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PROPERTIES OF THE SOLVENT-STIMULATED ATPase ACTIVITY OF CHLOROPLAST COUPLING FACTOR 1 FROM *CHLAMYDOMONAS REINHARDII*

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The effects of solvents on the ATPase activity of chloroplast coupling factor 1 (CF_1) isolated from wild-type *Chlamydomonas reinhardii* have been studied. Of the solvents examined, the following order summarizes their maximal ability to stimulate the ATPase activity of CF_1: ethanol > methanol > allyl alcohol > *n*-propanol > acetone ≈ dioxane > ethylene glycol. Glycerol inhibits the CF_1 activity at all concentrations. In the absence of organic solvents, 50% of the activity of the enzyme is irreversibly lost after a 10 min incubation at 65–70°C. Ethanol (23%) causes a 30°C drop in the temperature required for 50% inactivation. ATP partially stabilizes the CF_1 in the presence, but not in the absence, of ethanol. In the absence of organic solvents, both free Mg^{2+} and ADP inhibit the CF_1-ATPase. Mg^{2+} is a noncompetitive inhibitor with respect to MgATP, and the kinetic constants are: V, 6.3 μmol ATP hydrolyzed/mg protein per min; K_m(MgATP), 0.23 mM; $K_{ii}(Mg^{2+})$, 27 μM; and $K_{is}(Mg^{2+})$, 50 μM. In the presence of ethanol, double-reciprocal plots are no longer linear and have a Hill coefficient of about 1.8 ± 0.1. V increases about 10–12-fold. The pattern of inhibition by Mg^{2+} appears to change from noncompetitive to competitive with respect to MgATP. In addition, ADP no longer inhibits the MgATPase activity of CF_1.

Introduction

Recently, we described the purification and initial characterization of the chloroplast coupling factor 1 (CF_1) ATPase isolated from the green alga, *Chlamydomonas reinhardii* [1]. Our identification was based on: (i) the polypeptide subunit composition as revealed by SDS-polacrylamide gel electrophoresis, (ii) the immunological cross-reactivity of (a) the *C. reinhardii* ATPase with an anti (spinach) CF_1 antiserum preparation and (b) the spinach CF_1 with an anti (*C. reinhardii* ATPase) antiserum, (iii) our inability to isolate an active ATPase from photophosphorylation mutants lacking CF_1, and (iv) our ability to reconstitute phenazine methosulfate-dependent photophosphorylation with partially resolved *C. reinhardii* thylakoid particles and the purified *C. reinhardii* ATPase [1,2].

During the course of our studies, we found that the *C. reinhardii* CF_1 ATPase differed in several respects from the higher plant CF_1 and even from the cyanobacterial *Mastigocladus luminosus* and the *Spirulina platensis* coupling factors [3–5]. In particular, the *C. reinhardii* CF_1-ATPase is active as isolated (specific activity 2–4 units/mg protein, where 1 unit corresponds to 1 μmol ATP hydrolyzed/min) and cannot be further activated by either extensive proteolysis or heat. Thiol-reducing agents (e.g., β-mercaptoethanol and dithiothreitol) have only a marginal effect on the activity of the

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Abbreviations: CF_1, chloroplast coupling factor 1; Tricine, *N*-tris(hydroxymethyl)methylglycine.

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enzyme. And finally, the *C. reinhardii* CF_1-ATPase shows a much higher specific activity with either MnATP or MgATP than with CaATP.

We also discovered during our initial investigation that certain organic solvents markedly stimulated (6–25-fold) the activity of the *C. reinhardii* CF_1-ATPase [1]. This effect had previously been noted by Schuster [6] for mitochondrial F_1 and Tiefert [7] for spinach CF_1, but in neither report was the stimulatory effect as large as that observed for the *C. reinhardii* enzyme. If the enzyme is not irreversibly denatured by the solvents, the effects of solvents on the activity of the *C. reinhardii* CF_1-ATPase are completely reversible. We have studied the effect of the solvents (particularly ethanol) on the thermostability and the kinetics of the enzyme.

In this report we demonstrate that the *C. reinhardii* CF_1 is destabilized by ethanol, an activating solvent, but can be partially protected against this destabilization by ATP. Ethanol dramatically changes the kinetics of the enzyme, inducing positive cooperativity with respect to MgATP. The Mg^{2+} inhibition pattern of the *C. reinhardii* CF_1-ATPase, clearly noncompetitive with respect to MgATP in the absence of ethanol, becomes apparently competitive in the presence of ethanol. And, ADP, a potent inhibitor of the ATPase in the absence of ethanol, becomes quite ineffective in the presence of ethanol.

Materials and Methods

Purification of the C. reinhardii CF_1-ATPase. The *C. reinhardii* CF_1 was isolated from either wild-type strain 137c or 4j. The enzyme was released from chloroplast thylakoid particles by either chloroform or EDTA extraction followed by chromatography on DEAE-Sephadex A-50 [1]. The chloroform-released and EDTA-released ATPases were indistinguishable in these experiments.

ATPase assay. Reaction mixtures contained in a total volume of 0.1 ml: 20 mM Tricine-NaOH (pH 8.0), 0.05–1.0 mM EDTA, 0.05–5 mM $MgCl_2$, 0.1–5 mM [^{32}P]ATP (containing approx. 10^5 cpm), solvents (where indicated), 50 mM dithiothreitol, and 0.5 μg CF_1. Reaction mixtures were incubated at 37°C, and the ATPase reaction was terminated by the addition of 2.0 ml of a solution containing 0.8 M $HClO_4$ and 1% (w/v) ammonium molybdate. $^{32}P_i$ was determined as previously described [1]. The binding constants for MgATP and MgADP were assumed to be $1.58 \cdot 10^4$ and $1.27 \cdot 10^3$ M^{-1}, respectively. 1 unit of activity is defined as the amount of protein required to hydrolyze 1.0 μmol ATP/min.

Reaction mixtures for the colorimetric determination of nucleoside triphosphatase activity contained in a total volume of 0.1 ml: 20 mM Tricine-NaOH (pH 8.0), 1 mM EDTA, 5 mM $MgCl_2$, 23% (v/v) ethanol, 5 mM nucleoside triphosphate, and 0.5 μg CF_1. Reaction mixtures were incubated at 37°C for 15 min and terminated by the addition of 2.3 ml of a solution containing 1.43% (w/v) ascorbate and 0.36% (w/v) ammonium molybdate in 0.86 N H_2SO_4. P_i was determined colorimetrically [8].

Miscellaneous. Protein was determined by the Coomassie dye binding method using bovine serum albumin as a standard [9]. [^{32}P]ATP was prepared by the photophosphorylation of ADP [10] and purified by chromatography on PEI-cellulose [11].

The kinetic data shown in Fig. 4. were fitted to the equation:

$$v_i = \frac{VS_i}{S_i\left(1+\frac{M}{K_{ii}}\right)+K_m\left(1+\frac{M}{K_{is}}\right)}(1+e_i)$$

where v_i is the velocity at substrate concentration S_i in the presence of the free metal M, and e_i the variance in v_i. The best fits of the data were obtained by minimizing the Σe_i^2 with respect to V and K_m. V and K_m are the maximal velocity and Michaelis constant, respectively, and K_{ii} and K_{is} are the intercept and slope inhibition constants. Since the concentration of $M \neq 0$, V and K_m were determined from the y-intercepts of the slope (V/K_m) and intercept $(1/V)$ vs. M replots (the x-intercepts being $-K_{is}$ and $-K_{ii}$, respectively). The lines in the figure are the model fit and the points are the experimental data.

Reagents. [^{32}P]Orthophosphate was purchased from New England Nuclear. All nucleoside triphosphates were purchased from Sigma Chemical Co. All other materials were of reagent grade quality or better.

Results

Solvent effects on the activity of C. reinhardii CF_1-ATPase

Previously, we had demonstrated that the low MgATPase activity of the soluble *C. reinhardii* CF_1 could be substantially increased (6–20-fold) by the inclusion of ethanol (23%) in the reaction mixture [1]. Fig. 1 demonstrates that this stimulatory effect on the rate of ATP hydrolysis can also be obtained with (in decreasing order of effectiveness) methanol (30%), allyl alcohol (12%), *n*-propanol (10%), and acetone (30%). Other solvents tested, e.g., ethylene glycol and dioxane, also stimulate the ATPase activity of the *C. reinhardii* CF_1, whereas 2-chloroethanol and glycerol inhibit (data not shown).

Pick and Bassilian [12] have recently reported that the detergent β-octylglucoside induces an MgATPase activity with lettuce CF_1. β-Octylglucoside also stimulates the MgATPase activity of the *C. reinhardii* CF_1, and this activity is not further stimulated by ethanol (Pick, U. and Selman, B.R., unpublished results). It seems likely, therefore, that the mechanism of the β-octylglucoside activation of the higher plant CF_1 and the solvent action of the *C. reinhardii* ATPase are similar.

Fig. 2. shows the thermal stability of the *C. reinhardii* CF_1 preincubated for 10 min at various temperatures in either the absence (Fig. 2A) or presence (Fig. 2B) of ethanol (23%). The subsequent assay was performed at 37°C in 23% ethanol.

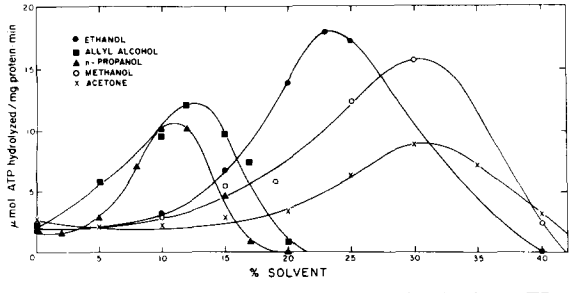

Fig. 1. Solvent stimulation of the *C. reinhardii* CF_1-ATPase. Reaction mixtures contained in 0.1 ml: 20 mM Tricine-NaOH (pH 8.0), 1 mM EDTA, 10 mM [^{32}P]ATP, 5 mM $MgCl_2$, 50 mM dithiothreitol, solvents as indicated (v/v), and 0.5 μg CF_1. Reaction mixtures were incubated for 15 min at 37°C, and [^{32}P]phosphate was determined as described in Materials and Methods.

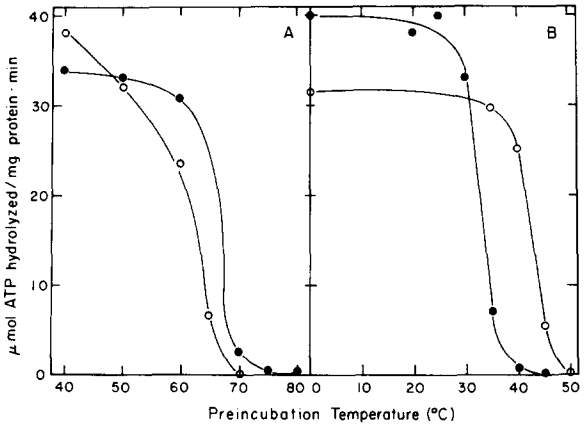

Fig. 2. Thermal stability of the *C. reinhardi* CF_1. Aliquots, 0.08–0.1 ml, of CF_1 were preincubated at the indicated temperatures for 10 min in buffer containing 20 mM Tricine-NaOH (pH 8.0), 1 mM EDTA, 50 mM dithiothreitol, and 0.5 μg CF_1. Some samples (O——O) contained in addition 10 mM [^{32}P]ATP. (A) No further additions. (B) Samples contained in addition 23% ethanol. After the preincubation, the samples were cooled to 0–4°C. The ATPase assay was started by warming the samples to 37°C and adding $MgCl_2$ to 5 mM. To the samples in A, ethanol was added to 23%, and the samples were assayed for 15 min. The samples in B were assayed for 4 min.

When preincubated in the absence of ethanol, 50% of the MgATPase activity of the *C. reinhardii* CF_1 is lost at about 65–70°C. However, when ethanol (23%) is present during the preincubation, the enzyme becomes quite heat labile, and 50% of the MgATPase activity is lost at about 35–40°C. ATP does not significantly protect the enzyme in the

TABLE I

SUBSTRATE SPECIFICITY FOR THE ETHANOL-STIMULATED *C. REINHARDII* CF_1-ATPase

Released P_i was determined as described in Materials and Methods. n.d., not determined.

Nucleoside (N)	μmol P_i released/ mg protein per min	
	NTP	dNTP
Adenosine	19.2	22.5
Guanosine	7.8	4.3
Inosine	3.3	4.5
Cytidine	1.2	0
Uridine	0.4	n.d.
Thymidine	0.7	n.d.

absence of ethanol (Fig. 2A), but it does increase the temperature for 50% denaturation in the presence of ethanol by 10°C to about 45–50°C (Fig. 2B).

Table I shows the nucleoside triphosphate specificity for the *C. reinhardii* CF_1 assayed in 23% ethanol. Clearly, the enzyme is most active with the purine base nucleoside triphosphates, the order of activity being dATP ≈ ATP > GTP > dGTP ≈ dITP > ITP. Little or no activity was observed with the pyrimidine base nucleoside triphosphates. These data are consistent with the nucleotide specificity observed for higher plant CF_1 [13].

Kinetics of the C. reinhardii CF_1-ATPase

Free Mg^{2+} is a very potent inhibitor of the ATPase activity of higher plant CF_1, to the extent that the enzyme is most conveniently assayed as a CaATPase rather than as an MgATPase [14,15]. The Mg^{2+} inhibition of the MgATPase activity of higher plant CF_1 is competitive with respect to MgATP [15]. Previously, we demonstrated that free Mg^{2+} also inhibits the MgATPase activity of the *C. reinhardii* CF_1 [1]. Fig. 3 shows the effect of free Mg^{2+} on the MgATPase activity of *C. reinhardii* CF_1 assayed in the presence or absence of ethanol (23%). Consistent with other CF_1-ATPases, free Mg^{2+} inhibits the MgATPase activity of the *C. reinhardii* enzyme both in the absence and presence of ethanol.

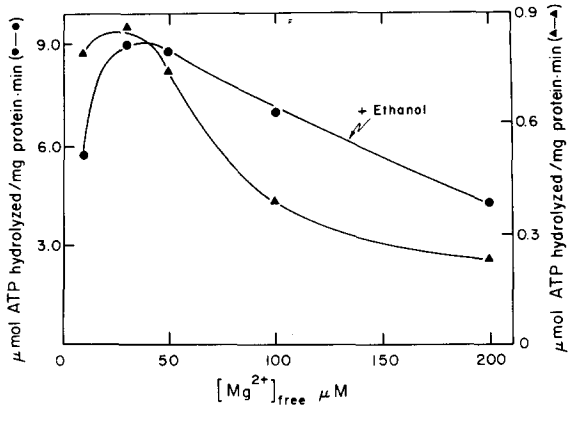

Fig. 3. Mg^{2+} inhibition of the *C. reinhardii* CF_1-MgATPase. Reaction mixtures contained in 0.1 ml, 20 mM Tricine-NaOH (pH 8.0), 50 mM dithiothreitol, variable amounts of [^{32}P]ATP and $MgCl_2$ such that the concentration of Mg[^{32}P]ATP was maintained at 0.27 mM and the concentration of free Mg^{2+} varied from 10 to 200 μM, 0.5 μg CF_1, and ethanol at 23% (v/v) where indicated. Reactions were terminated after 2.0 min and [^{32}P]phosphate was determined as described in Materials and Methods.

Fig. 4A shows a Lineweaver-Burk plot for the MgATPase activity of the *C. reinhardii* CF_1 in the presence of varying levels of constant free Mg^{2+}. In contrast to the higher plant CF_1 [15], Mg^{2+} is clearly a noncompetitive inhibitor of the ATPase with respect to MgATP. The K_{is} (slope effect) and K_{ii} (intercept effect) values for Mg^{2+} are 49.2 and 27 μM, respectively. From slope and intercept replots (extrapolated to zero free Mg^{2+}), the K_m for MgATP and the V can be determined to be 0.23 mM and 6.3 units/mg protein, respectively.

Fig. 4B shows a similar experiment performed in the presence of 23% ethanol. The double-reciprocal plots are no longer linear, but rather suggest positive cooperativity with respect to MgATP. Hill plots (not shown) indicate a Hill coefficient of about 1.8 ± 0.1 for all three lines shown in Fig. 4B. The inhibition pattern for Mg^{2+} becomes difficult to interpret; however, when extrapolated to infinite MgATP, it appears that the lines intersect on the y-axis. If this is the case, then, in the presence of 23% ethanol, Mg^{2+} appears to be a competitive inhibitor with respect to MgATP, consistent with the kinetics for the higher plant CF_1 [15]. The extrapolated V for the data shown in Fig. 4B is about 67 units/mg protein. This rate is about 11-fold higher than the V in the absence of ethanol.

ADP is a potent inhibitor of the ATPase activity of the higher plant and cyanobacterial coupling factor proteins [16,17]. The mode of inhibition is complex. ADP usually induces or exaggerates the sigmoidicity in the substrate-velocity relationship, and this has been interpreted as support for multiple active sites (two) regulated or modulated by nucleotides bound to control sites [3,16]. Fig. 5 demonstrates that ADP also severely inhibits the *C. reinhardii* CF_1-MgATPase activity, but only in the absence of ethanol. The rate in the presence of ethanol (23%) was only marginally (15%) inhibited by 0.4 mM ADP (where the initial concentration of MgATP was also about 0.4 mM), whereas in the absence of ethanol 0.4 mM ADP inhibited that rate 80%. In the absence of ethanol, ADP was a

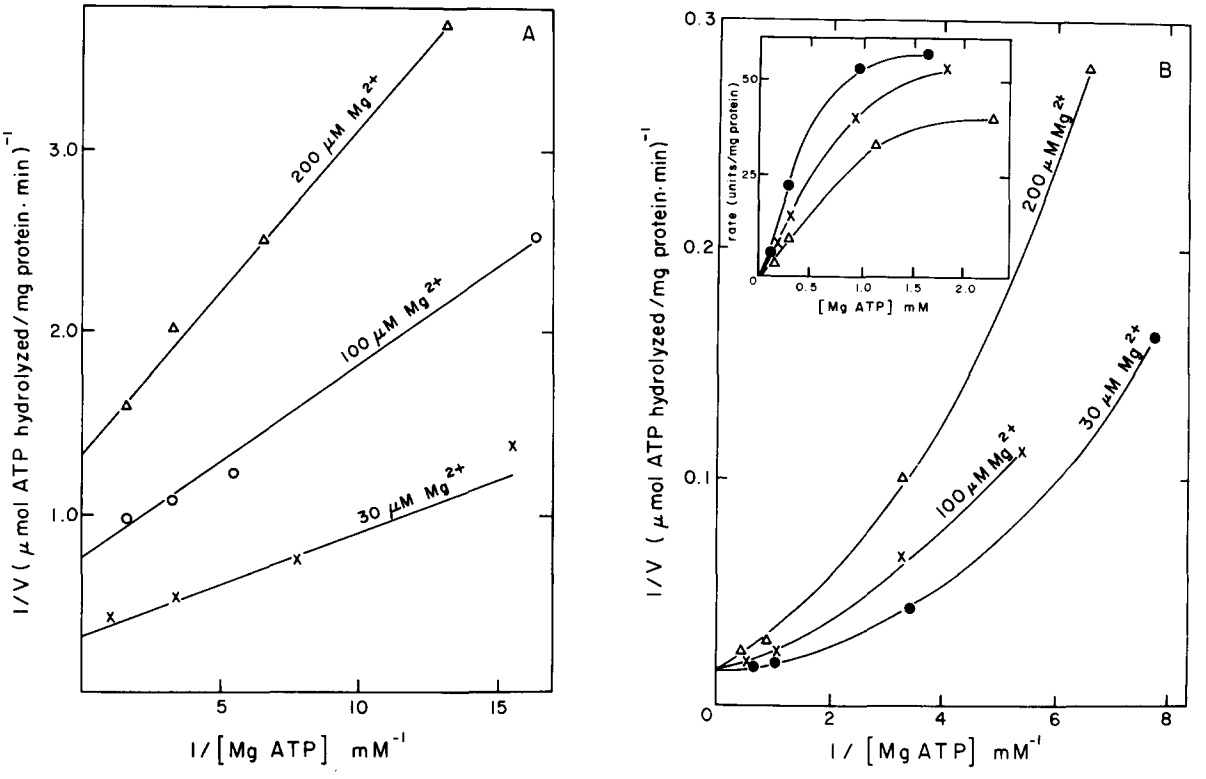

Fig. 4. The kinetics of the Mg^{2+} inhibition of the MgATPase activity of the *C. reinhardii* CF_1. Reaction mixtures contained in 0.1 ml, 20 mM Tricine -NaOH (pH 8.0), 50 mM dithiothreitol, and 0.5 μg CF_1. The concentrations of $MgCl_2$ and [^{32}P]ATP were varied in order to vary the concentrations of Mg[^{32}P]ATP at constant concentrations of free Mg^{2+} (30, 100, and 200 μM, as indicated). (A) No further additions. (B) Samples contained 23% ethanol in addition. (B, inset) The rate vs. the MgATP concentrations for the data in B. ATPase reactions were terminated after a 2.0 min incubation at 37°C and released $^{32}P_i$ was determined as described in Materials and Methods.

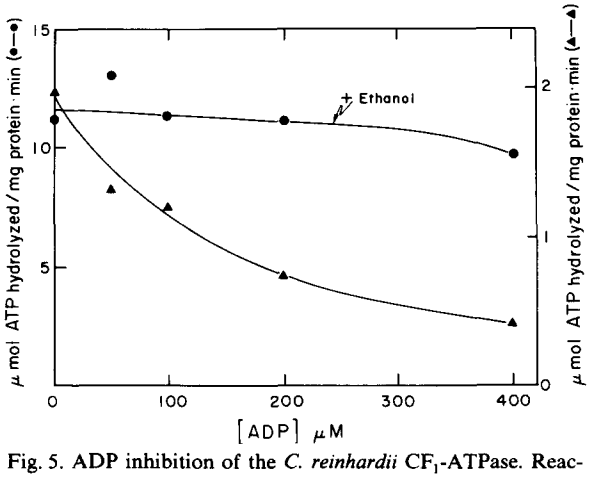

Fig. 5. ADP inhibition of the *C. reinhardii* CF_1-ATPase. Reaction mixtures contained in 0.1 ml: 20 mM Tricine-NaOH (pH 8.0), 50 mM dithiothreitol, 0.1 mM EDTA, 1 mM [^{32}P]ATP, 0.5 mM $MgCl_2$, 0.5 μg CF_1, and where indicated 23% ethanol. ATPase reactions were terminated after a 15 min incubation at 37°C, and the $^{32}P_i$ was determined as described in Materials and Methods.

competitive inhibitor of the ATPase with respect to MgATP. The apparent K_{is} was about 10 μM (data not shown).

Because of the relatively long assay time (15 min) used to collect the data for Fig. 5, it might have been possible that in the presence of ethanol ADP inhibited the initial rate of the ATPase but not the extent of ATP hydrolyzed (note that in Fig. 5 in the presence of ethanol, 85% of the total ATP was hydrolyzed after 15 min). Therefore, the effect of ADP (0–0.3 mM) over a range of MgATP concentrations (0.064–0.7 mM) was examined over a time period (0–2 min) where the assay in the presence of ethanol was approximately linear. At a concentration of MgATP of 0.064 mM, a concentration of MgATP at least 4-fold below the K_m for MgATP (see Fig. 4B), a 4.7-fold excess of ADP (300 μM) inhibited the initial rate of the ATPase 3% (see Fig. 6). Thus, in the presence of ethanol,

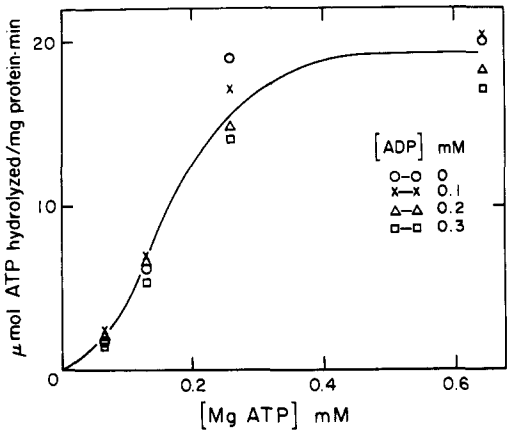

Fig. 6. Lack of effect of ADP on the *C. reinhardii* CF_1-ATPase in the presence of ethanol. Reaction mixtures contained in 0.1 ml: 20 mM Tricine-NaOH (pH 8.0), 50 mM dithiothreitol, 23% (v/v) ethanol, 0.5 μg CF_1 and variable amounts of $MgCl_2$ and [^{32}P]ATP to vary the concentration of Mg[^{32}P]ATP as indicated maintaining the free Mg^{2+} concentration at 30 μM. In addition, reaction mixtures contained ADP at the following concentrations: (O——O) 0 mM, (×——×) 0.1 mM, (Δ——Δ) 0.2 mM, (□——□) 0.3 mM. ATPase reactions were terminated after a 2.0 min incubation at 37°C, and ^{32}P was determined as described in Materials and Methods.

ADP is a very poor inhibitor of the *C. reinhardii* CF_1-ATPase.

Discussion

The stimulatory effect of various solvents on the activity of the *C. reinhardii* CF_1-ATPase can be quite pronounced, amounting to as much as a 20–30-fold increase in specific activity. Tan and Lovrien [18] have observed binary water-solvent stimulatory effects on the specific activity of a large variety of enzymes, while Schuster [6] and Tiefert [7] have observed solvent stimulation of the ATPase activity of mitochondrial F_1 and spinach chloroplast CF_1, respectively. To our knowledge, the solvent effects on the *C. reinhardii* CF_1-ATPase are the largest reported.

It seems likely that the primary effect of the solvents is to alter the conformation of the enzyme by lowering the dielectric constant of the medium. We have attempted to correlate the peak of activity in the various binary mixtures to the dielectric constant of the medium and have found no convincing relationship. It could very well be that there is a correlation between the medium dieletric constant and enzyme activity but that at each solvent concentration we are measuring a compromise between enzyme activity and enzyme denaturation. This could cause the peak of the activity of the enzyme to shift to an apparently higher dielectric constant than that required for optimal activity. Of the solvents that we have tried to correlate, acetone peaks at the lowest dielectric constant (63.5), followed by methanol (66.6), ethanol (67.4), *n*-propanol (71.5), and ethylene glycol (72.5) (using the values reported by Akerlof [19]).

Tan and Lovrien [18] also indicated that binary solvent mixtures enhanced the tendency for their enzymes to heat denature. This is clearly the case for the *C. reinhardii* CF_1 (Fig. 2). In the absence of ethanol, for example, 50% of the activity of the ATPase is lost after a 10 min preincubation at approx. 65–70°C. In the presence of 23% ethanol (the concentration of ethanol required to yield maximal activity at the assay temperature (37°C)), 50% of the activity is lost at about 35–40°C, a downward shift of about 30°C. ATP does not change the denaturation profile of the enzyme in any substantial way in the absence of ethanol, but it does tend to stabilize the protein in the presence of ethanol, causing a ten degree shift toward higher temperatures for 50% denaturation. Such substrate protection is certainly not unusual, and is even required for the heat and trypsin activation of the spinach CF_1 [13,20].

The effects of ethanol on the kinetics of the *C. reinhardii* ATPase are quite interesting and extremely complex. Firstly, in the absence of ethanol Lineweaver-Burk plots of activity vs. substrate are linear, indicating a V of approx. 6.3 units/mg CF_1 and a K_m for MgATP of approx. 0.23 mM (Fig. 4A). In the presence of 23% ethanol, the plot is no longer linear. It shows positive cooperativity with respect to MgATP and has a Hill coefficient of about 1.8. The extrapolated value for V is approx. 67 units/mg CF_1 (Fig. 4B).

Secondly, free Mg^{2+} is a potent inhibitor of the *C. reinhardii* CF_1-ATPase. In contrast, however, to the effect of free Mg^{2+} on the ATPase activity of higher plant CF_1 [15], free Mg^{2+} is very clearly a noncompetitive inhibitor with respect to MgATP for the *C. reinhardii* CF_1-ATPase (having K_{ii} and

K_{is} values of 27 and 50 μM, respectively) (Fig. 4A). Ethanol apparently causes the K_{ii} value to increase greatly and the resulting inhibition pattern appears to be competitive with respect to MgATPase (Fig. 4B).

Thirdly, ADP inhibits the ATPase activity of the *C. reinhardii* CF_1 ATPase. This is consistent with the ADP inhibition of higher plant and cyanobacterial CF_1-ATPases that have been studied [3,16,17]. However, in the presence of 23% ethanol, ADP no longer inhibits the ATPase (Fig. 5). Even at concentrations of MgATP 4–5-fold below below the K_m for MgATP and at concentrations of ADP 6-fold higher than the concentration of MgATP, ADP is a poor inhibitor (Fig. 6). Present studies on the kinetics of the heat-activated CaATPase activity of the higher plant spinach CF_1 have indicated that the release of product ADP from the enzyme is rate limiting (Frasch, W., Leirmo, S. and Selman, B.R., unpublished results). In addition, we have found that the ADP inhibition of the higher plant ATPase has an apparent pK around 9, below pH 9 ADP is a very effective apparently competitive inhibitor with respect to CaATP whereas above pH 9 ADP becomes a rather poor inhibitor. Because ethanol (23%) decreases the pH optimum for the maximal velocity of the ATPase about 0.5 pH units [1] and increases the maximal velocity about 10–12-fold, it is tempting to speculate that the net effect of ethanol (and other stimulating solvents) is to increase the rate of dissociation of product ADP from the enzyme. This might also explain why ADP is less effective as an inhibitor of the ATPase in the presence of ethanol. A similar suggestion has been offered for the much smaller stimulatory effects observed for methanol on the mitochondrial F_1-ATPase activity [21].

It could be argued that we are assaying two completely different ATPases in our preparation, one active in the absence of ethanol and one which dominates the reaction in the presence of ethanol. There are at least two lines of evidence which would suggest that this is not the case. Firstly, both activities are sensitive to our anti (spinach CF_1) antisera. And, secondly, photophosphorylation mutants of *C. reinhardii* lacking the CF_1-ATPase also lack the ethanol-stimulated ATPase [1,2].

At present, we cannot conclude how the solvents are affecting the *C. reinhardii* CF_1-ATPase. Experiments are currently in progress to determine which steps in the catalytic sequence of the enzyme are being altered by the solvents and how these solvent effects may relate to the in situ function of the ATPase.

Acknowledgements

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References

1 Selman-Reimer, S., Merchant, S. and Selman, B.R. (1981) Biochemistry 20, 5476–5482
2 Selman-Reimer, S., Merchant, S. and Selman, B.R. (1981) in Energy Coupling in Photosynthesis (Selman, B.R. and Selman-Reimer, S., Eds.), pp. 341–352, Elsevier/North-Holland, Amsterdam
3 Nelson, N. (1976) Biochim. Biophys. Acta 456, 314–338
4 Binder, A. and Bachofen. R. (1979) FEBS Lett. 104, 66–70
5 Owers-Narhi, L., Robinson, S.J., DeRoo, C.S. and Yocum, C.F. (1979) Biochem. Biophys. Res. Commun. 90, 1025–1031
6 Schuster, S.M. (1979) Biochemistry 18, 1162–1166
7 Tiefert, M.A. (1981) in Proceedings of the 5th International Congress on Photosynthesis Research (Akoyunoglou, G., ed.), Vol. 2, pp.893–902, Balaban International Science Services, Philadelphia
8 Chen, P.S., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756–1758
9 Bradford, N.M. (1976) Anal. Biochem. 72, 248–254
10 Selman, B.R. and Selman-Reimer, S. (1981) J. Biol. Chem. 256, 1722–1726
11 Magnusson, R.P., Portis, A.R., Jr. and McCarty, R.E. (1976) Anal. Biochem. 72, 653–657
12 Pick, U. and Bassilian, S. (1981) in Energy Coupling in Photosynthesis (Selman, B.R. and Selman-Reimer, S., Eds.), pp. 251–260, Elsevier/North-Holland, Amsterdam
13 Vambutas, V.K. and Racker, E. (1965) J. Biol. Chem. 240, 2660–2667
14 Hochman, Y., Lanir, A. and Carmeli, C. (1976) FEBS Lett. 61, 255–259

15 Hochman, Y. and Carmeli, C. (1981) Biochemistry 20, 6287–6292
16 Nelson, N., Nelson, H. and Racker, E. (1972) J. Biol. Chem. 247, 7657–7662
17 Wolf, M., Binder, A. and Bachofen, R. (1981) Eur. J. Biochem. 118, 423–427
18 Tan, K.H. and Lovrien, R. (1972) J. Biol. Chem. 247, 3278–3285
19 Akerlof, G. (1932) J. Am. Chem. Soc. 54, 4125–4139
20 Farron, F. and Racker, E. (1970) Biochemistry 9, 3829–3836
21 Flores, G.O., Acosta, A. and Payou, A.G. (1982) Biochim. Biophys. Acta 679, 466–473