CONTROL OF KINETIC CHANGES IN ATPase ACTIVITY OF SOLUBLE COUPLING FACTOR 1 FROM CHLOROPLASTS

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1. Introduction

Coupling factor 1 (CF_1) from chloroplasts is directly involved in the terminal steps of photophosphorylation. The enzyme can also catalyze ATPase activity which is probably a result of the reversal of ATP synthesis. However, ATPase activity is latent both in the membrane bound and in the isolated soluble protein. The activity in the membrane bound enzyme can be light-triggered in the presence of sulphydryl reagents [1] to hydrolyze ATP. It is possible that light-induced conformational changes in the protein control enzyme kinetics. Such conformational changes were suggested to be responsible for changes in hydrogen exchange [2], *N*-ethylmaleimide binding [4] and adenylate nucleotide exchange [4]. The regulatory function of this conformational change is examplified by the ability of ADP to quench the activated state of CF_1 [5].

The isolated soluble CF_1 is activated by heat treatment [6] or by a mild tryptic digestion [7] to catalyze ATPase activity. It was suggested that the soluble enzyme is also regulated through changes in conformation. The slow time-dependent changes in K_i of Co^{3+}–phenanthroline–ATP complex [8] is an indicator for conformational changes caused by the binding of the reagent to CF_1. Slow changes in the intrinsic fluorescence of CF_1 and the fluorescence of ethano-ADP upon its binding to the enzyme [9] might reflect the same process. Conformational changes in the coupling factor were also suggested [10,11] to be the route through which the energy released during electron transport is utilized for the synthesis of ATP. In this work rapid kinetic measurements show that ATPase activity in soluble CF_1 undergoes a transient state on addition of divalent metal ion–ATP as substrate. The kinetic change was of the order of 1 s^{-1} and was slowed down to 0.1 s^{-1} when divalent ions were added to the enzyme prior to ATP. The possible role of these changes in regulation of the enzymic activity was discussed. While this work was in progress changes in the transient state kinetics of soluble ATPase from yeast mitochondria were reported [12].

2. Materials and methods

Coupling factor 1 was prepared from lettuce chloroplasts according to the method used for spinach chloroplasts [13] and stored as ammonium sulfate suspension at 4°C. Following heat activation [6] the protein catalyzed ATP hydrolysis at a rate of 35 μmol $\times$ mg^{-1} $\times$ min^{-1}, indicating high purity of the enzyme.

The stored enzyme was freed from ammonium sulfate by passage on a Sephadex G-50 column (1 $\times$ 25 cm) equilibrated and eluted with 80 mM tricine–NaOH (pH 8) and 1 mM EDTA. The enzyme was activated in the eluting solution by 1 mg TPCK trypsin/35 mg protein, followed by addition of 2-fold excess of trypsin inhibitor. The enzyme solution was concentrated to 0.1 ml by forced filtration through an Amicon XM-100A membrane and diluted to final conc. 120 μg protein/ml, in 1 mM tricine–NaOH (pH 8) and 50 μM EDTA.

ATPase activity was measured spectrophotometrically using cresol red for monitoring the acidification of the low-buffered solution. Stoichiometry

of 0.96 mol protons released/mol ATP hydrolyzed at pH 8 [14] was used for quantitation of the catalytic rate. The difference in the $\Delta A_{580-630}$ were monitored by an Aminco DW-2 spectrophotometer equiped with a stopped flow apparatus with a time resolution of 5 ms. The reaction was started by mixing equal vol. 6 mM $CaCl_2$, 6 mM ATP and 50 μM cresol red (pH 8) with 120 μg protein of CF_1 in 1 mM tricine–NaOH (pH 8) and 50 μM EDTA at 37°C. Protein concentration was determined as in [15].

3. Results

A non-linear initial rate of ATPase activity was observed in rapid kinetic measurements (fig.1). The activity of trypsin treated CF_1 started at a slow rate on addition of $[CaATP]^{2-}$. The reaction was accelerated and reached maximal rate within 5 s. This can be seen more clearly in the expanded time scale of the ΔA trace (fig.1B). The same effect was obtained when EDTA concentration in the enzyme solution was increased to 0.5 mM. The time of acceleration varied from 4–6 s at various experiments. Similar acceleration was observed when CF_1 was activated by heat treatment (not shown). During the heat treatment CF_1 was incubated in the presence of 30 mM ATP. However, preincubation with ATP, either in the case of heat-treated or trypsin-activated CF_1, did not significantly alter the length of the acceleration period. It seems, therefore, that the presence of both ATP and Ca^{2+} was required in order to alter the rate of activity of ATPase. Measurements made in the ms range indicate that the same pattern holds from about 5 ms after addition of the substrate, which is within the time resolution in this stopped flow apparatus. Similar pattern of acceleration of the initial rate was also obtained when $[MgATP]^{2-}$ and $[MnATP]^{2-}$ were used as substrates.

Addition of Ca^{2+} to the trypsin-activated CF_1, prior to the addition of $[CaATP]^{2-}$ caused an increase in the period of acceleration (fig.1). The period was extended to about 50 s while the apparent maximal rate of activity was lower than the rate obtained

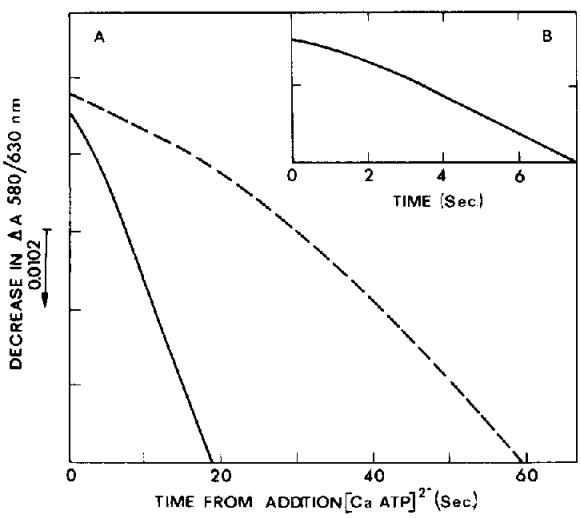

Fig.1. Non-linear initial rate of ATPase activity. The reaction was started by mixing $[CaATP]^{2-}$ with a solution of trypsin-treated CF_1 in a stopped flow apparatus. The enzyme was either preincubated with 25 μM $CaCl_2$ (– –) or no $CaCl_2$ (——) prior to the addition of $[CaATP]^{2-}$. The reaction mixture contained 3 mM ATP, 3 mM $CaCl_2$, 25 μM cresol red, 0.5 mM tricine–NaOH, 10 μM EDTA, 60 μg protein of CF_1, pH 8 at 37°C. The rate of the reaction was measured by following $\Delta A_{580-630}$ of the pH indicator cresol red.

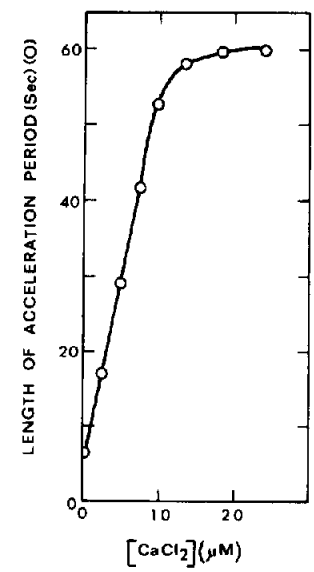

Fig.2. The effect of preincubation of CF_1 in the presence of Ca^{2+} on the length of the acceleration period. Various concentrations of $CaCl_2$ were added to trypsin-treated CF_1 prior to the beginning of the reaction by the addition of $[CaATP]^{2-}$. The length of the acceleration period was measured from traces similar to those presented in fig.1 and under experimental conditions as in section 2.

in the absence of Ca^{2+} during pre-incubation. The effect of Ca^{2+} on the length of the acceleration period was concentration dependent (fig.2). Saturation was reached at 10 μM of free Ca^{2+} calculated from the known stability constant of the Ca^{2+}–EDTA complex and the concentration of the Ca^{2+} and EDTA added to the medium. The effect of Ca^{2+} on the initial kinetics of ATPase activity was reversible. When pre-incubation of the enzyme with Ca^{2+} was followed by removal of these ions either by addition of excess of EDTA or by dilution, the effect of Ca^{2+} was reversed; the length of the acceleration period decreased and the apparent maximal rate of ATPase activity increased. Other divalent cations, such as Mg^{2+} and Mn^{2+} had similar effect on the length of the acceleration period.

The response of the enzyme to low concentrations of Ca^{2+} is either slowed down or prevented by added ATP. This can be concluded from the fact that 0.63 mM free Ca^{2+}, in the CaATP solution, did not have the same effect on the initial kinetics as observed by addition of 10 μM Ca^{2+} in the absence of ATP.

4. Discussion

It is well established that both membrane-bound [1] and soluble CF_1 [6,7] require conditioning of the protein in order to express its capacity to catalyze ATP hydrolysis. It was shown here that although the conditioning of the soluble enzyme is a prerequisite, it still induces a state which can catalyze only a slow rate of hydrolysis of ATP. Only after binding of the substrate, the kinetics of the enzyme is changed. It was shown that both divalent cations and ATP must be present in order to induce acceleration of the rate of catalysis. It is suggested that either the binding or the catalytic process of ATP hydrolysis induces a conformational change which results in the acceleration of the rate of catalysis.

The rate of acceleration was found to be a first order reaction with a kinetic constant of about 1 s^{-1}. This is much slower than the turnover rate of ATPase activity at steady state, calculated to be of the order of 160 s^{-1}. It is, therefore, possible that the rate of catalysis is fast enough to cause the slower change in the kinetic properties. Thus, the proposed change in conformation could represent a mode of regulation of this enzyme. However, this conformational change would be too slow if expected to be involved in the catalysis itself. Still, it can be assumed that similar but faster conformational changes occur in the membrane-bound enzyme during ATP synthesis and provide a means of energy transfer for catalysis of ATP [10,11]. It should be noted that the light-induced nucleotide exchange from CF_1, which was suggested to be a measure of the rate of the energy-induced conformational changes were also found to be much slower than the rate of catalysis [17].

Binding of divalent metal ions to the enzyme in the absence of ATP was found to have a potent effect on the rate of acceleration, decreasing it 10-fold to a rate constant of 0.1 s^{-1}. Saturation was reached at a concentration of 10 μM $CaCl_2$ which is in the range of the dissociation constant for binding of Mn^{2+} to CF_1 as measured by EPR [14]. These values are significantly lower than the K_i for competitive inhibition of free Ca^{2+} which is 7 mM [14]. This could indicate that the conformational changes that take place following the binding of the metal ion–ATP complex and the beginning of ATP hydrolysis are expressed also as a change in the dissociation constant for the binding of free Ca^{2+} or that both binding and release of Ca^{2+} are very slow. The results can be described by a model in which the enzyme can exist in two interchangeable forms, one that catalyzes a low rate of ATP hydrolysis and another which is highly activated. Divalent cations stabilize the low activity form while the binding of divalent metal ion–ATP to the enzyme stabilizes the high activity form. Thus, the ATPase activity is controlled by these two factors.

The nature of the effects of divalent cations and ATP and their effective concentrations are within the limits found in the intact chloroplast. Furthermore, it is well known [19] that upon light–dark transition the stroma's Mg^{2+} concentration varies as a result of ion transport through the thylakoid membrane. Thus it seems possible that these variations in divalent metal ions and metabolites might alter the concentration of the high turnover form of CF_1.

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