP hydrolysis by the corresponding total enzyme concentration assuming a molecular weight of 500 kD. Activation energies, E_a, were determined from plots of ln V against $1/T$, the slope being $-E_a/R$. Thermodynamic activation parameters were obtained according to transition

state theory. The free energy of activation $\Delta G\ddagger$ was determined from

$$K_c = (\overline{K}T/h)\, e^{-\Delta G\ddagger/RT}$$

where $\overline{K}$ and h are Boltzmann's and Plank's constants, respectively. The $\Delta S\ddagger$ was calculated from the relationship

$$T \cdot \Delta S\ddagger = \Delta H\ddagger - \Delta G\ddagger \quad \text{and} \quad \Delta H\ddagger = E_a - RT$$

Results

The temperature dependence of the mitochondrial ATPase activity is analyzed in the presence of different phospholipids. Experiments have been performed with preparations of submitochondrial particles, isolated complex V, and complex V reconstituted into pure DMPC and DOPC vesicles.

The interval of temperatures investigated ranges approximately between 10 and 37°C.

The kinetic parameters K_m and V are evaluated using substrate concentrations of 50–500 μM that show straight lines in the Lineweaver–Burk plots. In submitochondrial particles, V increases more than 10-fold from 0.27 to 3.44 μmol/min/mg protein when the temperature is brought from 10 to 37°C. The relative increase is rather large in the range up to 25°C where the rate increases 7-fold, whereas in the range from 25 to 37°C the increase is less than 2-fold. The Arrhenius plot is shown in Fig. 1a. From the slopes of the straight lines interpolating the data, two activation energies are calculated: 12.4 and 21.8 kcal/mol above and below the break point, respectively.

The K_m values also change with temperature, being 0.11 mM at 10°C and 0.33 mM at 35°C. The increase is steeper below 25°C, as shown in the Van't Hoff plot of Fig. 1b.

Experiments have been performed with various preparations of isolated complex V, and one example covering the temperature range from 7 to 35°C is shown in Fig. 2a. The ATP hydrolysis rate increases about 100-fold from 0.025 to 2.66 μmol/min/mg. The relative increase is extraordinarily large in the range up to 18°C where the rate increases 15-fold, whereas in the range from 20 to 35°C the increase is only 7-fold. The Arrhenius plot indicates clearly the change in the temperature dependence with a distinct discontinuity at 18°C. Below the break point, the temperature increase corresponds to an activation energy of 39.7 kcal/mol, and beyond that range E_a is only 21.0 kcal/mol.

The temperature dependence of K_m reported in Fig. 2b shows an increase of K_m with increasing temperature, with a sharp change at 20–23°C.

Complex V has been reconstituted into pure monoacyl lecithin vesicles to

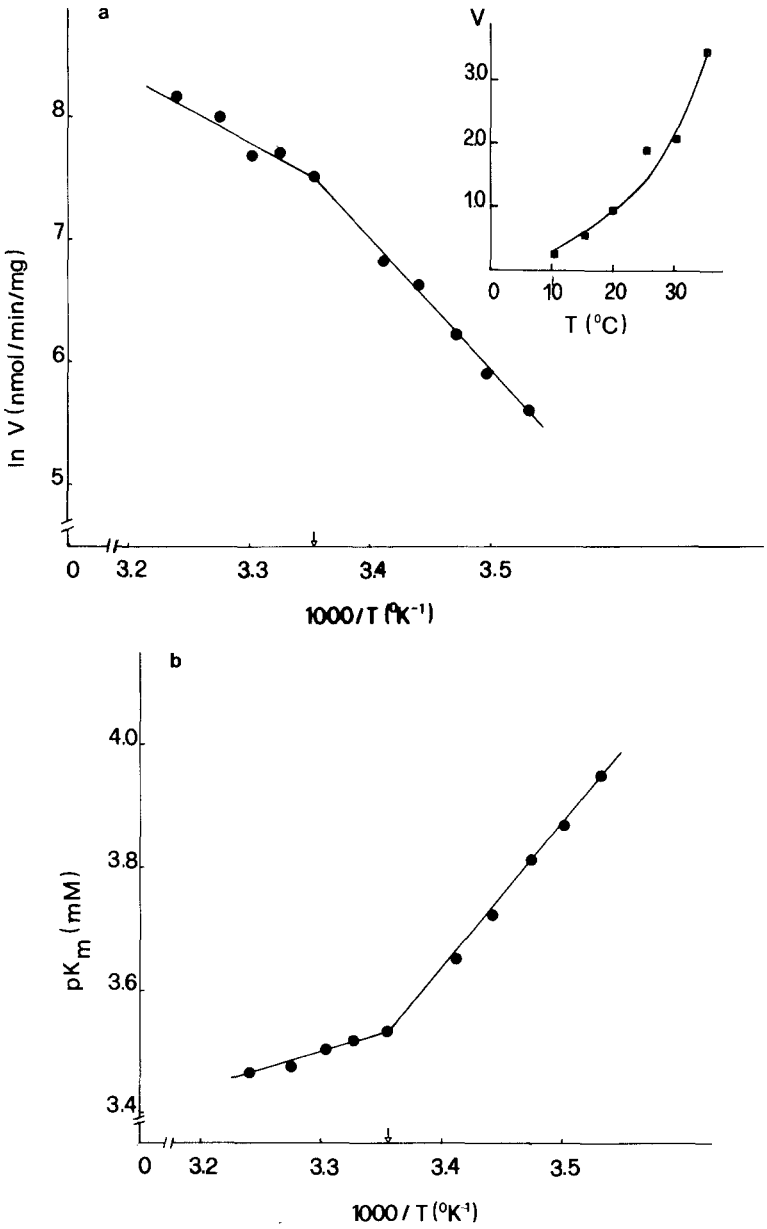

Fig. 1. The effect of temperature on the kinetics of ATPase in submitochondrial particles. (a) Arrhenius plot of maximal ATP hydrolysis rate; (b) Van't Hoff plot for the K_m. The experimental conditions and the method of calculation are described under Materials and Methods.

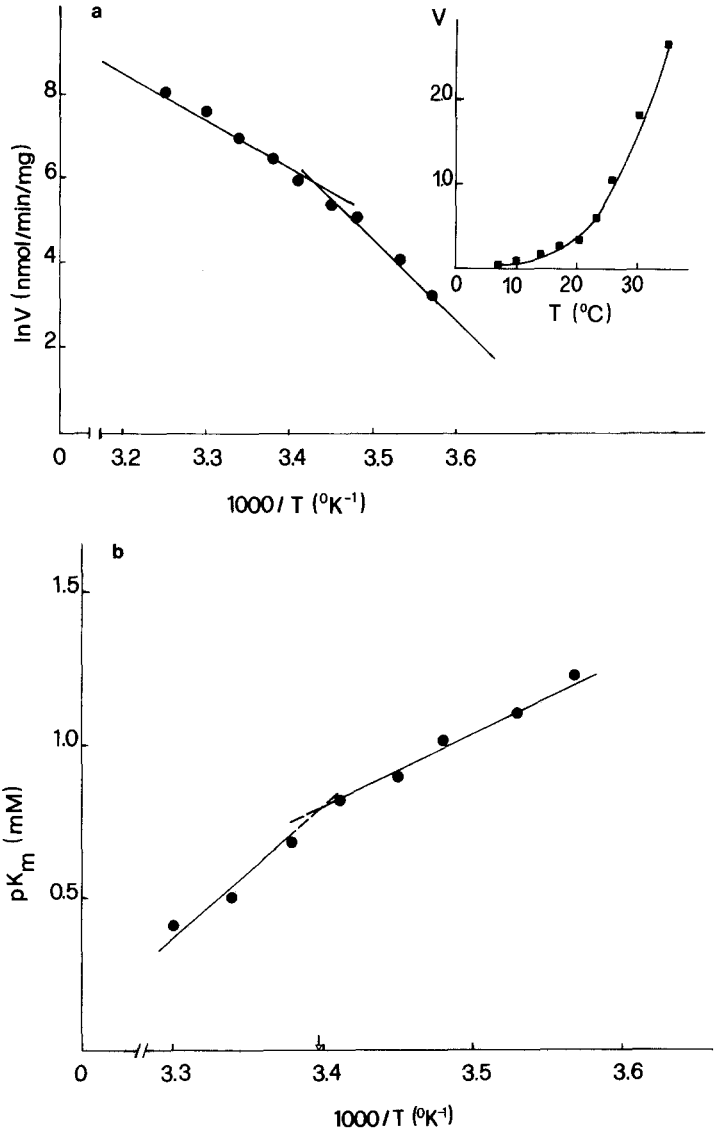

Fig. 2. The effect of temperature on the kinetics of complex V ATPase activity. (a) Arrhenius plot of maximal ATP hydrolysis rate; (b) Van't Hoff plot for the K_m. The experimental conditions and the method of calculation are described under Materials and Methods.

a final lecithin:protein ratio of about 1 mg phospholipid/mg protein; other phospholipids are absent according to GLC analysis (not shown). When the kinetic properties of these ATPase preparations are investigated, we observe different behaviors from SMP when the lecithin contains either oleoyl or mirystoyl fatty acyl chains, as would be expected after Bruni *et al.* (1975). Complex V reconstituted into DMPC vesicles appears poorly active, and it is only 40% oligomycin-sensitive. These characteristics might be due to the unsuitable short fatty acyl chain of the phospholipid, which induces an unfavorable structure of the bilayer, according to Montecucco *et al.* (1982).

The temperature dependence study reported in Fig. 3a shows that a sharp break occurs between 24 and 28°C in the Arrhenius plot of the DMPC–enzyme, indicating a steep drop in the ATPase activity below 24°C. The calculated activation energies are reported in Table I. The DOPC–complex V Arrhenius plot (Fig. 4a) shows a linear decrease of ln V vs $1/T$, from which an activation energy of 17.8 can be calculated. Below the transition temperature the activation energy for DMPC–enzyme, 41.4 kcal/mol, is considerably higher than those of the other preparations.

Above the transition temperatures, however, all activation energies are similar and are in the range of 12 to 14 kcal/mol (only isolated complex V shows an exceptionally high E_a, which we cannot explain at present, although two possibilities might be considered: the low lipid content of the enzyme preparation and the presence of a presumably high amount of detergent that might affect the kinetic parameters).

As shown in Table I, the differences in activation energy are compensated by differences in entropy of activation, resulting in values of free energy of activation roughly independent of variations in temperature and phospholipid composition.

The Van't Hoff plot for the DOPC–enzyme reported in Fig. 4b shows a nearly unchanged value of pK_m with temperature. The plot for the DMPC–enzyme (Fig. 3b) is biphasic with a discontinuity at 24–28°C. The K_m values are more sensitive to temperature changes below such break, indicating a major temperature dependence of ATPase affinity for ATP below 24°C.

Discussion

Significance of the Thermodynamic Parameters of Activation

In most cases where nonlinear Arrhenius plots have been observed, the nonlinearity was expressed as a break of two intersecting lines, with an abrupt increase of activation energy (slope) below the break (Raison, 1972). The mitochondrial ATPase under the conditions of this study exhibits such a pattern. The Arrhenius plot shows a break with two intersecting lines, the rate

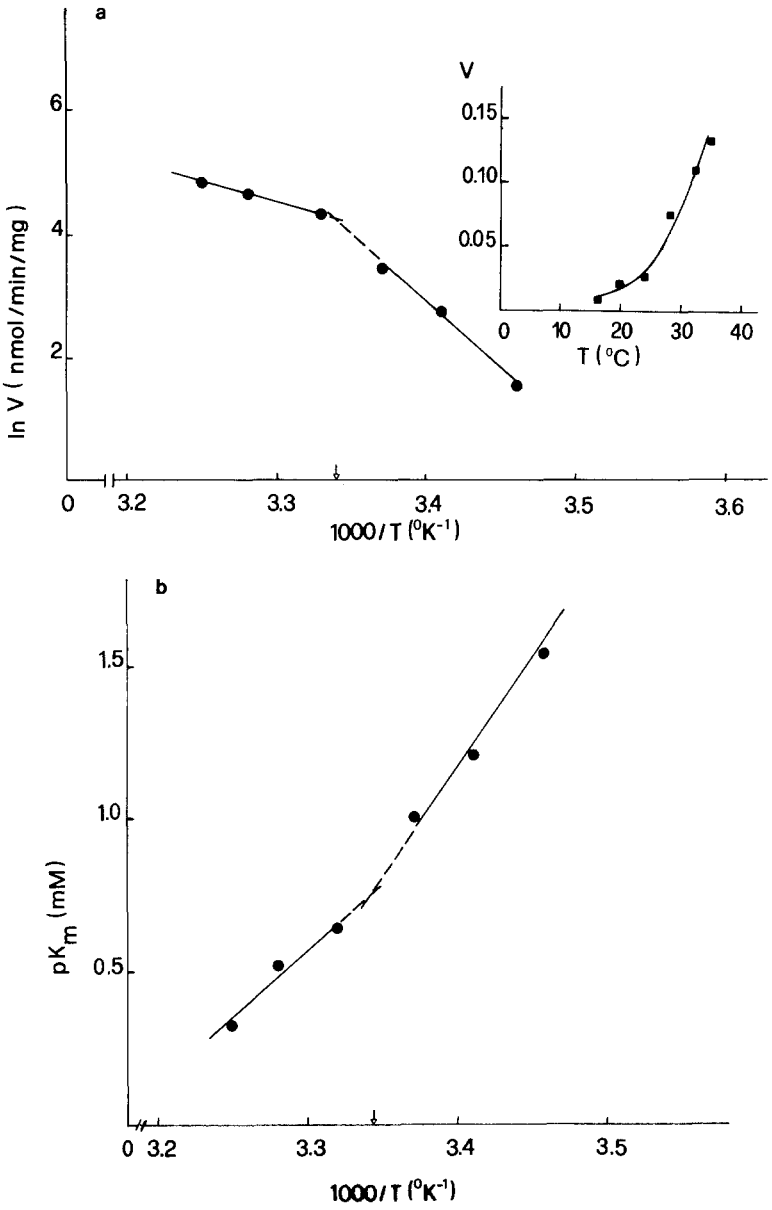

Fig. 3. The effect of temperature on the kinetics of DMPC–complex V ATPase activity. (a) Arrhenius plot of maximal ATP hydrolysis rate; (b) Van't Hoff plot for the K_m. The experimental conditions and the method of calculation are described under Materials and Methods.

Table I. Kinetic and Thermodynamic Activation Parameters of the ATP Hydrolysis Reaction for Various ATPase Preparations

Preparation	Temperature (°C)	K_c (sec^{-1})	$\Delta G‡$ (kcal · mol^{-1})	E_a (kcal · mol^{-1})	$\Delta H‡$ (kcal · mol^{-1})	$\Delta S‡$ (entropy units)
SMP	30	287.50[a]	14.3	12.4	11.8	−8.25
	10	37.50[a]	14.5	21.8	21.2	23.70
Complex V	30	15.20	16.1	21.0	20.4	14.35
	10	0.50	16.9	39.7	39.2	78.60
DMPC–complex V	32	0.90	17.9	13.9	12.5	−17.70
	16	0.06	18.5	41.4	40.9	77.40
DOPC–complex V	32	2.55	17.3	17.8	17.9	2.1

[a]A content of 0.12 nmol ATPase/mg mitochondrial protein was considered, according to Bertina *et al.* (1973).

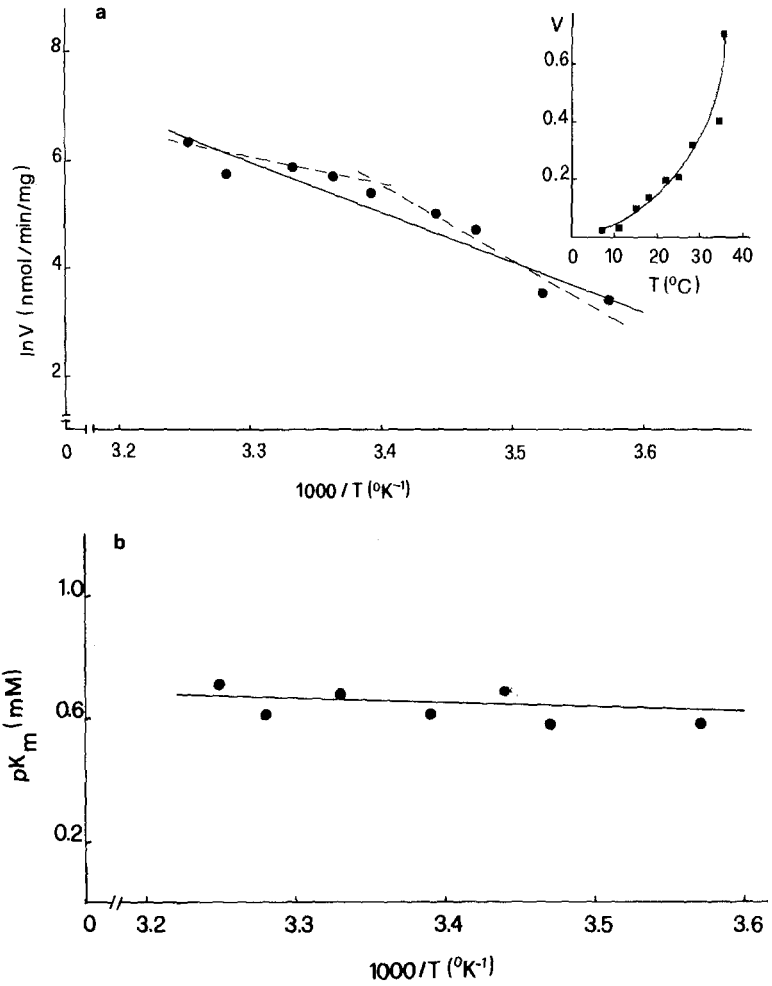

Fig. 4. The effect of temperature on the kinetics of DOPC–complex V ATPase activity. (a) Arrhenius plot of maximal ATP hydrolysis rate; (b) Van't Hoff plot for the K_m. The experimental conditions and the method of calculation are described under Materials and Methods. However, the data in the Arrhenius plot are here analyzed using the linear regression method both in the whole temperature range studied, where a correlation coefficient (r^2) of 0.92 is calculated, and in the temperature ranges 7–20°C and 20–35°C separately, where r^2 of 0.91 and 0.76, respectively, are calculated.

constants immediately above and below the point of intersection (isokinetic point) are equal, and, therefore, according to the transition state theory, they have the same free energy change $\Delta G\ddagger$ for formation of the activated complex. However, as reasoned by Kumamoto *et al.* (1971), the two independent processes on either side of the isokinetic point have different activation

energies, as determined experimentally, and therefore different enthalpy values; therefore, since $\Delta G‡ = \Delta H‡ - T \cdot \Delta S‡$, the change in $\Delta H‡$ requires a corresponding and compensating change in $\Delta S‡$ to maintain a constant $\Delta G‡$. Such compensation has been demonstrated for H^+-ATPase by calculating the thermodynamic parameters of the activated state even at temperatures far away from the isokinetic point.

The break in the Arrhenius plot may therefore be well defined in thermodynamic terms. Its mechanistic significance, however, is unclear, since there is no theoretical reason *a priori* why the transition entropy should compensate the transition enthalpy in all cases. Interpretation of the activation parameters is difficult since they involve different types of enzyme–substrate interaction, solvation and ionic effect, and also the viscosity and specific nature of the solvent (Biosca *et al.*, 1983; Beece *et al.*, 1980). The nature of the lipid environment is considered important in determining the sign and value of the activation parameters for the sarcoplasmic reticulum ATPase (Hidalgo *et al.*, 1976).

Causes for Nonlinear Arrhenius Plots

Several reasons may be given for a nonlinear Arrhenius plot (Dixon and Webb, 1964; Londesborough, 1980; Kumamoto *et al.*, 1971).

The first cause of a nonlinear Arrhenius plot may be purely kinetic in origin; when a composite rate constant is a function of two or more rate constants having very different energies of activation, the contribution of each constant may change significantly as the temperature is varied, so that the rate-limiting step changes with temperature. This is probably the most common cause of a nonlinear Arrhenius plot.

Another cause is a temperature-induced conformational change in the enzyme. A theoretical Arrhenius plot of an enzyme existing in two catalytically active forms having different activation energies and in a temperature-dependent equilibrium will show a sharp break centered at the temperature in which the two forms are in equal amounts (Massey *et al.*, 1966); Biosca *et al.*, (1983) have shown that a break in the myosin subfragment 1 at 12°C in the kinetic constant of formation of the activated myosin–ATP complex is accompanied by a temperature-induced phase transition (aggregation) of the protein.

A third cause of a nonlinear Arrhenius plot is that the solvent structure undergoes a structural transition. Such relationships have been proposed for water at 0–4°C (Biosca *et al.*, 1982; Raison, 1972), and also for the so-called "vicinal" water adjacent to interfaces such as membranes at higher temperatures (Drost-Hansen, 1972).

On the other hand, Raison (1972) reasons that breaks in Arrhenius plots for membrane-bound enzymes are affected by the properties of membrane

lipids, irrespective of the enzyme species considered: in terms of a temperature-induced conformational change, it is unlikely that each of the enzymes tested would undergo an intrinsic change at the same temperature under one condition, and at a different temperature under another condition.

The main objection against the interpretation of the discontinuities as resulting from a phase change of the lipids is the fact that no phase transition is detected by differential scanning calorimetry in the lipids at the temperatures where the breaks occur. It was found that mitochondrial lipids undergo a broad phase transition below 0°C (Hackenbrock *et al.*, 1976; Höchli and Hackenbrock, 1977), whereas most breaks in Arrhenius plots of mitochondrial activities occur at temperatures ranging between 18 and 25°C (Lenaz, 1977). On the other hand, the mobility of spin probes in mitochondrial membranes (Raison *et al.*, 1971; Raison and McMurchie, 1974; Lenaz *et al.*, 1983) undergoes abrupt changes in the same temperature region where the enzyme activity breaks are detected. The significance of such breaks in the motion parameters of spin labels is obscure, although some are related to the lipids per se, and some others to the presence of proteins (Minetti and Ceccarini, 1982; Lenaz *et al.*, 1983). These anomálies could reflect either a phase transition occurring in relatively small domains of the lipids (McMurchie *et al.*, 1983; Minetti and Ceccarini, 1982) or alternatively temperature-dependent changes in the protein distribution, either lateral (Chapman *et al.*, 1979) or vertical (Shinitzky, 1979) leading to a nonhomogeneous fluidity change with temperature. Another objection to a phase change in the lipids as the cause of nonlinear Arrhenius plots has been the finding that Ca–ATPase from sarcoplasmic reticulum delipidated to one phospholipid molecule per molecule of protein and solubilized in detergent still shows a break at 20°C (Dean and Tanford, 1978); the same enzyme, however, shows a break at 29°C when incorporated in dipalmitoyl lecithin and no break when in dioleoyl lecithin (Hidalgo *et al.*, 1976). It is reasonable that the enzyme may undergo an intrinsic change with temperature, but this change is affected by the properties of lipids.

The structural transitions which form the basis of the kinetic changes are not understood. One possibility deserving consideration is that some temperature-dependent properties of the lipids other than a phase transition induce the structural transition in the proteins. One such property may be membrane viscosity; the catalytic properties of a protein are affected by viscosity of the medium (Beece *et al.*, 1980), by hindrance of the transition between two conformational states involved in catalysis. For this purpose Swann (1983) has investigated the temperature dependence of brain (Na^+,K^+)-ATPase and found that a break at 17°C results from stabilization of the E_2 conformation at lower temperatures and of the E_1 conformation at higher temperatures. The increased order ($\Delta S < 0$ when $E_1 \rightarrow E_2$) accompanying the E_2 conformation

might be favored by increased lipid viscosity. Another property of lipid bilayers that can be affected by temperature is the state of the lipid–water interface (Quinn, 1981). Davis *et al.* (1976) found by NMR spectroscopy that the mobility of the choline methyl groups of lecithin in sarcoplasmic reticulum undergoes a change with temperature which fits the breaks in the Arrhenius plots of Ca-ATPase better than the changes in the methylene groups of the hydrocarbon chains. According to Sandermann (1972, 1974, 1976, 1978), lipid hydration is the crucial factor for activity of C_{55}-isoprenoid alcohol phosphokinase.

Temperature Dependence of Mitochondrial H^+-ATPase

In analogy with several enzymes bound to membranes, the mitochondrial oligomycin-sensitive proton transport ATPase has a nonlinear Arrhenius plot. Breaks have been observed in ATPase from beef heart (Lenaz *et al.*, 1975), yeast (Bertoli *et al.*, 1976), and other sources (Lee and Gear, 1974), ranging between 10 and 25°C depending on the systems and the experimental conditions used. The breaks are in some way related to the lipid composition of the membrane. Studies in mitochondria from yeast cells grown with different fatty acid supplementations (Bertoli *et al.*, 1976) showed that the temperature of the break in the Arrhenius plots of ATPase activity increased with increasing saturation of the fatty acids incorporated into the mitochondrial membranes. There is, however, no correlation with the calorimetric gel-to-liquid phase transition, as this in mitochondria usually occurs at temperatures below 0°C (Hackenbrock *et al.*, 1976). As already discussed, however, in mitochondria, discontinuities have been observed in the mobility parameters of spin labels incorporated into the membranes (Lenaz *et al.*, 1983; Raison *et al.*, 1971; Benga *et al.*, 1978). A break detected in the Arrhenius plot of the ATPase activity of isolated OS-ATPase is accompanied by a break in the order parameter of the spin label 5-NS incorporated into the lipid surrounding the enzyme (Solaini and Bertoli, 1981).

Our studies reported in the present paper point out that the breaks in the Arrhenius and Van't Hoff plots of the isolated ATPase appear related to the lipid composition of the membrane. The reaction activation energy changed at the same temperature as the slope in the Van't Hoff plot of the apparent K_m for ATP both in submitochondrial particles and in the isolated enzyme with its natural phospholipid complementation or when incorporated into dimyristoyl lecithin vesicles. When, however, the enzyme was inlayed in dioleoyl lecithin liposomes the activation energy as well as the K_m for ATP in such system remain steady in the whole temperature range. In a complex system when many steps are involved in catalysis the meaning of the K_m is also complex and is probably better related to the Briggs–Haldane relationship K_m =

$(K_{-1} + K_2)/K_1$ than to the Michaelis–Menten relationship $K_m = K_s = K_{-1}/K_1$ (Dixon and Webb, 1964). Therefore, if K_2 is large compared with K_{-1}, the change of V could also induce a change in K_m. It is likely that, at least in the F_0F_1 ATPase, K_2 is limited by the H^+ conductance in F_0 and by other factors (Pedersen, 1975). Breaks in Van't Hoff plots have been reported for other membrane enzymes (Sullivan *et al.*, 1974; Blazyk *et al.*, 1981). Under experimental conditions in which a break in the activity of isolated complex V is detected, no break was found in the isolated soluble moiety of the enzyme, F_1 (Solaini and Bertoli, 1981). On the other hand, Gomez-Puyou *et al.* (1978) observed a break in F_1 at 18°C in water but no break in D_2O; Harris *et al.* (1981) found a break in F_1 at 18°C with ATP, but not using ITP as the substrate. It should be noted that the kinetic and thermodynamic properties of F_1 are drastically different from those of the total ATPase; when H^+ ions are not conducted through F_0 the rate-limiting step could be changed [since ATPase activity in F_1 is usually enhanced severalfold (Tzagoloff *et al.*, 1968)], and a break in the Arrhenius plot could have a different origin. Moreover, cold lability of isolated F_1 (Penefsky and Warner, 1965) but not of the F_0F_1 complex might indicate that the break in the ATPase activity of F_1, when found, is a different phenomenon from the break observed in intact mitochondrial membranes or in the isolated oligomycin-sensitive ATPase. That the break in the oligomycin-sensitive enzyme is a property related to the membrane sector of the enzyme is also supported by other observations, as the absence of the break by treatment with detergents or organic solvents (Parenti Castelli *et al.*, 1979). A study of the temperature dependence of the membrane sector TF_0 of the thermophilic bacterium PS3 has shown a break in the Arrhenius plot of the H^+ conductance of the factor inlayed in phospholipid vesicles (Okamoto *et al.*, 1977).

Our data cannot demonstrate the mechanism by which lipids influence the properties of ATPase; the thermodynamic parameters of activation follow the general pattern already discussed for other enzymes showing breaks in Arrhenius plots (Hidalgo *et al.*, 1976), but little mechanistic implication can be derived from such data. It could be speculated that a constraint by increased lipid viscosity or some other factors in the F_0 moiety (the proton channel) could limit essential conformational changes between different structural states involved in the catalytic activity. The interpretation given by Hidalgo *et al.* (1976) for sarcoplasmic reticulum ATPase could be valid in the case of mitochondrial ATPase. When lipids assume a more rigid state, a less efficient catalysis (higher positive ΔH for activation) is accompanied by a greater loss of order during activation (higher positive $\Delta S‡$). Phospholipids in a rigid state may stabilize a preactivation state by the increased viscosity around the enzyme. Accordingly, the values of free energy of activation are independent of variations of the viscosity of the lipid phase.

The overall data suggest that a temperature-dependent modification in the lipid–protein interactions occurs in the F_0F_1 ATPase; such modification appears to be accompanied by a conformational change in the protein, as shown by the circular dichroism data indicating a decrease of α-helix content below 20–25°C (Curatola *et al.*, 1983) and by the discontinuity in the temperature dependence of intrinsic tryptophan fluorescence (Parenti Castelli *et al.*, 1983).

The drastic decrease of α-helix content shown by the CD spectra of ATPase below 20°C is in line with the suggestion that changes in the hydrophilic–hydrophobic balance of the lipid–protein contacts could occur; no experimental data are, however, available on the possible existence of vertical displacements of this enzyme in the lipids.

Acknowledgments

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