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Temperature-induced states of isolated F_1-ATPase affect catalysis, enzyme conformation and high-affinity nucleotide binding sites

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Isolated, nucleotide-depleted bovine-heart F_1-ATPase exhibits a break in Arrhenius plot with a 2.7-fold increase in activation energy of ATP hydrolysis below 18–19 °C. Analysis of intrinsic tyrosine fluorescence and of the circular dichroism of F_1-ATPase showed an abrupt and reversible conformational change occurring at the break temperature, characteristic of a structural tightening at low temperature. Analysis of catalytic nucleotide binding sites using fluorescent ADP analog, 3′-*O*-(1-naphthoyl)adenosine diphosphate did not show any significant change in affinity of nucleotide binding around the transition temperature but the bound fluorophore exerted a more restricted motion and slower rotation at temperatures below the break, indicating a change in the mobility of groups in the close neighbourhood. It is concluded that, as a result of temperature, two kinetically distinct states of F_1-ATPase are induced, due to a change in enzyme conformation, which influences directly the properties of catalytic nucleotide binding sites.

Introduction

Proton-translocating F_0F_1-ATPase (H^+-ATPase) of mitochondria, chloroplast and bacteria consists of a membrane sector (F_0) which conducts protons and of a peripheral part (F_1), which is the site of catalysis [1,2]. Both parts of enzyme are flexible, oligomeric proteins capable of various conformational states, which can be either static – stable in time, or dynamic – short lived, e.g., occurring during catalysis. Particularly suited for changes in conformation appears to be the catalytic part F_1, which is in fact a water-soluble protein, easily detachable from the membrane [1,2]. It consists of five types of subunits with stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$ [1,2]. Both slow and fast conformational changes of F_1 were proposed and described, e.g., in response to binding of substrate, binding of inhibitors, binding of F_1 to F_0, or energization of the membrane (for references, see Refs. 1–7). Particular attention is, however, paid to all the changes in F_1 quaternary structure related to reversible and probably cyclic conformational states of intra- and intersubunit character that could participate in enzyme catalysis, ion translocation and coupling of these two processes [8–12].

It was shown before that isolated F_1 of different origin exhibits a nonlinear temperature dependence of ATP hydrolysis [13–17]. As isolated F_1 is free of any bound lipid, the nonlinearity of Arrhenius plots is most probably related to conformational changes in the enzyme molecule [18], as observed by measurements of intrinsic fluorescence [16], circular dichroism [16] or small angle neutron scattering [17].

The aim of the present study was to analyze whether the above temperature-induced changes influence directly the catalytic sites on F_1. For this purpose we used the nucleotide depleted enzyme and a fluorescent naphthoyl derivative of ADP, which can bind with high affinity up to all three catalytic sites [19,20] and represents a potentially suitable probe to sense changes in nucleotide binding sites on F_1.

Abbreviations: F_1, F_1-ATPase (catalytic part of H^+-translocating ATP synthase of F_0F_1-type); Nd-F_1, nucleotide-depleted F_1-ATPase; F_0, membrane sector part of H^+-translocating ATP synthase of F_0F_1-type; N-ADP, 3-*O*-(1-naphthoyl)adenosine diphosphate; TNP-ADP, 2′,3′-*O*-(2,4,6-trinitrophenyl)adenosine diphosphate; DMAN-ADP, 3′-*O*-1-(5-dimethylamino)naphthoyladenosine diphosphate; CD, circular dichroism.

Enzymes: F_1-ATPase (EC 3.6.1.3); pyruvate kinase (EC 2.7.1.40); lactic dehydrogenase (EC 1.1.1.27).

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Materials and Methods

Preparation of F_1-ATPase and removal of nucleotides. Bovine heart submitochondrial particles prepared as described in Ref. 21 and suspended in 0.25 M sucrose, 4 mM ATP, 1 mM EDTA, 10 mM Tris-HCl (pH 9.2) were incubated at a protein concentration 30 mg/ml at room temperature for 12–16 h. The pH was then adjusted to 8.0 with 1 M HCl and crude F_1-ATPase was isolated by chloroform treatment as described by Beechey et al. [22]. The aqueous phase was centrifuged for 30 min at $105\,000 \times g$ at 20 °C and the obtained supernatant was brought to 0.6 saturation with saturated ammonium sulfate solution (pH 7.0). After 10 min it was centrifuged at 20 °C for 15 min at $25\,000 \times g$ and the sedimented enzyme was dissolved in 100 mM Tris-SO_4, 5 mM EDTA (pH 8.0). A second precipitation was performed the same way at 0.5 saturation of ammonium sulfate.

Nucleotides were removed by chromatography on Sephadex G-50, using as the elution buffer 100 mM Tris-SO_4, 5 mM EDTA, 50% glycerol, pH 8.0 as described by Garrett and Penefsky [23]. The fractions with the highest A_{280}/A_{260} ratio, indicative of nucleotide depletion [23] were pooled and concentrated on a Centricon 30 Microconcentrator (Amicon). The specific activity of the enzyme was 60–80 U/mg protein (at 20 °C) and this nucleotide depleted F_1-ATPase is referred to further as Nd-F_1. To prepare a control enzyme with bound nucleotides, the Sephadex chromatography was performed with elution buffer containing 100 mM Tris-SO_4, 1 mM $MgCl_2$, 10 μM ATP, 50% glycerol (pH 8.0). This enzyme is referred to further as F_1.

Protein and ATPase assay. Submitochondrial particles protein was determined by the biuret method [24] in the presence of 1% deoxycholate. F_1-ATPase protein was measured according to Lowry et al. [25]. Bovine serum albumin was used as a standard.

ATPase activity was measured with ATP-regenerating system [16] by following the decrease of absorption of NADH at 340 nm in a Zeiss PMQ III spectrophotometer equipped with a thermostating system. The medium contained 25 mM Tris-acetate, 25 mM KOH, 0.3 M sucrose, 4 mM ATP, 5 mM $MgCl_2$, 200 μM NADH, 1.5 mM phospho*enol*pyruvate, 10 U of lactate dehydrogenase, 7 U of pyruvate kinase, pH 7.5 and the reaction was started by addition of 4 μg protein of enzyme.

Fluorescence measurements. For the determination of intrinsic fluorescence 1 ml of 1.2 μM enzyme in 40 mM Tris-SO_4 (pH 8) was used and the fluorescence spectra were recorded between 290 and 450 nm or the intensity of emission at 307 nm was measured after excitation at 276 nm in a Jasco FP-550 spectrofluorometer equipped with a thermostating system.

The temperature dependence of fluorescence anisotropy ($\lambda_{exc} = 298$, $\lambda_{em} = 392$ nm) of naphthoyl-ADP (N-ADP) bound to the nucleotide depleted F_1-ATPase (Nd-F_1) was carried out incubating 0.3 μM N-ADP with 0.5 μM Nd-F_1 in 50 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$ (pH 8). The same sample was analyzed at each temperature in the range 12–30 °C taking the temperature from the lowest to the highest value and vice versa. The sample was constantly stirred magnetically. The anisotropy value reported at each temperature is the average of 5–10 measurements obtained on the same sample. The experiment was repeated at least with five different enzyme preparations. The subnanosecond spectrofluorometer SLM 4800S was used for both the steady-state and the dynamic measurements. Limiting anisotropy r_∞ and the rotational relaxation rate R, were computed according to Weber's theory of hindered rotations [26] from the values of steady-state anisotropy r, fluorescence life-time τ and from dynamic depolarization Δ. The quantities, fulfilling equation

$$r = \frac{r_0 - r_\infty}{1 + 6R\tau} + r_\infty$$

where r_0 is the fluorescence anisotropy observed in the absence of depolarizing rotations, were determined according to ref. 27.

The fluorescence titrations of N-ADP with Nd-F_1 were carried out at different temperatures in the range 8–30 °C. In 1 ml of 50 mM Tris-Cl, 50 mM KCl, 2 mM $MgCl_2$ (pH 8.0), 0.45 μM N-ADP was titrated with Nd-F_1. To complete the association reaction and attain the state of equilibrium, as suggested in Ref. 19, after the addition of each aliquot of the enzyme to the probe, the reaction mixture was left in incubation for 2 min.

The apparent dissociation constant (K_d') value of the binding reaction was evaluated through the measurements of fluorescence polarization (anisotropy) changes as described by Tiedge et al. [19], using a computer-supported fit of theoretical binding curves to N-ADP/Nd-F_1 titration data by a non-linear least-square analysis [28,29]. The binding sites were considered identical and independent.

Circular dichroism measurements. The circular dichroism spectra were taken at 14 and 28 °C on a Jasco J-500A spectropolarimeter equipped with a thermostating system, using cuvettes of 0.1 cm path length. Samples of 0.05–0.2 mg protein of F_1 or Nd-F_1 in 1 ml of 40 mM Tris-SO_4 (pH 8.0) were used for scanning. Several samples were analyzed at both temperatures; some of them were taken from the lower to the higher temperature, others from the higher to the lower. The molar ellipticities were obtained from the mean of two spectra recorded on one sample and at each temperature six samples were examined. The experiment was repeated at least three times with different enzyme preparations. The ellipticities were calculated in deg · cm^2 · $dmol^{-1}$ on

the basis of a mean molecular weight of 115 per aminoacid [30]. The contribution of α-helix was evaluated according to Ref. 31.

Materials. N-ADP was a generous gift by Prof. G. Schäfer. ATP, NADH, phospho*enol*pyruvate, pyruvate kinase and lactic dehydrogenase were purchased from Sigma (St. Louis, U.S.A.). All other chemicals were of analytical grade.

Results

Temperature dependence of ATP hydrolysis

With the aim of analyzing in detail the effect of temperature on catalytic activity and conformational states of F_1-ATPase, two enzyme preparations were used: purified, chloroform released F_1-ATPase (further indicated as F_1) which is analogous to the enzyme used in previous studies [16]; and purified, chloroform released F_1-ATPase, further depleted of endogenous nucleotides (further indicated as Nd-F_1) which permits the characterization of catalytic nucleotide binding sites with the aid of adenine nucleotide analogs [19,20].

As shown in Fig. 1, both enzyme preparations, F_1 and Nd-F_1, exhibit a discontinuous Arrhenius plot of ATP hydrolysis at saturating substrate concentration (4 mM ATP). In case of F_1 the intersection of two straight lines corresponds to 18°C, in case of Nd-F_1 the break point is found at 17.5°C. Both F_1 and Nd-F_1 exhibit equally low activation energy (E_a) above the break at higher temperatures, which amounts 37 and 38 kJ/mol, respectively. This value is in fact slightly higher than the value previously obtained [16] with F_1-ATPase prepared according to Ref. 32. The higher E_a values at lower temperatures below the break are, in contrast, significantly different in the two enzymes, being lower in Nd-F_1 (100 kJ/mol) than in F_1 (150 kJ/mol). Thus it is evident that the two temperature-induced states of F_1-ATPase, which differ in catalytic properties and might be connected with different conformational states, are preserved even in Nd-F_1. The difference in E_a below the break for the two forms of the enzyme further indicates that nucleotide binding sites can be directly involved in temperature-induced changes, because the only difference between F_1 and Nd-F_1 is the removal of bound nucleotides from the catalytic site(s).

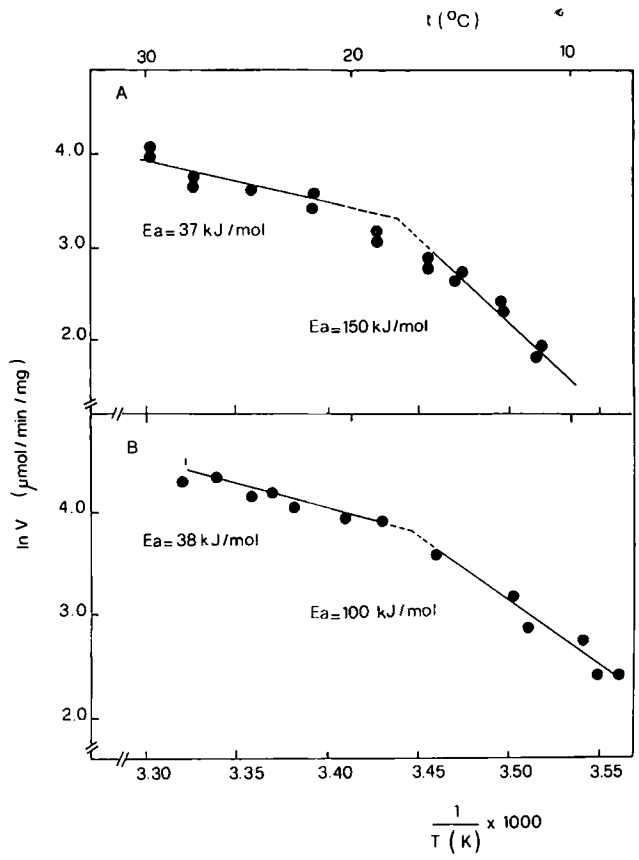

Fig. 1. Arrhenius plot of the rate of ATP hydrolysis by F_1 ATPase (A) and nucleotides-depleted F_1-ATPase (B). The ATPase activity was measured with an ATP-regenerating system using 4 mM ATP and 4 μg protein of enzyme. For details, see Materials and Methods.

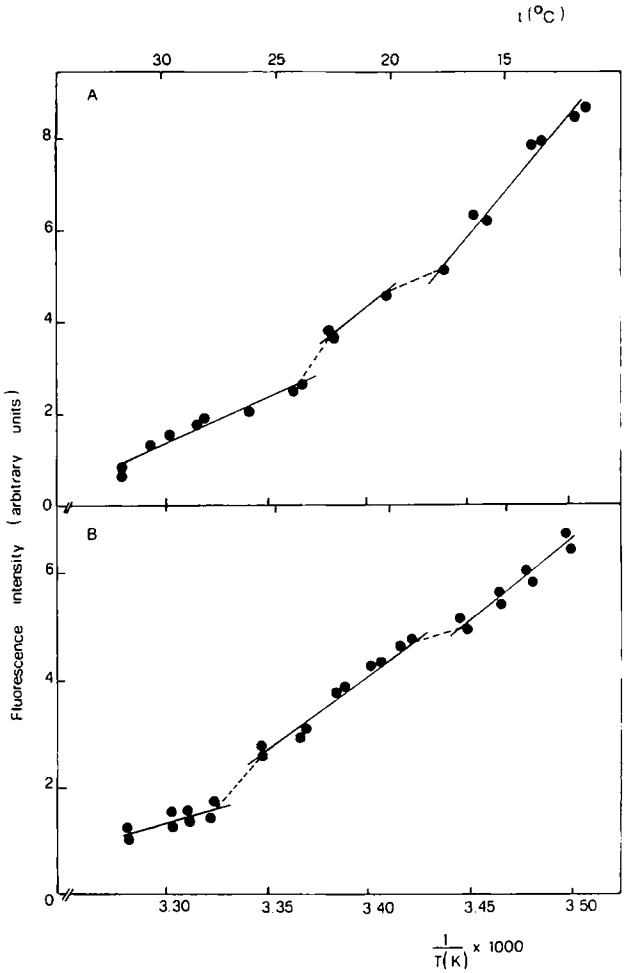

Fig. 2. Temperature dependence of the intrinsic fluorescence of F_1-ATPase (A) and nucleotide depleted F_1-ATPase (B). The fluorescence intensity at 307 nm, reported in arbitrary units, was measured after excitation at 276 nm.

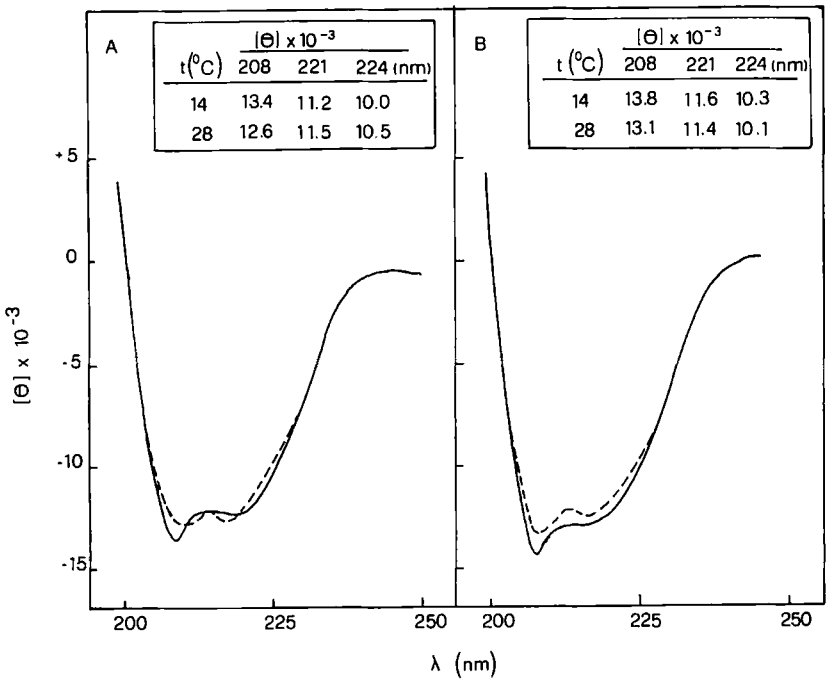

Fig. 3. Circular dichroism spectra of F_1-ATPase (A) and nucleotide depleted F_1-ATPase (B). Mean residual molar ellipticity of F_1-ATPase (A) and nucleotide-depleted F_1-ATPase (B) in 40 mM Tris-SO_4 (pH 8) at 14 (———) and 28° C (- - -). Spectra were recorded in 0.1 cm cells at a protein concentration of 0.1 mg/ml. In the inserts the mean residual molar ellipticities at three representative wavelengths are reported.

The effect of temperature on intrinsic fluorescence of F_1-ATPase

Fluorescence changes of protein tyrosine and tryptophan can be indicative of structural changes in the protein. Numerous tyrosyl, but not tryptophan residues [33,34], are responsible for the intrinsic fluorescence of F_1 and Nd-F_1.

In Fig. 2 the intrinsic fluorescence was measured over a wide range of temperature using both the F_1 and Nd-F_1. The intrinsic fluorescence (intensity of emission at 307 nm) of F_1 (Fig. 2A) decreased with increasing temperature nonlinearly as two discontinuities are present at 18–20° C and at 22–24° C, respectively. Similarly, in Nd-F_1 (Fig. 2B) the dependence was nonlinear

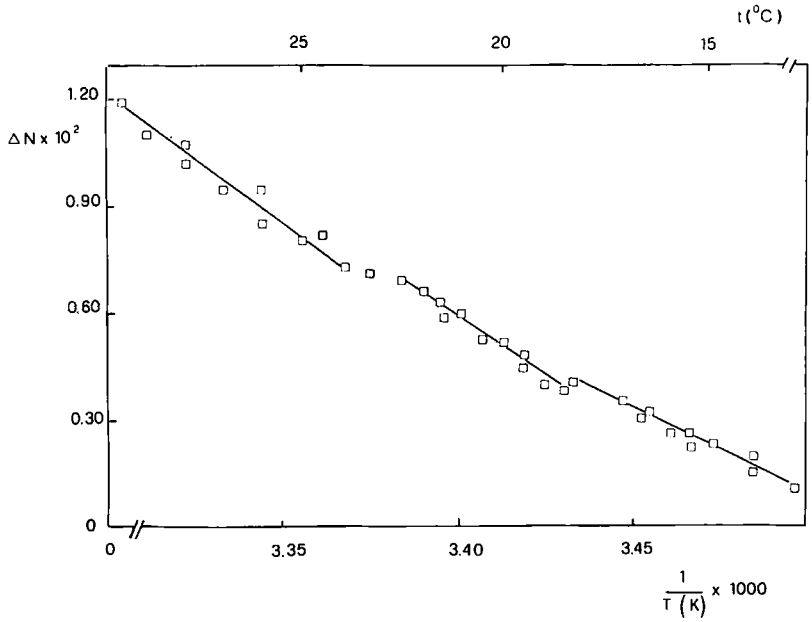

Fig. 4. Temperature dependence of fluorescence anisotropy of N-ADP bound to nucleotide depleted F_1-ATPase. ΔN represents the average of the anisotropy difference between the maximal anisotropy (at 12° C) and the anisotropy value measured at any other temperature in a typical experiment. Each anisotropy value was obtained as the average of 10 measurements and the standard error was never greater than 1%. Measurements were performed in the range of 10–30° C using 0.3 μM N-ADP and 0.5 μM Nd-F_1 at $\lambda_{exc} = 298$ nm and $\lambda_{em} = 392$. Lines were derived by regression analysis. For details, see Materials and Methods.

and two discontinuities were found at 17–19 °C and 26–28 °C, respectively. Fluorescence measurements indicate a significant change in enzyme structure due to changing temperature. As the temperature dependence is nonlinear, it is probable that distinct conformational states which change the behaviour of some tyrosines are induced in both forms of enzyme rather abruptly. In both enzymes thus the discontinuity-conformational change at approx. 17–20 °C corresponds well with the breaks in Arrhenius plots (see Fig. 1), suggesting that the change in catalytic properties might be related to a forgoing structural change of enzyme molecule. The importance and functional relevance of the discontinuity at higher temperature is unclear; however, the two transitions are of different character. While the transition at lower temperature shows a conservation of fluorescence intensity within the temperature interval of the transition, the transition at higher temperature shows a drop in fluorescence intensity (Fig. 2).

Influence of temperature on circular dichroism spectra of F_1-ATPase

Structural properties of the enzyme were then investigated by comparing circular dichroism (CD) spectra of F_1 and Nd-F_1 at different temperatures. The spectra were recorded at 200–250 nm at two temperatures, 14 °C and 28 °C, which should be indicative of the enzyme states differing in activation energy (below and above the break: Fig. 1). As shown in Fig. 3 the CD spectra obtained are characterized by a negative band at 208 nm, characteristic for relatively high α-helix contribution. At higher temperature the CD spectra of both F_1 and Nd-F_1 become significantly less intense.

Thus at 14 and 28 °C the molar ellipticities (in deg · $cm^2 \cdot dmol^{-1}$) of F_1 at 208 nm/221 nm/224 nm were 13441/11200/10015 and 12650/11464/10542, respectively. In case of Nd-F_1 the corresponding values 13812/11643/10339 and 13076/11385/10133 were observed at 14 and 28 °C, respectively. The approximation according to Ref. 31 showed that in F_1 the α-helix content decreased from 32.5 to 29.5% between 14 and 28 °C, whilst in case of Nd-F_1 it decreased from 34 to 31%, respectively.

This indicates that both enzyme preparations exhibit different conformations at temperatures below and above the break in Arrhenius plots (see Fig. 1) which are pronounced enough to be detected by the CD.

The effect of temperature on nucleotide binding sites

As in the above experiments the two forms of the enzyme, F_1 and Nd-F_1, behaved essentially the same way, although not completely identically, the Nd-F_1 was used further to assess whether the conformational changes are directly connected with the enzyme catalytic sites and their interaction with nucleotides. For this purpose we used the fluorescent analog of ADP, 3-*O*-(1-naphthoyl)adenosine diphosphate [19,20], which binds tightly to three equivalent nucleotide binding sites

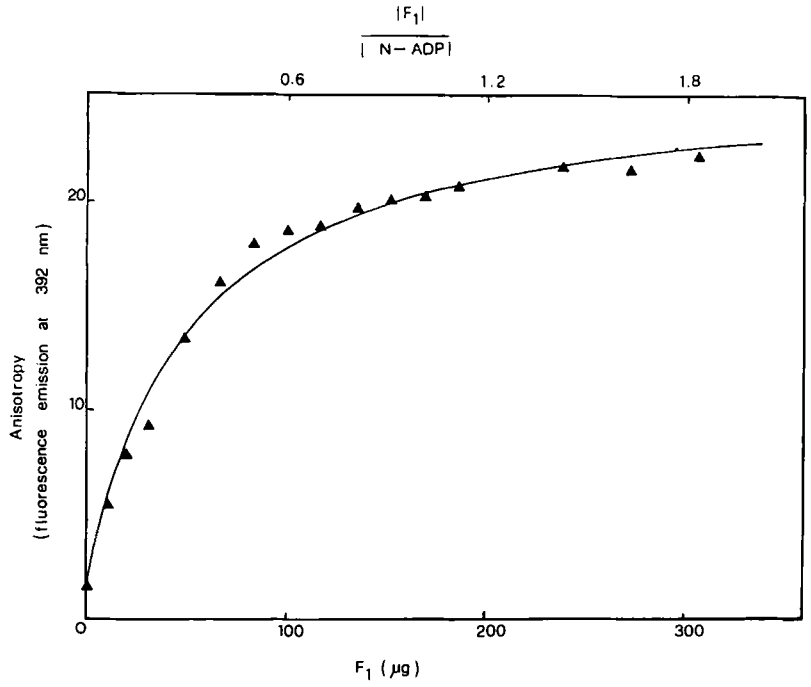

Fig. 5. Fluorescence anisotropy titration of N-ADP with Nd-F_1. The anisotropy of the fluorescence of N-ADP (0.45 μM) is shown as a function of the total Nd-F_1 added at 24 °C. Analogous plots (not shown) were obtained at different temperatures in the range 8–30 °C. After each addition, the sample was incubated for 2 min before the measurements were done. For details, see Materials and Methods.

on Nd-F_1 with similar affinities (K_d around 100 nM in the presence of Mg^{2+}), the binding being accompanied by the large decrease of fluorescence intensity and increase of fluorescence anisotropy due to immobilization of naphthoyl residue [19,20].

Nd-F_1 was incubated in the presence of Mg^{2+} with N-ADP at a molar ratio 0.5/0.3, i.e., under the condition where practically all N-ADP should be bound preferentially to one site/enzyme, and fluorescence anisotropy of the N-ADP/F_1 complex was measured as a function of temperature. As shown in Fig. 4, the fluorescence anisotropy decreases as the reciprocal of the temperature decreases. However, the relationship is nonlinear as only very slight differences in the anisotropy have been measured between 22 and 24 °C and between 18 and 19 °C, respectively. The presence of the two discontinuities, which reflect the change in properties of the nucleotide binding site tested, could be due to conformational changes of the enzyme causing either a change of the overall motion of the bound adenine nucleotide analog, and/or a change of the enzyme catalytic site(s) affinity for the probe.

To distinguish between these possibilities, the temperature dependence of the enzyme affinity for the probe was evaluated from the N-ADP/Nd-F_1 titration curves as exemplified in Fig. 5 for the temperature of 24 °C. The data were fitted by computer analysis to a single hyperbolic function which appears well in line with the experimental points and the K_d' values were determined by the computer program. Any attempt to interpolate the experimental data by assuming that the two binding sites experimentally found be characterized by different affinities did not significantly improve the goodness of the fit, and the resulting affinities of the two sites were always approximately equal within a few percent. The apparent dissociation constants (K_d') of the N-ADP/Nd-F_1 complex were calculated at different temperatures, and measurements were performed with four different preparations of Nd-F_1, whose characteristics are indicated in Table I. The observed K_d' values and number of binding sites approaching 2 per

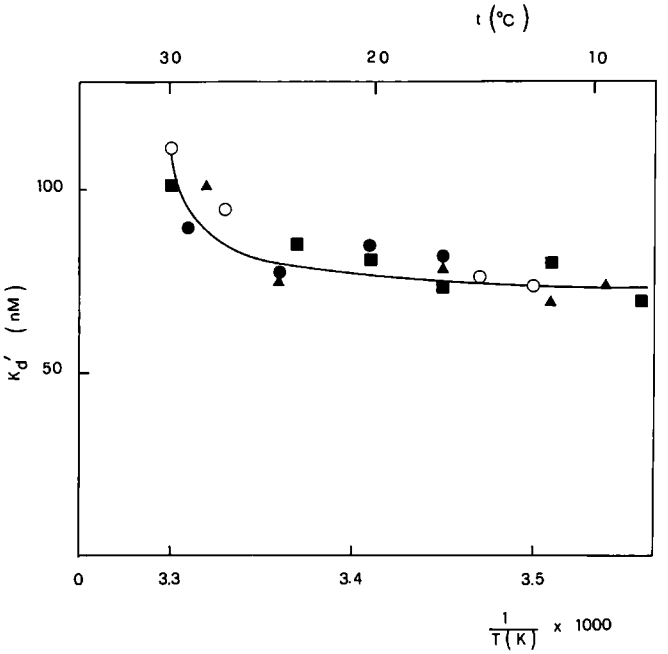

Fig. 6. Temperature dependence of apparent dissociation constant (K_d') for N-ADP. Measurements were performed as in Fig. 5. For each temperature tested, a new aliquot of N-ADP was used. K_d' values of four different enzyme preparations (I, II, III, IV) were calculated as described in Materials and Methods.

F_1 are in agreement with Ref. 19, although the method based on the fluorescence anisotropy measurements may lead to incorrect calculations possibly due to depolarization by energy transfer from tyrosine to naphthoyl residues as suggested in Ref. 20.

As is apparent from Table I and Fig. 6, within the temperature range 8–30 °C the K_d' increases from 71 to 102 nM (mean from four experiments) with increasing temperature. The dependence is, however, not linear and the significant change occurs only at higher temperatures, between 23 and 30 °C. The temperature range of K_d' changes may correspond to the discontinuity in fluorescence anisotropy of Nd-F_1/N-ADP at higher temperatures but not to the discontinuity at lower temperatures where the break in Arrhenius plots is also found (Fig. 1).

As shown in Table II, the two temperature-induced kinetics states of enzyme exhibit different dynamics of bound N-ADP. When the Nd-F_1/N-ADP complex was analyzed at 13 °C and 23 °C, both the order parameter

TABLE I

Characteristics of nucleotide depleted F_1-ATPase preparations

Specific activity was measured at 20 °C. A_{280}/A_{260} ratio indicates the content of endogenous nucleotides [23]. The apparent dissociation constants (K_d') and the number of binding sites (n) for N-ADP represent the minimum and maximum values determined within the temperature range 8–30 °C. For details see Materials and Methods.

Preparations	Specific activity (μmol·min^{-1}·mg^{-1})	A_{280}/A_{260}	K_d' (10^{-8} M)	n (mol/mol)
I	60	1.45	6.9– 9.0	1.5–1.7
II	70	1.50	7.0–10.2	1.6–1.8
III	60	1.48	7.4–10.3	1.7–2.0
IV	80	1.53	7.2–11.2	1.6–2.2

TABLE II

Temperature-induced changes of order parameter and rotational relaxation rate of Nd-F_1/N-ADP complex

Temperature (°C)	Order parameters (arbitrary units)	Rotational relaxation rate (rad/ns)
13	0.89	1.70
23	0.78	2.50

(S) and rotational movements indicated by rotational relaxation time (τ_c) were significantly changed, suggesting that at the temperatures above the break in Arrhenius plot the mobility of N-ADP is less restricted than at the temperatures below the break, while its rotational rate is increased.

Discussion

The main conclusion that can be drawn from the present study is that previously described [13–17] temperature induced changes in kinetics of hydrolytic activity of F_1-ATPase can be related to differences in mobility of groups which form, or are in close vicinity of, the high affinity nucleotide binding sites involved in catalysis. As a result of temperature, two states of F_1 are induced below and above approx. 18–19°C, and the higher activation energy of ATP hydrolysis at lower temperatures is accompanied by more restricted motion of the bound fluorescent adenine nucleotide.

To be able to analyze whether the temperature affects in any way the nucleotide binding sites involved in catalysis, the F_1 was depleted of nucleotides (Nd-F_1) using chromatography on Sephadex G-50 [23]. Comparison of Nd-F_1 and control F_1 from bovine heart showed that both enzymes behave very similarly with respect to changing temperature. In both cases the break in Arrhenius plots of ATP hydrolysis was found at similar temperature (17.5–18°C) and also the activation energy at higher temperatures was equally low: 37–38 kJ/mol. Both enzymes also showed that their conformation is similarly influenced by the temperature as judged from CD measurements (Fig. 3). CD spectra, similar to those shown by Roux et al. [35] and for the chloroplast F_1, by Younis et al. [36], demonstrated that at higher temperatures the content of α-helical structures decreases in F_1 and Nd-F_1; however, using this technique it was not possible to find whether this change occurs abruptly around the temperature of the break in Arrhenius plots. In contrast, the analysis of intrinsic fluorescence of F_1 due to tyrosyl residues showed a temperature dependence which was clearly nonlinear with two transitions present in both F_1 and Nd-F_1. Of these the transition at lower temperature (18–20°C in F_1 and 17–19°C in Nd-F_1) apparently correlated with the break in Arrhenius plot. The quantum yield of F_1 fluorescence indicates that tyrosine residues in F_1 contribute differently to observed intrinsic fluorescence [19] and the signal recorded certainly includes tyrosyl residues which are functionally and structurally related to the catalytic sites because significant quenching is observed after binding of ADP and especially after binding of its fluorescent analogs: N-ADP, DMAN-ADP or TNP-ADP [19,37]. Therefore, the temperature-induced changes in intrinsic fluorescence suggest that a conformational change takes place close to, or in the catalytic site. In fact, the transition in fluorescence of Nd-F_1 was at somewhat lower temperature than that of F_1 (17–19°C vs. 18–20°C) similarly as was the break in Arrhenius plot of Nd-F_1 with respect to that of F_1.

Fluorescence anisotropy of naphthoyl ADP (N-ADP) is increased after binding to F_1 [19,20] where it binds with similar affinity to all three catalytic sites [20]. A direct demonstration of temperature induced change in catalytic domain of F_1 is based on fluorescence anisotropy measurements of Nd-F_1/N-ADP complex, which exhibits a nonlinear dependence on temperature, again with one of the two transitions occurring at 18–19°C. The change in anisotropy of Nd-F_1/N-ADP was accompanied with changes in affinity of N-ADP binding occurring only at high temperatures, possibly in relation to the above-described discontinuities in F_1 fluorescence intensity and N-ADP fluorescence anisotropy found at higher temperatures (above 23–24°C). The change in fluorescence anisotropy of Nd-F_1/N-ADP around the temperatures corresponding to break in Arrhenius plots, where the change in K_d' is negligible, could thus mirror the effect of temperature on other properties of the nucleotide binding/catalytic site. Therefore we tried to characterize a microenvironment of bound N-ADP by determining the order parameter and rotational relaxation time on theoretical basis developed by Weber [26].

As documented in Table II, both the order parameter (S) and rotational rate (R) of Nd-F_1/N-ADP were significantly altered by the temperature change which induces the two states of F_1 described above. Although the two temperatures tested were rather close to the break point, going from 13 to 23°C, S decreased to 88% and the angular velocity increased to 147%. At the lower temperature state of F_1 the fluorophore was therefore more restricted in its motion and rotated more slowly than at higher temperatures.

It is suggested that the abrupt decrease of the activation energy of ATP hydrolysis by isolated bovine heart F_1-ATPase is connected with a conformational change of the enzyme which takes place close to or in the catalytic site and affects its rotational mobility. In contrast, the temperature-induced conformational change which affects the affinity of nucleotide binding site occurs at different, higher temperatures.

Acknowledgements

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