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Activation of F_1-ATPase isolated from potato tuber mitochondria

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The ATP-hydrolyzing activity of F_1-ATPase purified from potato tubers mitochondria was stimulated 2- and 3.5-fold by anions, chloride and bicarbonate, respectively, and 5.5- and 6.5-fold by detergents, octyl glucoside and lauryl dimethylamine oxide (LDAO), respectively. The maximal specific activity of the activated F_1, 129 μmol/min per mg protein is the highest activity of plant mitochondrial F_1 hitherto reported and exceeds several-fold values reported earlier. In the absence of activators F_1-catalyzed ATP hydrolysis exhibits non-linear double-reciprocal plots of $[ATP]^{-1}$ vs v^{-1} indicative of negative cooperativity, while in the presence of activators, linear plots are observed. It is suggested that the activators reduce the cooperativity originating from the interaction between different subunits of the enzyme.

F_1-ATPase; Anion activation; Detergent activation; Cooperativity; (Plant mitochondrion)

1. INTRODUCTION

The mitochondrial H^+-ATPases (also called F_0F_1-ATPases) of animals and yeast as well as those of chloroplasts and bacteria have been extensively characterized. However, little is known about the plant mitochondrial enzyme. This is in large part due to the difficulties involved in isolating large quantities of plant mitochondria. Plant mitochondrial F_1-ATPases have been recently purified from such sources as maize [1,2], fava beans [3], sweet potatoes [4], oat roots [5], pea cotyledons [6], and cuckoo-pint [7]. The enzyme has been shown to consist of five polypeptides, designated α, β, γ, δ, ϵ in order of decreasing molecular mass comparable to other F_1-ATPases [1,2,5,7]. In some preparations [3,4,6], an additional polypeptide of molecular mass slightly lower than the δ-subunit has been found.

Strikingly, the ATP-hydrolyzing activity of both membrane bound and isolated plant mitochondrial F_1 has been found to be several-fold lower than the activity of mammalian F_1 [8,9]. A low ATP-hydrolyzing activity has also been found for the isolated chloroplast CF_1 [10], cyanobacterial F_1 [11] and F_1 from thermophilic bacterium PS3 [12]. The ATPase activity of F_1 in these systems can, however, be activated several-fold by different agents. There are so far no reports available indicating high level activation of the isolated plant mitochondrial F_1-ATPase.

In the present paper we describe activation of F_1-ATPase isolated from potato tubers mitochondria. Several-fold activation of the ATP hydrolyzing activity of the enzyme is obtained by a number of chemical compounds resulting in a maximal stimulated activity of the same magnitude as found with the mammalian enzyme. The

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Abbreviations: F_1, F_0, catalytic respectively H^+-translocating coupling factor of H^+-ATPase; Mops, 3-(*N*-morpholine)propanesulfonic acid

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stimulation of the activity is analyzed kinetically and discussed in terms of changes in cooperativity between the subunits of the oligomeric enzyme.

2. MATERIALS AND METHODS

Potato tubers mitochondria were purified according to the procedure involving Percoll gradient centrifugation in the presence of polyvinylpyrrolidone as a terminal purification step [13].

Submitochondrial particles were prepared by sonication of the mitochondria with a Branson Sonifier (model B-30, setting 5) five times for 20 s at 4°C, in a medium containing 0.25 M sucrose, 5 mM Mops (pH 7.5), 20 mM $MgCl_2$, at a protein concentration of about 7 mg/ml. The disrupted mitochondria were centrifuged at 10000 × *g*, 4°C for 10 min. The precipitate was discarded, the supernatant was sedimented at 105 000 × *g*, for 50 min and washed once in a medium containing 0.25 M sucrose, 5 mM Mops (pH 7.5) and twice in a medium containing 0.3 M sucrose, 0.2 mM EDTA, 5 mM $MgCl_2$, 2 mM Mops (pH 7.5), 20% (v/v) ethylene glycol.

The soluble F_1-ATPase of potato tubers mitochondria was isolated by shaking of ethylene glycol washed submitochondrial particles suspended at a protein concentration of 10 mg/ml in 20% ethylene glycol with 220 mM chloroform at 37°C for 10 s at pH 7.5. The extraction was followed by centrifugation on a linear glycerol gradient (20–50%) as described by Fisher et al. [14].

ATPase activity was determined spectrophotometrically by coupling the reaction to the pyruvate kinase and lactate dehydrogenase reactions and following the oxidation of NADH at 340 nm [15].

Protein was measured according to Peterson [16] after precipitation with 7.2% trichloroacetic acid.

Octyl glucoside was purchased from Sigma. Lauryl dimethylamine oxide (LDAO) purified with mixed-bed ion-exchange chromatography was a kind gift of Dr Timo Penttilä.

3. RESULTS

The specific ATP-hydrolyzing activity of F_1-ATPase isolated from potato tubers mitochondria is 20.9 μmol/min per mg protein. This activity

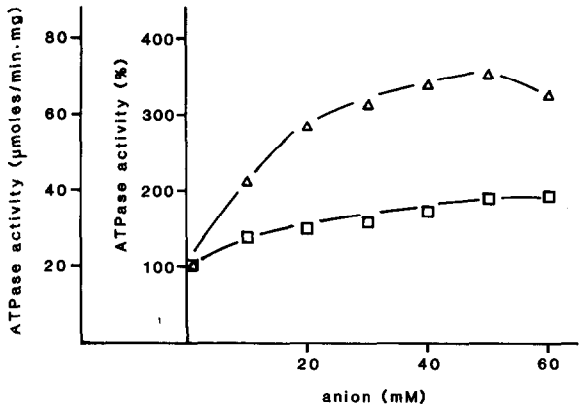


Fig.1. Activation of potato F_1-ATPase by anions, Cl^- (□—□) or HCO_3^- (Δ—Δ). Activation was performed by the addition of the anion directly to the cuvette prior to the measurement of ATPase activity.

is stimulated by $NaHCO_3$ and NaCl as shown in fig.1. The maximal activation is obtained at 50 mM and results in about 2-fold and 3.5-fold activation with chloride and bicarbonate, respectively. The effect of the two types of anions was not additive.

As shown in fig.2A, octyl glucoside, a non-ionic but polar detergent is able to induce stimulation of the ATPase in a linear manner up to 15 mM, where an activation of 5.5-fold is reached, resulting in a specific activity of 115 μmol/min per mg. Since the critical micelle concentration (CMC) of octyl glucoside is 20 mM, the monomeric form of the detergent is responsible for the activation. Higher concentrations of octyl glucoside inhibit the F_1-catalyzed ATPase activity.

Fig.2B shows stimulation of the ATPase activity of F_1 by LDAO. The maximal activation (6.2-fold) is obtained at 0.65 mM, i.e. at concentrations higher than CMC (CMC = 0.125 mM). The specific ATPase activity is enhanced from 20.9 μmol/min per mg protein to 129 μmol/min per mg protein. Cholate and deoxycholate inhibit potato mitochondrial F_1 (not shown). In the presence of bicarbonate or chloride, a lower concentration of octyl glucoside (10 mM) or LDAO (0.5 mM) is needed to achieve the optimal activation. The maximal activity is the same or slightly lower in the presence of both an anion and a detergent (cf. fig.2).

Activation of F_1 by different activators results in

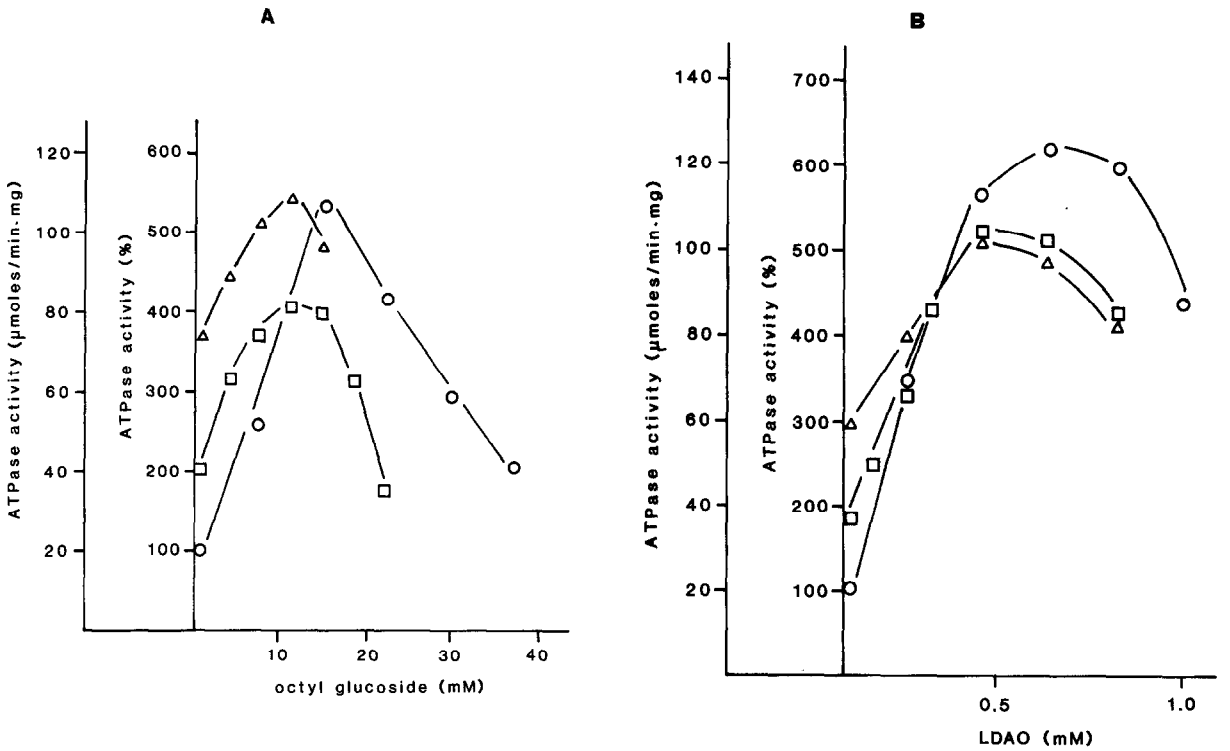


Fig.2. Activation of potato F_1-ATPase by detergents. (A) Octyl glucoside or (B) LDAO in the absence (○—○) and in the presence of Cl^- (□—□) or HCO_3^- (△—△). Conditions as in fig.1.

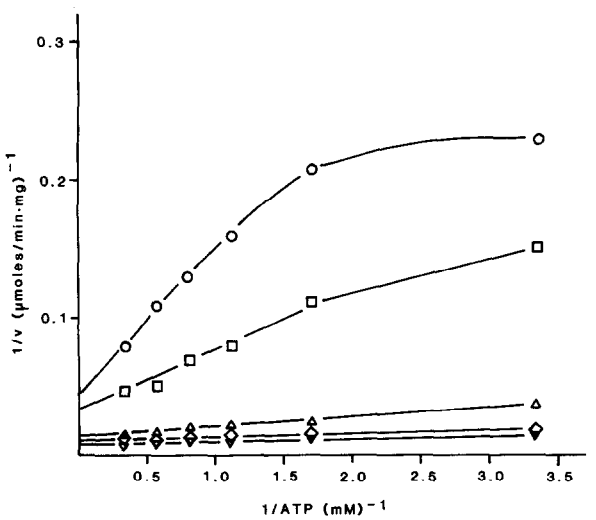


Fig.3. Double-reciprocal plots of the initial velocity of ATP hydrolysis vs ATP concentration of potato F_1-ATPase in the absence (○—○) and in the presence of activators, Cl^- (□—□), HCO_3^- (△—△), octyl glucoside (◇—◇) and LDAO (▽—▽). Conditions as in figs 1 and 2.

a lowered K_m value and increased V_{max} value (fig.3). K_m value of 2.10 mM in the absence of activators is decreased to 1.40, 0.33, 0.30 and 0.26 mM in the presence of NaCl, $NaHCO_3$, octyl glucoside and LDAO, respectively. The double-reciprocal plot of non-activated F_1 shows a marked curvature indicative of negative cooperativity. The Hill coefficient calculated in the concentration range of ATP of 0.18 mM–1.2 mM is 0.4 (not shown). The curvature of the double-reciprocal plots was almost completely abolished in the presence of 50 mM NaCl. In the presence of other F_1-activators the double-reciprocal plots were linear. Hill coefficients calculated for kinetics in the presence of activators were 0.91, 0.95, 0.80 and 0.82 for NaCl, $NaHCO_3$, octyl glucoside and LDAO, respectively. The linear kinetics of double-reciprocal plots in the presence of activators as well as the change of Hill coefficients indicate reduced cooperativity between subunits in the presence of activators.

4. DISCUSSION

Previous reports have shown that the ATP-hydrolyzing activity of both the membrane bound and the isolated F_1-ATPase of plant mitochondria was low, ranging between 0.02 and 0.3 μmol/min per mg protein [8,9] and 3 and 30 μmol/min per mg protein [1,4–6], respectively. These values are several-fold lower than the values reported for the mammalian enzyme or for the activated latent H^+-ATPases.

Treatments commonly known to remove the endogenous ATPase inhibitor protein, as trypsin digestion or exposure to elevated temperature, increased ATPase activity of the membrane bound F_1-ATPase of plant submitochondrial particles [8,9], indicating that the low activity of the membrane bound enzyme is at least partially due to the inhibitory effect of the ATPase inhibitor protein. These treatments, however, did not increase the ATPase activity of the isolated enzyme [17].

Slight activation of the isolated plant mitochondrial F_1-ATPases by anions has been reported [5,17], however there are no reports available indicating activation of the plant mitochondrial F_1 to a high level. In the present work we show that the isolated potato F_1-ATPase can be highly stimulated by detergents, octyl glucoside and LDAO. Octyl glucoside activates also the latent Mg-dependent CF_1-ATPase of chloroplasts [18–20] and F_1-ATPase of thermophilic bacterium PS3 [21]; LDAO has been shown to enhance relatively low ATPase activity of *E. coli* F_1-ATPase [22]. In contrast, the mammalian F_1-ATPase which exhibits high ATP-hydrolyzing activity, is inhibited by these detergents [21,23]. It may be thus concluded that potato F_1-ATPase shares some common intrinsic properties with the chloroplast and bacterial F_1-ATPase. The activity of an oligomeric enzyme such as F_1-ATPase is controlled by both the hydrophobic and ionic interactions between the subunits of the enzyme. It has been proposed that the low ATPase activity may be indicative of a rigid subunit interaction [24]. The possible effect of detergents or anions may be thus to weaken both the hydrophobic or ionic interactions between the subunits of the enzyme and to increase their flexibility. This suggestion is strongly supported by the kinetic analysis data (cf. fig.3) which indicate that the negative cooperativity, characteristic of the non-activated enzyme is greatly reduced in the presence of activators.

The physiological significance of the strong inhibition of the ATPase activity in plant mitochondria as well as its possible activation in situ remains to be elucidated.

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