Properties of the Cyanobacterial Coupling Factor ATPase from *Spirulina platensis*

II. Activity of the Purified and Membrane-Bound Enzymes

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Cyanobacterial (*Spirulina platensis*) photosynthetic membranes and isolated F_1 ATPase were characterized with respect to ATP activity. The following results indicate that the regulation of expression of ATPase activity in *Spirulina platensis* is similar to that found in chloroplasts: (a) the ATPase activity of *Spirulina* membranes and isolated F_1 ATPase is mostly latent, a characteristic of chloroplast ATPase activity; (b) treatments that elicit ATPase activity in higher plant chloroplast thylakoids and isolated chloroplast coupling factor (CF_1) greatly stimulate the activity of *Spirulina* membranes and F_1, and (c) the cation specificity of chloroplast ATPase activity, e.g., light-induced membrane activity that is magnesium dependent and trypsin-activated CF_1 activity that is calcium dependent, is also observed in *Spirulina*. Thus, an 8- to 15-fold increase in specific activity (to 13-15 μmol P_i min^{-1} mg chl^{-1}) is obtained when *Spirulina* membranes are treated with trypsin (CaATPase) or with methanol (MgATPase); a light-induced, dithiothreitol-dependent MgATPase activity is also found in the membranes. Purified *Spirulina* F_1 is a CaATPase when activated with trypsin (endogenous activity increases from 4 to 27-37 μmol P_i min^{-1} mg protein^{-1}) or with dithiothreitol (5.6 μmol P_i min^{-1} mg^{-1}), but a MgATPase when assayed with methanol (18-20 μmol P_i min^{-1} mg^{-1}). The effects of varying calcium and ATP concentrations on the kinetics of trypsin-induced CaATPase activity of *Spirulina* F_1 were examined. When the calcium concentration is varied at constant ATP concentration, the velocity plot shows a marked sigmoidicity. By varying Ca–ATP metal–nucleotide complex concentration at constant concentrations of free calcium or ATP, it is shown that the sigmoidicity is due to the effect of free ATP, which changes the Hill constant to 1.6 from 1.0 observed when the free calcium concentration is kept constant at 5 mM. Therefore not only is ATP an inhibitor but it is also an allosteric effector of *Spirulina* F_1 ATPase activity. At 5 mM free calcium, the K_m for the Ca–ATP metal–nucleotide complex is 0.42 mM. © 1986 Academic Press, Inc.

The latent ATPase activity in photosynthetic membranes of oxygen-evolving organisms is strikingly different from the ATPase activity observed in energy-transducing membranes of nonoxygenic photosynthetic and heterotrophic bacteria and mitochondria, which is expressed without treatment. Over two decades ago, Petrack and Lipmann (1) found that ATP hydrolysis in membranes of the cyanobacterium *Anabaena variabilis* and spinach chloroplasts could be induced by illumination in the presence of an electron transport mediator and a sulfhydryl reagent, and suggested that this activity represented a reversal of ATP synthesis. Since that landmark work, the enzyme that catalyzes ATP hydrolysis in chloroplasts, chloroplast

coupling factor or CF_1,[2] has been studied in order to understand how its catalytic activity is regulated [reviewed in (2)]. When isolated from the membrane, the latency of CF_1 is overcome by dithiothreitol in a reaction that probably mimics the light modulation of membrane-bound CF_1 activity (3). However, the cation specificity is altered when CF_1 is removed from the membrane. Light modulation activates MgATPase activity in thylakoids while DTT stimulates CaATPase activity in isolated CF_1 (4). Other treatments that enhance CF_1 activity also show a cation specificity: trypsin (CaATPase) (5), solvents (MgATPase) (6, 7), or detergents (Mg-ATPase or CaATPase, depending on method of activation) (8, 9).

In this report, we show that latent cyanobacterial ATPase activity in *Spirulina* membrane vesicles and F_1 is elicited by treatments that stimulate chloroplast activity. We also show that ATP acts both as an inhibitor and as an allosteric effector of CaATPase activity in *Spirulina* F_1. The accompanying paper (13) discusses the purification of the five-subunit reconstitutively active F_1 ATPase from photosynthetic membranes of *Spirulina platensis*.

MATERIALS AND METHODS

Trypsin-activated ATPase activity was assayed routinely in a reaction mixture containing 50 mM Tricine, pH 8.0, 10 mM $CaCl_2$, and 5 mM ATP. Substrate concentrations were varied where indicated. Assays were initiated by the addition of 2–5 µg *Spirulina* F_1 or membrane vesicles equivalent to 5–10 µg chl a. Liberated phosphate was determined spectrophotometrically as the reduced phosphomolybdate complex, according to the method developed by LeBel *et al.* (10), as described by Jagendorf (11). The reaction was terminated after 5 min at 37°C by the addition of 1 ml of color reagent containing 2% (w/v) trichloroacetic acid, 2.4 M acetic acid, 0.4 M sodium acetate, 12 mM $CuSO_4 \cdot 5H_2O$, 1% (w/v) ammonium molybdate, 1% (w/v) Na_2SO_3, 0.4% (w/v) elon, and 1% SDS. Color development was stopped after 5 min by the addition of

0.1 ml of 34% sodium citrate (12). Reaction vessels, containing the reaction mixture plus or minus phosphate standards, were incubated alongside the sample assays; in the case of the membrane ATPase assays, an equivalent volume of membranes was added to these tubes after addition of the color reagent. Concentrations of the CaATP metal nucleotide complex were calculated using a dissociation constant of 50 µM at pH 8.0 for the complex (W. Frasch, personal communication). The calculations were not corrected for the weak binding of calcium by Tricine.

Trypsin activation was accomplished either by adding trypsin (5 mg/ml in 1 mM H_2SO_4) to the reaction mixture (trypsin:ATPase, 10–20:1 w/w) or by incubating the ATPase or membranes at room temperature with the amounts of trypsin indicated in the appropriate figure, in a solution containing 50 mM Tricine (pH 8.0) and 0.2 mg/ml ATPase or 0.1 mg chl a/ml membrane vesicles. The digestion was terminated at the specified intervals by a threefold excess of soybean trypsin inhibitor. Controls were treated identically except that trypsin inhibitor was added prior to the trypsin. Methanol-stimulated MgATPase assays contained 25 mM Tricine, pH 8.6, 2.5 mM $MgCl_2$, 5 mM ATP, and the indicated amount of methanol (v/v). Reactions were initiated by 2–5 µg of *Spirulina* ATPase or membrane vesicles equivalent to 5–10 µg chl a. The reaction was terminated after 2 min at 37°C as above, and color development was allowed to proceed for 10 min.

The procedure for light activation of membrane MgATPase was that of McCarty and Racker (4). Membranes (70 µg chl a/ml) were illuminated (approximately 3×10^6 ergs cm^{-2} s^{-1}) for 5 min in a solution containing 50 mM Tricine, pH 8.0, 50 mM NaCl, 5 mM $MgCl_2$, 0.1 mM PMS, and 5 mM DTT. Aliquots containing 1.4 µg chl a were transferred to reaction mixtures containing 50 mM Tris-Cl, pH 8.0, 5 mM $MgCl_2$, and 5 mM ATP and assayed at 37°C for 30 min in the dark. Dithiothreitol activation of isolated *Spirulina* F_1 followed that of Nalin and McCarty (3). *Spirulina* F_1 was incubated at 1 mg/ml in 50 mM Tris-Cl, pH 8.0, 1 mM ATP, 1 mM EDTA, and 50 mM dithiothreitol at room temperature and aliquots containing 5 µg were assayed at 15-min intervals over a 2-h period. *Spirulina* F_1 is maximally stimulated by 15 min incubation with DTT; CF_1 activity continues to increase up to 60 min (3).

Procedures for isolation of photosynthetic membrane vesicles and the coupling factor ATPase of the membranes of *S. platensis* are detailed in (13).

Protein was estimated by the method of Lowry *et al.* using bovine serum albumin as the standard (14). Chlorophyll was determined by the procedure of Arnon (15).

RESULTS

Membrane ATPase. Untreated photosynthetic membrane vesicles from *S. pla-*

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[2] Abbreviations used: F_1, the hydrophilic portion of the proton-translocating ATPase complex; CF_1, chloroplast F_1; PMS, phenazine methosulfate; chl, chlorophyll; DTT, dithiothreitol; Tricine, *N*-tris(hydroxymethyl)methylglycine; SDS, sodium dodecyl sulfate.

tensis catalyze low levels of ATP hydrolysis, 0.6–2.3 μmol P_i min^{-1} mg chl^{-1} for CaATPase activity, and 1–1.2 μmol P_i min^{-1} mg chl^{-1} for Mg-dependent ATPase activity. CaATPase activity is increased approximately fivefold by trypsin digestion prior to ATPase assay (Fig. 1A), a procedure Vambutas and Racker discovered to be effective in activating chloroplast thylakoid ATPase activity (5). Optimal rates are obtained with short periods of exposure to relatively high concentrations of trypsin. More effective activation occurs when trypsin is added directly to the reaction mixture (Fig. 1B). An 8- to 10-fold increase in CaATPase activity relative to untreated membranes is observed under these conditions. The CaATPase activity of the membranes ranged from 13 to 16, and averaged 14.5 μmol P_i min^{-1} mg chl^{-1}. ATPase activity is stimulated 30 to 50% by trypsin treatment simultaneous with ATPase assay relative to trypsin treatment prior to assay.

The procedure of McCarty and Racker (4) for light activation of chloroplast thylakoids was used to assay light-modulated ATPase activity of *Spirulina* membrane vesicles. As shown in Table I, a light-activated, dithiothreitol-dependent Mg-ATPase activity could be demonstrated in *Spirulina* membrane vesicles. Light activation with DTT stimulates activity about

sixfold relative to dark controls without DTT. However, the stimulation is only twofold relative to dark controls with DTT, suggesting that DTT by itself can partially activate the membrane-bound ATPase.

The basal levels of MgATPase in *Spirulina* membranes under our usual MgATPase assay conditions (2 min at 37°C) are 1–1.2 μmol P_i min^{-1} mg chl^{-1}. Varying the concentrations of Mg and ATP or altering the ratio of Mg to ATP did not increase the activity. Methanol has been shown to be a rapid, reproducible, and highly effective stimulator of MgATPase activity in spinach chloroplast thylakoid membranes (7). Methanol proved to be a potent stimulator of Mg-dependent ATPase activity in *Spirulina* membranes. In contrast to spinach chloroplasts, which are maximally stimulated at 35% methanol, optimal stimulation of *Spirulina* membranes occurs when 25% methanol is included in the reaction mixture (Fig. 2). The specific activity of the membrane MgATPase increases 12- to 15-fold to about 15 μmol P_i min^{-1} mg chl^{-1} with methanol stimulation.

Catalytic activity of purified Spirulina ATPase. As detailed in the preceding paper (13), the coupling factor ATPase has been isolated and purified from *Spirulina* membrane vesicles. While the MgATPase activity of untreated *Spirulina* F_1 is barely de-
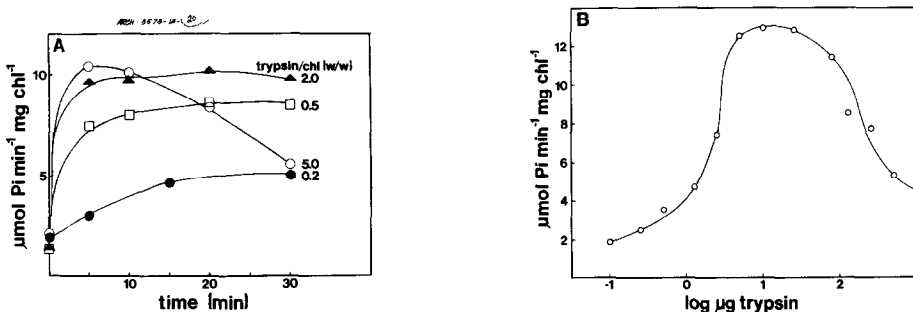

FIG 1. Trypsin activation of *Spirulina* membrane CaATPase activity. (A) Time course of activation by trypsin digestion prior to ATPase assay. *Spirulina* membrane vesicles (0.1 mg chl/ml) were incubated at room temperature in the presence of 50 mM Tricine, pH 8.0, and the ratio of trypsin to chl is indicated. Digestion was terminated at the indicated times by the addition of a threefold excess of soybean trypsin inhibitor. Aliquots of the activation mixture containing 10 μg chl were assayed for 5 min at 37°C in a reaction mixture containing 50 mM Tricine, pH 8.0, 10 mM CaCl$_2$, and 5 mM ATP. (B) Activation by inclusion of trypsin in the ATPase assay. *Spirulina* membrane vesicles equivalent to 8 μg chl were assayed as described above except that the indicated amount of trypsin was included in the reaction mixture. The rate without trypsin was 1.6 μmol P_i min^{-1} chl^{-1}.

TABLE I

LIGHT ACTIVATION OF *Spirulina* MEMBRANE ATPase ACTIVITY

Conditions	MgATPase activity (μmol P_i min^{-1} mg chl^{-1})
+DTT, light	1.7–2.0
+DTT, dark	0.8–1.0
−DTT, light	0.4–0.6
−DTT, dark	0.2–0.4

Note. Spirulina membranes were kept in the dark or illuminated in a solution containing 50 mM Tricine, pH 8.0, 50 mM NaCl, 5 mM MgCl$_2$, 0.1 mM PMS, with or without DTT (5 mM). After 5 min, aliquots were assayed for MgATPase activity in the dark for 30 min as described under Materials and Methods. The ranges of two independent experiments with two different membrane preparations are given. Each value is the average of duplicate determinations.

tectable, *Spirulina* F$_1$ catalyzes measurable levels of calcium-dependent hydrolysis, averaging about 4 μmol P_i min^{-1} mg protein^{-1}. Under conditions that increase spinach CF$_1$ activity 8- to 10-fold, dithiothreitol treatment of F$_1$ increases the specific activity (CaATPase) about 40% to 5.6 μmol P_i min^{-1} mg^{-1} (data not shown). That DTT treatment is much more effective with spinach CF$_1$ may be at least partially due to the lower specific activity of untreated CF$_1$, around 1 μmol P_i min^{-1} mg^{-1}. When trypsin treated, as described below, *Spirulina* F$_1$ CaATPase activity increases to 27–37 μmol P_i min^{-1} mg^{-1} which is equal

to or greater than reported values for spinach CF$_1$, but again the extent of activation is lower with *Spirulina* F$_1$. Thus, *Spirulina* F$_1$ behaves catalytically as if it is partially activated, at least in comparison to spinach CF$_1$. Although this could be due to a partial loss of ϵ subunit (an ATPase inhibitor (16)) content during purification which is not detectable on Coomassie-stained polyacrylamide gels, it is more probably an inherent property of the enzyme, since the membrane-bound and soluble enzyme are activated to about the same extent (8- to 10-fold) by trypsin.

Digestion of *Spirulina* F$_1$ by trypsin prior to ATPase assay elicits a five- to sixfold increase in CaATPase activity (20–25 μmol P_i min^{-1} mg^{-1}); we have shown that optimal trypsin activation requires higher concentrations of trypsin and longer digestion times than are observed with spinach CF$_1$ (17). Higher specific activities, averaging 33.4 μmol P_i min^{-1} mg protein^{-1}, are observed when trypsin activation and ATPase assays are carried out simultaneously (data not shown). As was found for membrane CaATPase activity, soluble *Spirulina* F$_1$ is stimulated 30 to 50% by trypsin treatment simultaneous with assay relative to trypsin treatment prior to assay.

The nucleotide and metal specificities of the purified *Spirulina* coupling factor were

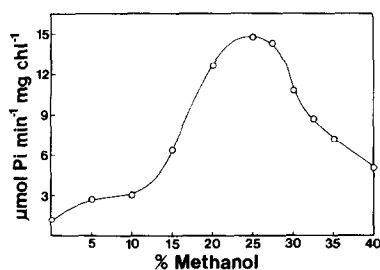

FIG. 2. Methanol stimulation of *Spirulina* membrane MgATPase activity. *Spirulina* membrane vesicles equivalent to 8 μg chl were assayed for 2 min at 37°C in a reaction mixture containing 25 mM Tricine, pH 8.6, 2.5 mM MgCl$_2$, 5 mM ATP, and the indicated amount of methanol (v/v).

TABLE II

NUCLEOTIDE SPECIFICITY OF TRYPSIN-ACTIVATED *Spirulina* F$_1$

Substrate	Specific activity[1] (μmol P_i min^{-1} mg protein^{-1})	% of control
ATP	23.6	100%
GTP	7.9	33.5
ITP	6.9	29.2
CTP	1.3	5.5
TTP	0	0
UTP	0.4	1.7
ADP	0.6	2.5
PP_i	0.8	3.4

Note. Spirulina F$_1$ was activated for 15 min at room temperature, as described under Materials and Methods, and assayed for 5 min at 37°C in a reaction mixture containing 50 mM Tricine, pH 8.0, 10 mM CaCl$_2$, and 5 mM nucleotide.

determined for the trypsin-activated enzyme. As expected, the preferred nucleotide is ATP, with other purine nucleoside triphosphates producing lower, but significant, levels of hydrolytic activity (Table II). The pyrimidine nucleoside triphosphates, as well as ADP and pyrophosphate, are essentially inactive as substrates. These results are similar to those reported for isolated spinach CF_1 (5) and for chloroplast thylakoid ATP synthetic and hydrolytic activities (18). The trypsin-activated enzyme shows a marked preference for calcium over Mg, Mn, or Zn (Table III) and in this regard is much more metal specific than is spinach CF_1 (19).

Although, as noted above, the endogenous MgATPase activity of *Spirulina* F_1 is quite low (0.2–0.3 μmol min^{-1} mg^{-1}), very significant MgATPase activity is revealed in the presence of certain organic solvents. Three solvents which were particularly effective in stimulating spinach CF_1 MgATPase activity (6) were tested for their effects on *Spirulina* F_1. As shown in Fig. 3, methanol (25%) is the most effective solvent, increasing the specific activity of the *Spirulina* MgATPase over 50-fold to 18–20 μmol P_i min^{-1} mg protein^{-1}. Ethanol (20%) raises the specific activity to 5.0 μmol P_i min^{-1} mg protein^{-1}, while acetone completely inhibits activity at all concentrations tested.

FIG. 3. Effect of solvents on the MgATPase activity of purified *Spirulina* F_1. *Spirulina* F_1 (5 μg) was assayed for 2 min at 37°C in a reaction mixture containing 25 mM Tricine, pH 8.6, 2.5 mM $MgCl_2$, 5 mM ATP, and the indicated amount of solvent (v/v).

Kinetics of the trypsin-activated Spirulina CaATPase. When we examined the Ca^{2+} dependence of ATPase activity in trypsin-activated *Spirulina* F_1 at a constant ATP concentration (5 mM) we found that the velocity curve is markedly sigmoidal (Fig. 4A). Optimal rates are observed at 10–20 mM calcium; higher concentrations are only slightly inhibitory. The reciprocal experiment, where the ATP concentration is varied, at a constant calcium concentration of 10 mM, produces a hyperbolic velocity curve except at high concentrations of ATP, which are very inhibitory (Fig. 4B). It has been shown for CF_1 that the true substrate for ATP hydrolysis is a divalent cation–nucleotide complex (19). In view of this finding, the sigmoidicity observed when calcium concentration is varied may be explained by the effect of free ATP present in excess of CaATP metal–nucleotide complex. This hypothesis was tested by varying the concentration of the CaATP complex at constant concentrations of free ATP or free calcium. Figure 5 summarizes the results. Concentrations of 1 or 5 mM free ATP are inhibitory; the inhibition at 5 mM free ATP is particularly strong. Not only is free ATP an inhibitor; it also is an allosteric effector that yields a sigmoidal velocity plot. This is similar to the effect of ADP on MgATPase activity of CF_1 observed by Nelson *et al.* (20). The Lineweaver–Burk plot of 1 mM free ATP, shown in Fig. 6, is concave, as expected for a velocity plot exhibiting positive cooperativity. The Hill plot of these data also is curved; the slope of the

TABLE III

CATION SPECIFICITY OF TRYPSIN-ACTIVATED
Spirulina F_1

Metal	Metal (mM)	Specific activity (μmol P_i min^{-1} mg protein^{-1})	% of control
Calcium	20	23.0	100%
Magnesium	50	3.5	15
Manganese	2	1.2	5
Zinc	20	0.9	4

Note. Spirulina F_1 was activated for 15 min at room temperature, as described under Materials and Methods, and assayed for 5 min at 37°C in a reaction mixture containing 50 mM Tricine, pH 8.0, 5 mM ATP, and varying concentrations (1–50 mM) of the metals indicated. The concentration of metal that gave the highest activity is listed.
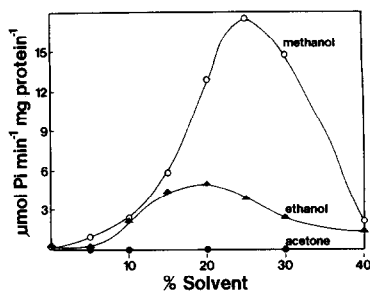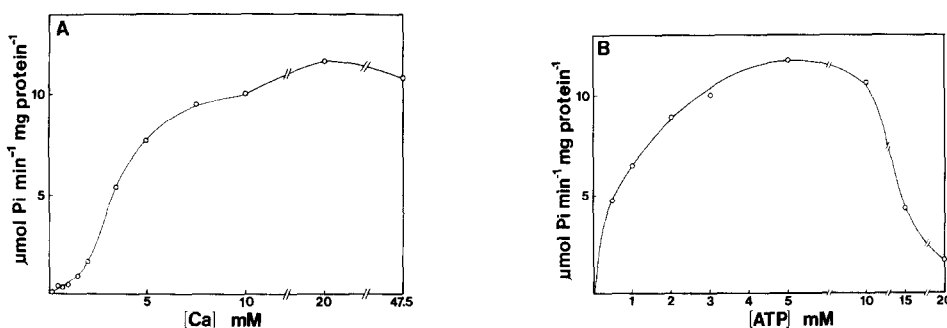
FIG. 4. Effect of varying calcium and ATP concentration on the ATPase activity of trypsin-activated *Spirulina* F_1. (A) Effect of varying calcium concentration. *Spirulina* F_1 (0.2 mg/ml) was activated for 15 min at room temperature in the presence of 50 mM Tricine, pH 8.0, and trypsin (1 mg/ml). The activation was terminated by a threefold excess of soybean trypsin inhibitor. Aliquots of the activation mixture containing 5 μg F_1 were assayed for 5 min at 37°C in a reaction mixture containing 50 mM Tricine, pH 8.0, 5 mM ATP, and the indicated concentrations of $CaCl_2$. (B) Effect of varying ATP concentration. Trypsin-activated F_1 was assayed in the presence of 50 mM Tricine, pH 8.0, 10 mM $CaCl_2$, and the indicated concentrations of ATP.

line, i.e., the apparent Hill constant, in the region near 0.5 V, is 1.64. The average Hill constant of three experiments is 1.72 ± 0.08. Other methods for determining the n_{app} value, as outlined in (21), yielded values of 1.5–1.8. In contrast, at 5 mM free calcium the velocity plot obeys Michaelis–Menten kinetics. The Hill plot for 5 mM calcium is linear, except at high [CaATP], and the calculated Hill constant is 1.02 (Fig. 7). The K_m for CaATP at 5 mM free calcium, determined from Lineweaver–Burk plots, is 0.43 ± 0.24 (n = 3) (Fig. 8).
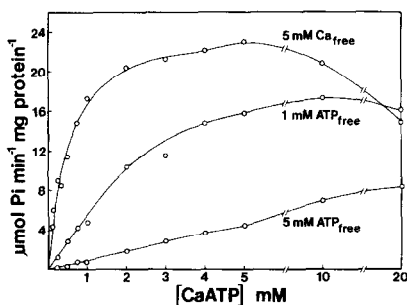
DISCUSSION

The ATPase activity of *Spirulina* F_1, whether in the membrane-bound or isolated form, is greater than 80% latent as defined by the ratio of endogenous activity to the activity observed when activated. The endogenous activity of *Spirulina* membranes is substantially increased by light modulation in the presence of dithiothreitol, a procedure that has been found in chloroplasts to follow the same apparent
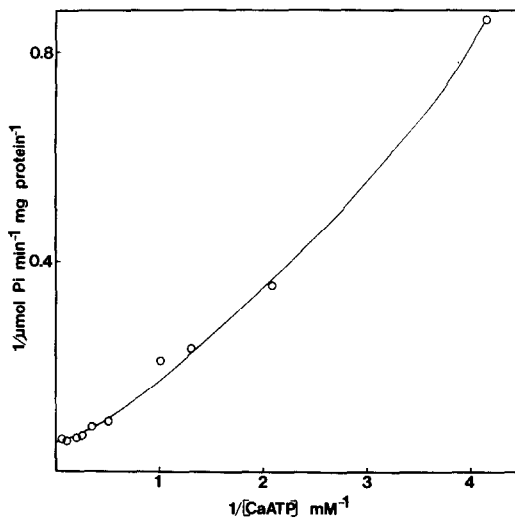

FIG. 5. Effect of varying Ca–ATP metal–nucleotide complex concentration on ATPase activity in trypsin-activated *Spirulina* F_1 with different constant concentrations of free calcium or ATP. Trypsin-activated F_1 was assayed in the presence of 50 mM Tricine, pH 8.0, and the indicated concentrations of Ca–ATP complex with the concentrations of free calcium (5 mM) or free ATP (1 and 5 mM) held constant.

FIG. 6. Plot of $1/v$ versus $1/[CaATP]$ with free [ATP] held constant at 1 mM. Conditions were as described in Fig. 5 with the free ATP concentration set at 1 mM.
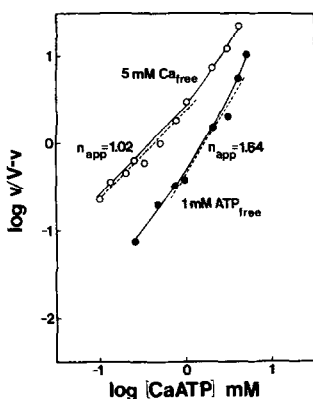
FIG. 7. Hill plot of effect of free calcium and free ATP on CaATPase activity in trypsin-activated *Spirulina* F_1. Conditions were as described in Fig. 5 with the free calcium concentration set at 5 mM and the free ATP concentration set at 1 mM. The lines were determined by linear regression using points in the region of $v = 0.5$ V (8 points in the case of free calcium, 5 points in the case of free ATP).

mechanism as the *in vivo* activation of chloroplast ATPase activity by illumination of leaves (22) or intact chloroplasts (23, 24). Dithiothreitol also stimulates *Spirulina* F_1 activity, though to a lesser extent than is observed with CF_1. Whether the lower stimulation is due to suboptimal activation conditions or whether the enzyme undergoes changes upon solubilization that make it less sensitive to dithiothreitol is not known. *Spirulina* F_1 does undergo a shift in metal preference from Mg (light modulation) to Ca (dithiothreitol) when removed from the membrane just as observed with CF_1. Furthermore, the γ subunit of *Spirulina* F_1 cross-reacts with antibodies to the γ subunit of spinach CF_1 (N. Nelson, personal communication). Thus, we believe that *Spirulina* F_1 is probably subject to the same light/dark modulation correlated with reduction of a dithiol group in the γ subunit that appears to regulate CF_1 activity (2, 22–24). Other procedures that activate CF_1 also stimulate *Spirulina* F_1 activity in the same cation-specific manner. While these latter procedures (trypsin, alcohols) are certainly unphysiological, they suggest that key molecular properties relating to catalytic control are shared by the cyanobacterial enzyme and its chloroplast counterpart.

The purified *Spirulina* ATPase has some interesting kinetic properties. At 5 mM ATP, a plot of varying calcium concentration against specific activity shows pronounced sigmoidal character. Similar published plots for CF_1 and for the *Mastigocladus laminosus* ATPase, although not discussed in those reports, also appear to be sigmoidal, but to a much lesser degree (19, 25). The origin of the sigmoidicity we observe is probably not from cooperative binding of calcium, but rather is due to inhibition by the free ATP present in excess of the metal-nucleotide complex concentration. Evidence for this is seen in Figs. 5, 6, and 8, where the CaATP complex concentration is varied at constant concentrations of free ATP or free calcium. When excess free ATP is present, the kinetics no longer obey the Michaelis–Menten kinetics that are observed when excess free calcium is present. Thus, binding of substrate (CaATP metal-nucleotide complex) is noncooperative when free ATP is absent, but changes to cooperative binding in the presence of the allosteric effector, free ATP. Free ATP does not appear to be an allosteric effector of spinach CF_1 since $1/v$ versus $1/S$ plots at constant free ATP are linear (26); instead, ATP is a competitive inhibitor of spinach CF_1. The effect of free ATP on the *Spirulina* ATPase resembles the effect of ADP observed with spinach CF_1 (MgATPase) (20), with *Chlamydo-*
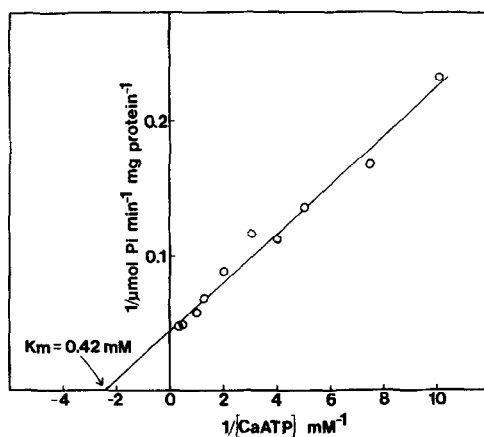

FIG. 8. Plot of $1/v$ versus $1/[CaATP]$ with free [Ca] held constant at 5 mM. Conditions were as described in Fig. 5 with the free calcium concentration set at 5 mM.

monas CF_1 in the presence of ethanol (27), and with the *M. laminosus* F_1 (28).

Regulation of ATPase activity in chloroplasts appears to be very complex [reviewed in (2)]. In addition to the control exerted by proton gradients generated by light-driven electron transport or ATP hydrolysis, regulation of ATPase activity probably involves conformational changes in CF_1 correlated with the reduction of a disulfide group in the γ subunit. The effects of ADP and certain anions such as bicarbonate and phosphate on the kinetics of CF_1 suggest that these small molecules are likely to have a physiological role in controlling ATPase activity. Free ATP, because it is only a weak competitive inhibitor of CF_1, is not thought to be a significant factor in ATPase regulation. The latency and activation of ATPase activity in both the membrane-bound and soluble *Spirulina* F_1 suggest that the membrane ATPase activity of *Spirulina* may be subject to the same regulation found in chloroplasts. Our finding that ATP is both a potent inhibitor and an allosteric effector of soluble *Spirulina* F_1 CaATPase activity, however, would indicate that, unlike the situation with CF_1, ATP may be involved in regulating ATPase activity in *Spirulina* membranes. Since these data were collected using the soluble F_1, it will be necessary to extend these studies to an examination of the effects of ATP on ATPase activity in *Spirulina* membranes, especially on the light-activated activity, to determine what role ATP has in controlling the hydrolysis of ATP *in vivo*.

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