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CONFORMATIONAL CHANGES OF SOLUBLE MITOCHONDRIAL ATPase AS CONTROLLED BY HYDROPHOBIC INTERACTIONS WITHIN THE ENZYME

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SUMMARY

At 30° C soluble mitochondrial ATPase from baker's yeast shows non-linear kinetics with respect to Mg-ATP; the apparent Km values for Mg-ATP are 0.6 and 2.0 mM. At lower temperatures, 5°C and 12° C, the kinetics of the enzyme are linear with a Km for Mg-ATP of approximately 0.6 mM. Octylguanidine induces non-linear kinetics at 12° C. As octylguanidine and increases in temperature augment hydrophobic interactions within the enzyme, it is concluded that the strength of hydrophobic bonding within the protein regulates its conformational changes. Methanol activates the enzyme only at relatively high temperature which further indicates that the protein may exist in two active conformations.

INTRODUCTION

Pullman et al (1,2) were the first to isolate a soluble oligomycin insensitive ATPase (F_1) from the inner membrane of the mitochondria. The importance of this enzyme in oxidative phosphorylation became apparent when it was found that F_1 restored oxidative phosphorylation in mitochondrial membranes devoid of this factor (2). It is now established that F_1 possesses the catalytic site(s) for ATP formation and hydrolysis and thus, its mechanism of action is being extensively studied (for review see ref. 3). In this respect it has been proposed that during oxidative phosphorylation, the enzyme may undergo conformational changes (4,5) that are directly related to the formation of ATP, however at the present this possibility is a question of considerable controversy (3).

In this work it is shown that F_1 may exist in two forms, which may be distinguished by their distinctive kinetics toward Mg-ATP, and

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by their different sensitivity to methanol. In addition the results indicate that the change from one state to another is controlled by variations in the strength of the hydrophobic interactions that exist within the enzyme.

MATERIAL AND METHODS

Mitochondria from baker's yeast (La Azteca, S. A.) were obtained according to the procedure described by Tzagoloff (6) in 0.4 M sucrose, 50 mM tris- HCl (pH 8.2) and 1mM EDTA. The mitochondria were suspended in 0.25 M sucrose, 10 mM tris -HCl (pH 7.5) and 1 mM phenylmethylsulfonyl fluoride (PMSF) and sonicated for 1 min; the submitochondrial particles were isolated by centrifugation at 105,000 x g for 60 min. F_1 was solubilized by the method of Beechey et al (7), the water phase contained 0.15M sucrose, 2mM ATP, 2 mM EDTA and 1 mM PMSF (pH 7.3). Further purification was achieved by passage of the enzyme through a column of Sepharose-hexyl-ammonium (8), except that the elution medium contained 1 mM PMSF. The enzyme was stored as a 70% $(NH_4)SO_4$ precipitate. It was collected by centrifugation and the precipitate dissolved in 0.15M sucrose, 2mM ATP and 2mM EDTA (pH 7.4) and the ATPase activity measured in the conditions described under Results. After stopping the reaction with 5% trichloroacetic acid, inorganic phosphate was determined according to Sumner (9).

RESULTS

In agreement with Recktenwald and Hess (10) and Takeshige et al (11), the ATPase activity of yeast F_1 at various concentrations of Mg-ATP and at incubation temperature of 30° C shows a biphasic kinetic behavior in a Lineweaver-Burk plot (Fig. 1 A). The two Km values for Mg-ATP are approximately 0.6 mM and 2.0 mM. The kinetic pattern at 30° C has been consistently and repeatedly observed in twelve different preparations of soluble F_1 with almost identical results. However it has been found also that variations in temperature drastically alter the kinetic behavior of F_1. As shown in Figure 1A, at an incubation temperature of 12°C, and more clearly at 5°C, the Lineweaver-Burk plot is monophasic with a Km for Mg-ATP of approximately 0.6 mM, which apparently corresponds to one of the Km values of the enzyme incubated at 30°C.

It is known that the strength of hydrophobic bonding in a given system increases as the temperature is raised (12). Therefore one of the possible

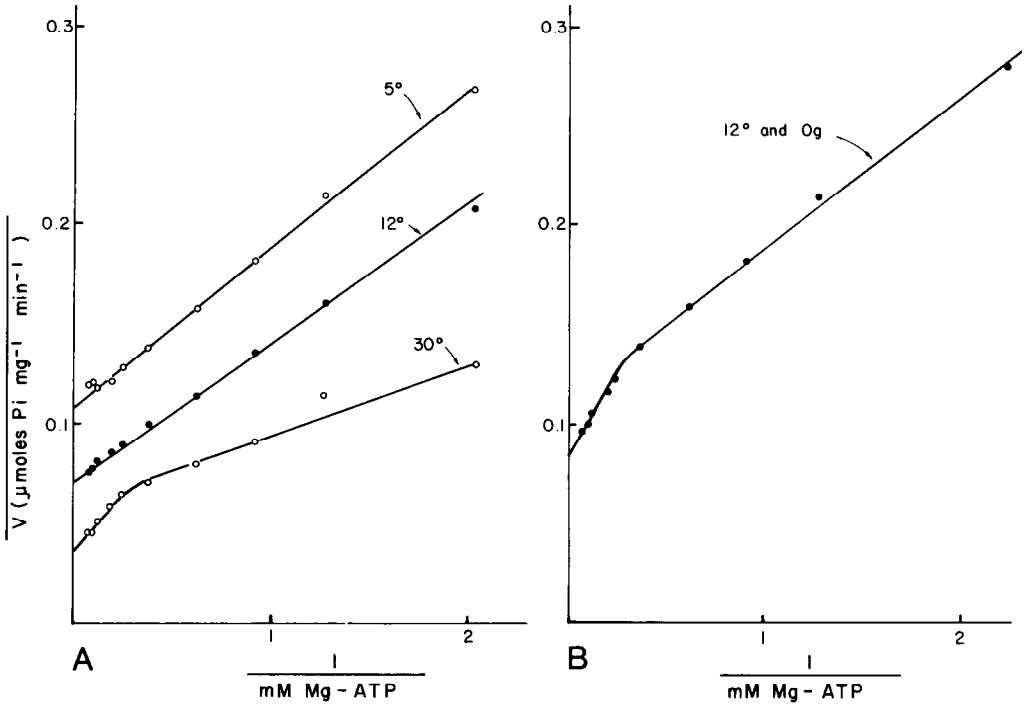

Figure 1. Lineweaver-Burk Plot for Mg-ATP of Soluble F_1. The experimental conditions were 10 mM tris-HCl (pH 8.5), the indicated concentrations of Mg-ATP shown maintaining Mg-ATP/free Mg^{2+} = 1. The incubation time was 1 min, at the indicated temperatures. The reaction was started by the addition of F_1 after temperature equilibration of the reaction mixture. The experiments at 30°C, 12°C, and 5°C were carried out with 15 μg 30 μg and 35 μg of enzyme respectively. In the experiment with octylguanidine (Og) 35μgm of enzyme were employed; the concentration of octylguanidine was 180 μM and the incubation temperature was 12°C

causes of the distinctive kinetic characteristics of F_1 at the temperatures assayed could be the different strength of hydrophobic interactions that exist within the enzyme (either in the interaction between its subunits or within one or another of its subunits). Indeed the sensitivity to low temperatures of soluble mitochondrial F_1 has been shown to be due to the weakening and rupture of the hydrophobic bonding that maintains the native structure of the enzyme (13). Accordingly it was thought that if the strength of hydrophobic bonding within F_1 controls the kinetics of the enzyme, any agent or condition that modifies the magnitude of hydrophobic interactions in the enzyme should also alter its catalytical properties.

Octylguanidine inhibits the hydrolytic activity of F_1 and protects the enzyme against the inactivating action of low temperatures, the latter effect of octylguanidine being due to an increase in hydrophobic interactions within the protein (14). Figure 1 B shows that at an incubation temperature of 12°C, octylguanidine inhibits ATPase activity, but also induces a change from linear to non-linear kinetics. Thus relatively high temperatures as well as octylguanidine at low temperatures induce non-linearity of enzyme kinetics. Apparently the common action of these two agents is to increase the strength of hydrophobic interactions in the enzyme.

The results of Figure 1 show that the enzyme may exist in two conformations i.e. a form characterized by a low Km for Mg-ATP (the low temperature form), and another that possesses a low and a high Km for Mg-ATP (the 30° C form). Therefore, it might be expected that these two forms could differ not only in their Km values toward Mg-ATP, but also in their response to agents that modulate the ATPase activity of F_1. Thus, the response to methanol of the enzyme incubated at various temperatures was explored, because methanol activates the enzyme through the removal of ADP, or via an alteration in the binding of ADP to F_1 (15), which suggests that the stimulation of the activity by methanol requires the presence of a binding site for ADP.

Table 1 shows that methanol fails to stimulate the ATPase activity of F_1 at temperatures below 20° C; at higher temperatures methanol induces a definite stimulation of ATPase activity. Apparently the appearance in the sensitivity of F_1 to methanol coincides with the change of monophasic to biphasic kinetics, which suggests that methanol affects only the form of the enzyme with the higher Km for Mg-ATP.

DISCUSSION

The results show that there are two forms of soluble F_1 one

Table I

Effect of Methanol on the ATPase Activity of Soluble F_1 at Various Temperatures

Temperature °C	μmoles Pi formed min^{-1} mg^{-1} - methanol	+ methanol	% of Activity in methanol
10	10.3	9.0	87
20	45.7	44.6	98
30	55.4	66.8	121
40	64.2	97.2	151
50	81.3	115.1	142

The experimental conditions were 5.5 mM ATP, 4 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.5), 15μg of F_1 and 15% methanol where shown. Incubation time was one min at the indicated temperature.

characterized by a low Km toward Mg-ATP and insensitivity to the activating action of methanol (the low temperature enzyme) and the other showing both low and high Km for Mg-ATP and the property of being activated by methanol. Although these findings can be explained solely on the basis of conformational changes, the results may also be consistent with the possibility that the enzyme in each of its two states possesses a different number of catalytic sites. The 30°C form would apparent possess two sites, while the low temperature form would possess only one site. The latter suggestion is reinforced by the observed lack of effect of methanol on the latter form of the enzyme. Since the activating action of methanol involves an alteration in the binding of ADP to F_1 (15), the absence of a stimulating action of methanol on the low temperature form would suggest that in this enzyme a binding site for adenine nucleotides is missing.

The data also indicate that F_1 undergoes conformational changes as a consequence of changes in the magnitude of hydrophobic bonding within the

enzyme. This is of interest because the solvent strikingly affects the kinetic and structural properties of F_1 (16). Therefore if during oxidative phosphorylation the enzyme undergoes changes in its location within the membrane (3), effects on the magnitude of hydrophobic bonding within the enzyme may induce reversible conformational changes accompanied by the appearance and disappearance of an adenine nucleotide binding site. Studies on the location of F_1 in the mitochondrial membrane will be presented elsewhere.

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