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METHANOL-INDUCED RELEASE OF TIGHTLY BOUND ADENINE NUCLEOTIDES FROM THYLAKOID-ASSOCIATED CF_1

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Incubation of thylakoids in 33% methanol causes a release of the tightly bound nucleotides from CF_1. This methanol effect is not a stimulation of nucleotide exchange, since no medium ATP or ADP is incorporated into CF_1 during the methanol treatment. While the optimal conditions for stimulating the release of tightly bound ADP were similar to those for activating the ATPase, a direct relationship between the effects was not found. The tightly bound ADP does not represent a catalytic intermediate in this system, since (a) its rate of release is much slower than enzyme turnover, and (b) the substrate specificity for hydrolysis is different from that which promotes ADP release. A regulatory role for the tightly bound ADP in methanol-activated ATPase is also not indicated, since (a) activation of the ATPase occurs much more rapidly than ADP release, and (b) after the tightly bound ADP has been lost, high rates of ATP hydrolysis still require the presence of methanol, and (c) the small ATPase activity which persists after the removal of the methanol is not correlated with the loss of bound ADP. These results show that significant rates of ATP hydrolysis can occur with ADP still tightly bound to CF_1. This argues against any model in which ADP regulates ATPase activity by binding directly to the catalytic site.

Introduction

The thylakoid membranes of the chloroplast have attached to their surface the coupling factor protein (CF_1) which catalyses the synthesis of ATP in photophosphorylation. Like the coupling factors of the mitochondria and bacteria, this protein has adenine nucleotides tightly bound to it. These nucleotides do not readily exchange with medium nucleotides unless the thylakoids are energized either by illumination or by imposing a pH transition [1–3].

Illumination of thylakoids not only causes the release of bound ADP but also activates the ATPase. This correlation and the finding that added ADP accelerates the dark decay of ATPase activity have led to the proposal that tightly bound ADP serves a regulatory function [4,5]. Alternatively, it has been proposed that the tightly bound nucleotides directly participate in ATP synthesis via a 'binding change' mechanism. In this scheme, the energy input in photophosphorylation is for the release of the tightly bound nucleotides rather than for ATP synthesis directly [6,7].

We have recently shown that high concentrations of methanol will activate the ATPase of thylakoids [8]. Given the close association between ATPase activation and ADP release which occurs

Abbreviations: CF_1, chloroplasts coupling factor 1; Chl, chlorophyll; Ado*PP*[NH]*P*, 5′-adenylyl-β, γ-imidodiphosphate; arabino-ATP, Adenine-9-β-D-arabinofuranoside 5′-triphosphate; ϵ-ATP, 1 N^6-ethenoadenosine 5′-triphosphate; Tricine, *N*-[2-hydroxy-1, 1-bis(hydroxymethyl)ethyl]glycine; Taps, 3-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}-1-propanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

with illumination, it was of interest to look at the effect of methanol on the tightly bound nucleotides. The relationship between ATPase activation and methanol-induced nucleotide release should yield further information on the role of the tightly bound adenine nucleotides. The results we report here argue against their direct participation in either regulation or catalysis, at least when the ATPase has been activated by methanol.

Materials and Methods

Spinach thylakoid preparation and ATPase measurements were performed as described previously [8]. Labeling of the tight ADP binding site was performed as described by Shavit and Strotmann [9]. Thylakoids were illuminated at 1 mg Chl/ml at 10 °C for 2 min in a solution which contained 25 mM Tricine (pH 8.0), 25 mM NaCl, 50 μM PMS and 10 μM [^{3}H]ADP at 150 μCi/μmol. After 2 additional minutes in darkness the thylakoids were washed three times and resuspended in 25 mM Tricine (pH 8.0)/25 mM NaCl. Labeling of the tight site varied between 0.4 and 1.1 nmol/mg Chl in different experiments.

Measurement of the methanol-induced ADP release was done in a volume of 0.3 ml. The incubation medium contained 25 mM Taps-NaOH (pH 8.8), 25 mM NaCl, 33% (v/v) methanol and other additions as indicated. Solutions were preheated to 37 °C and the reaction started by the addition of the thylakoids (to a final concentration of about 0.5 mg Chl/ml). Reactions were stopped by the addition of 0.7 ml of cold 25 mM Tricine (pH 8.0)/25 mM NaCl. This lowered the methanol concentration to 10%, which completely stopped ADP release. The samples were then centrifuged for 10 min at 12000 × *g* and aliquots of the supernatant were either counted for radioactivity of assayed colorimetrically for P_i. In some experiments the pellets were then resuspended and washed three times to determine the nucleotides remaining with the thylakoids.

To determine the amount of ADP and ATP bound to the thylokoids, perchloric acid was added to a final concentration of 4% and after 5 min at room temperature the samples were centrifuged to removed the sediment. Aliquots of the clear supernatant were then either counted for radioactivity or neutralized for the determination of total nucleotide content via the luciferase method. Neutralization was done by the trioctylamine method of Khym [10], except that chloroform was substituted for freon.

The luciferase assay for total nucleotide content was based on that used by Keifer and Spanswick [11]. To 2.5 ml of a 25 mM Hepes (pH 7.5)/5 mM $MgSO_4$ solution was added either 0 to 100 μl of 1 μM ATP (0 to 100 pmol) or 5 to 50 μl of neutralized thylakoid extract. To start the assay, 10 μl of firefly extract (5 mg/ml) was added. Starting 30 s after the addition of the firefly extract the samples were counted for 30 s in a Packard liquid scintillation spectrometer. For the determination of ADP, the ADP was first converted to ATP by incubation with pyruvate kinase and phospho*enol*pyruvate. In 0.5 ml of the Hepes-$MgSO_4$ buffer was added 20 μl of 5 mM phospho*enol*pyruvate and 20 μl of pyruvate kinase at 0.1 unit/μl. After 1 h at room temperature an additional 2.0 ml of the Hepes-$MgSO_4$ was added and the samples were assayed for ATP as above. Conversion of ADP to ATP was between 90 and 100% in various experiments.

Results

When thylakoids were incubated with methanol at 33%, a release of the tightly bound preloaded [^{3}H]ADP was observed (Fig. 1). This release was greatly accelerated by the addition of MgATP but inhibited by free Mg^{2+}. Unlike the case when bound nucleotides are removed during illumination, the release of [^{3}H]ADP in methanol was not stimulated by ADP (Table II). The acceleration of ADP release by ATP is apparently not due to a simple exchange reaction; when thylakoids were incubated in methanol with 0.45 mM [^{3}H]ATP no incorporation of ATP by the thylakoids could be detected (data not shown). A similar lack of incorporation was also observed with ADP. The effect of methanol is thus to cause a net release of tightly bound ADP rather than an exchange.

The methanol concentration dependence for this ADP release is shown in Fig. 2. This curve is very similar to that which was observed for activation of the ATPase.

The loss of ADP is not readily reversible. When methanol-pretreated thylakoids were subsequently

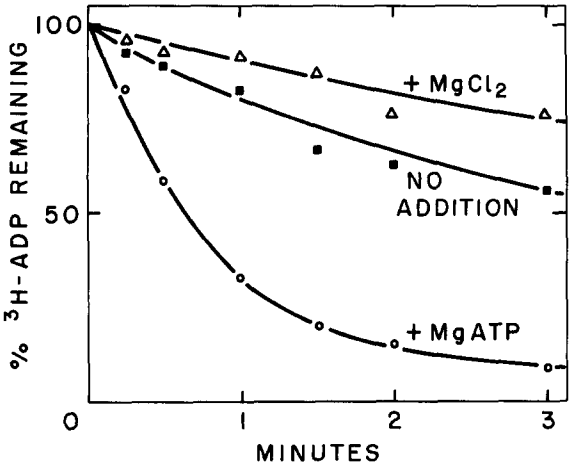

Fig. 1. Time-course for [^{3}H]ADP release. The tight ADP-binding site was loaded to 0.50 nmol [^{3}H]ADP/mg Chl by prior illumination as described in Materials and Methods. Methanol-induced release was done by incubating the thylakoids at 37 °C in 33% methanol/25 mM NaCl/25 mM Taps (pH 8.8) with the additions as indicated. These additions were either nothing (■, 5 mM $MgCl_2$ (△), or 1.0 mM ATP, 0.5 mM $MgCl_2$ (○). At the indicated times the incubations were stopped by dilution of the methanol and centrifugation of the thylakoids. The thylakoids were then washed twice more and the amount of radioactivity remaining with the membranes was determined.

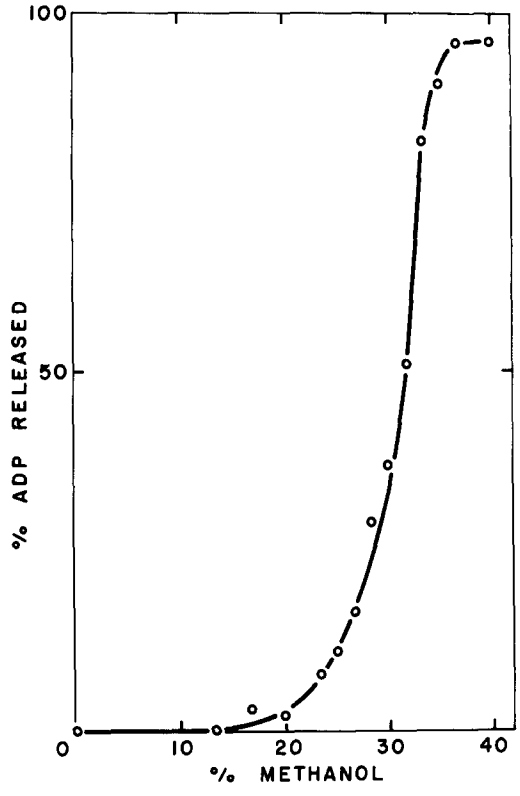

Fig. 2. Methanol concentration dependence for [^{3}H]ADP release. Thylakoids were incubated as in Fig. 1 with 1.0 mM ATP, 0.5 mM $MgCl_2$ and the concentration of methanol indicated. After 2 min the methanol was diluted 3-fold and the thylakoids removed by centrifugation. Aliquots of the supernatant were counted for radioactivity to determine the extent of ADP release. Total [^{3}H]ADP loaded was 0.72 nmol/mg Chl.

incubated for 30 min in the dark with either [^{3}H]ATP or [^{3}H]ADP, even less incorporation of nucleotide was observed than with non-pretreated, control thylakoids (Table I). This rebinding was measured by incubation in the dark. It is well known that the capacity of thylakoids to bind nucleotides is greatly enhanced by illumination [3]. It is possible that if methanol-pretreated thylakoids could be brought to the high energy state the CF_1 would then be able to rebind ADP. This possibility could not be tested, however, since the methanol pretreatment rendered the thylakoids incapable of either light-induced H^+ uptake or acid-base transition phosphorylation (data not shown).

A trivial explanation for the irreversible loss of bound ADP would be a simple denaturation of CH_1 by methanol. This possibility can be ruled out since the ATPase activity of the treated thylakoids was not substantially less than that of the control membranes when assayed in a subsequent 2 min methanol incubation (Table I). When assayed for ATPase in the absence of methanol, the activity of the treated thylakoids was actually several times greater than that of the controls (see below).

The divalent cation requirement for the ATP stimulation of ADP release shows a strong similarity to the requirement for ATPase activity. The ATP stimulation requires Mg^{2+} but is inhibited by excess free Mg^{2+} (Fig. 3). As with ATPase activity, Mn^{2+} but not Ca^{2+} may substitute for Mg^{2+} (data not shown). The inhibition by high concentrations of Mg^{2+} can be overcome by including 50 mM Na_2SO_3. The effect of SO_3^{2-} on ADP release is more complicated than for ATPase, since even in the absence of Mg^{2+}, SO_3^{2-} stimulates ADP release.

TABLE I

EFFECT OF A METHANOL PRE-TREATMENT ON SUBSEQUENT ATPASE ACTIVITY AND NUCLEOTIDE BINDING

Thylakoids were pretreated for 2 min under the same conditions as in Fig. 1. The methanol was then diluted 20-fold and thylakoids washed twice with 50 mM NaCl. The thylakoids were then assayed for ATPase activity either in the presence of methanol as described previously [8] or under the identical conditions but in the absence of methanol. Nucleotide rebinding was measured by incubating the thylakoids at 1 mg Chl/ml for 30 min in the dark at room temperature with 25 mM Taps (pH 8.8) 25 mM NaCl, 30 μM $MgCl_2$ and 60 μM [^{3}H]ATP or [^{3}H]ADP. Samples were then washed three times with 50 mM NaCl and the amount of nucleotide with the thylakoids was determined.

Pretreatment	Nucleotide bound (nmol/mgChl)		ATPase activity (μmol/mg Chl per h)	
	ADP	ATP	+ methanol	− methanol
Control	0.63	0.42	649	16.4
+ methanol	0.28	0.14	544	46.2
+ methanol + 1 mM ATP + 0.5 mM $MgCl_2$	0.20	0.12	502	46.1
+ methanol + 5 mM $MgCl_2$	0.20	0.11	615	59.4

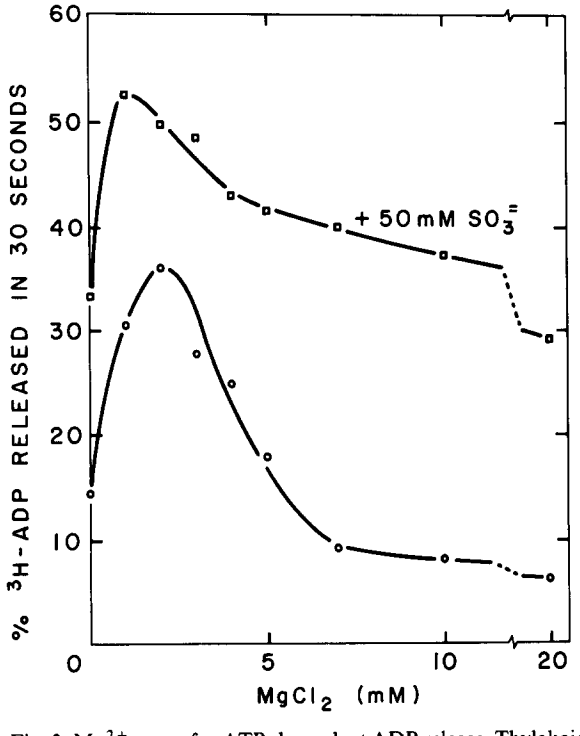

Fig. 3. Mg^{2+} curve for ATP-dependent ADP release. Thylakoid were incubated in 33% methanol under the same conditions as in Fig. 2, except the ATP concentration was 5 mM and the amount of $MgCl_2$ added was an indicated. Where indicated, 50 mM Na_2SO_3 was also added. An incubation time of 30 s was used to approximate better the initial rate of ADP release. Total [^{3}H]ADP loaded was 0.72 nmol/mg Chl.

While both the divalent cation and methanol requirements are very similar for ATPase and ADP release, the ADP release is apparently not directly related to ATP hydrolysis for several reasons. The initial rate of ADP loss shown in Fig. 1 is only 0.031 μmol/mg Chl per h. This is less than 0.1% of the initial rate of ATP hydrolysis under the same conditions. The ATP concentration necessary for half maximal stimulation of ADP release is 80 μM while the K_m for ATPase activity is 1 mM. Additionally, the substrate specificity for hydrolysis and ADP release are quite different (Table II). The stimulation of ADP release is considerably more specific for ATP than is hydrolysis. Other nucleoside triphosphates, such as GTP, ϵ-ATP or arabino-ATP, which were nearly as good as ATP as substrates for hydrolysis, gave much smaller stimulation of bound ADP release. This smaller stimulation was apparently unrelated to their affinity as substrates, since CTP, which was a very poor substrate, gave the same stimulation. The ATP binding site involved in promoting ADP release is apparently distinct from the catalytic site.

Numerous proposals have been made in which the tightly bound ADP acts to regulate ATPase activity [4,5,12]. The possibility that ATPase activation by methanol was indirect due to the loss of regulatory ADP was thus investigated. A direct

comparison of the time-course for ADP release and ATP hydrolysis is shown in Fig. 4. If CF_1 were active as an ATPase only after release of bound ADP, then one would expect a slow increase in the net ATPase activity in parallel with the loss of the ADP from more and more of the CF_1 present. This is not the case. While ADP release was not complete even at 4 min, the ATPase achieved a steady rate after only a few seconds. Occasionally, a more pronounced lag could be detected at the outset of ATP hydrolysis, but in no case was it greater than about 15 s.

Previously, it had been reported by ourselves and others [8,13,14] that the solvent stimulation of both soluble and thylakoid-bound CF_1 is completely reversible. However, as the data in Table I show, this assertion is not strictly correct. While removal of the methanol does cause the ATPase rate to revert to a low level, it does not cause a complete return to the control rate. Rather, following a methanol pretreatment, the ATPase rate is usually 2- to 3-fold higher than that of the controls (this rate is still only 10% of the rate measured in the presence of methanol). An irreversible activation of about the same magnitude also occurs with the Ca^{2+}-ATPase of methanol-pretreated soluble CF_1 (not shown). To test whether this smaller irreversible activation might be due to the loss of regulatory ADP, the time-course of activation was

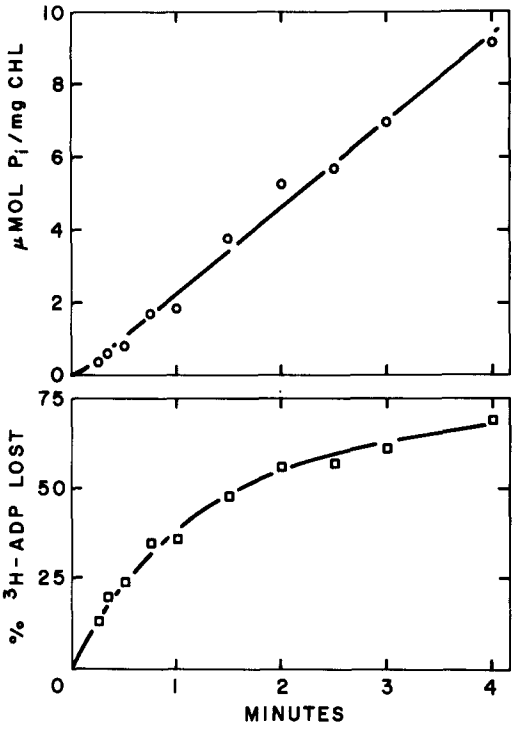

Fig. 4. Time-course for ADP release and ATP hydrolysis. Conditions were as in Fig. 1 except that the incubation was done at 25 °C with ATP at 10 mM and $MgCl_2$ at 5 mM. This lower temperature was used because it slowed the rate of ADP release and permitted more samples to be taken prior to complete ADP release. Qualitatively similar results were obtained at 37 °C. At the time points indicated the incubation was stopped by dilution of the methanol and centrifugation of the thylakoids. Aliquots of the supernatant were then analysed for radioactivity (to determine the extent of ADP release) or colorimetrically for P_i (to determine the extent of ATP hydrolysis). Total [^{3}H]ADP loaded was 0.69 nmol/mg Chl.

TABLE II

SUBSTRATE SPECIFICITY FOR HYDROLYSIS AND STIMULATION OF BOUND ADP RELEASE

Methanol-induced ADP release and NTPase activity were measured as in Fig. 4. All nucleotides were added at 1.0 mM along with 0.5 mM $MgCl_2$. Reaction time was 30 s. Total [^{3}H]ADP loaded was 0.65 nMol/mg Chl.

Addition	ADP release (nmol ADP/ mg Chl per 30 s)	NTPase activity (nmol P_i/mg Chl per 30 s)
None	0.024	–
ATP	0.284	801
Arabino-ATP	0.129	742
ϵ-ATP	0.087	663
GTP	0.091	604
CTP	0.090	58
Ado*PP*[NH]*P*	0.072	–
ADP	0.030	–

examined under conditions which either enhance (plus MgATP) or reduce (plus $MgCl_2$) bound ADP release (Fig. 5). For this experiment, thylakoids were first pre-incubated in 33% methanol with various additions and, at the times indicated, aliquots were diluted 12-fold directly into an assay medium which lacked methanol. From a comparison of the results in Figs. 1 and 5, it is apparent that the activation is not related to ADP loss. While ATP greatly accelerates ADP loss it does not accelerate the ATPase activation. On the other hand, Mg^{2+}, which inhibits ADP release, induces the most rapid irreversible activation. The irreverisble change in CF_1 responsible

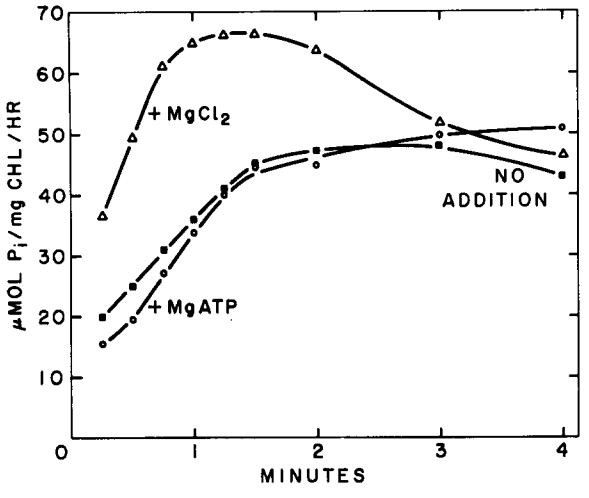

Fig. 5. Time-course for methanol-induced irreversible activation. Thylakoids were preincubated under the same conditions as in Fig. 1. At the indicated times, aliquots were removed and diluted 12-fold into an assay medium as in Ref. 8 except that it lacked methanol. ATP hydrolysis was allowed to proceed for 15 min before the reaction was terminated and P_i determined colorimetrically. Separate experiments showed that the rate of ATP hydrolysis in the absence of methanol was linear for at least 20 min. Control activity (no methanol pretreatment) was 20 μmol/mg Chl per h.

for the activation must be something other than simply the loss of ADP.

Besides the one ADP per CF_1 which readily exchanges in the light, thylakoid membranes have been shown to contain an additional two tightly bound ATP per CF_1 [7]. Labeling studies show that this ATP does not exchange as readily as the ADP; little is known about its function. The effect of methanol on the total endogenous adenylate content is shown in Table III. While the total nucleotide content was found to be quite variable and somewhat higher than reported previously, the ratio of ATP to ADP was usually about 2:1. For five different thylakoid preparations the average ATP to ADP ratio was 1.96 ± 0.2 (mean ± S.D.). The variability in the total adenylate content on a chlorophyll basis probably results from the variability in chlorophyll-to-CF_1 ratio [16]. In the three experiments shown in Table III, the thylakoids were incubated with and without 33% methanol for 2 min then washed three times and acid-extracted. The extract was neutralized and analysed for ATP and ADP by the luciferase method. These

TABLE III

TOTAL NUCLEOTIDE CONTENT OF THYLAKOID BOUND CF_1 BEFORE AND AFTER METHANOL TREATMENT

Thylakoids were pretreated as in Table I in the presence of 1.0 mM ATP and 0.5 mM $MgCl_2$. The thylakoids were then acid-extracted and analysed for their ATP and ADP content by the luciferase method as described in Materials and Methods.

	ATP (nmol /mg Chl)	ADP (nmol mg Chl)	ATP /ADP
Control	5.40	2.40	2.25
Treated	1.44	0.68	2.11
%lost	74	72	
Control	2.40	1.30	1.85
Treated	0.84	0.49	1.71
% Lost	65	62	
Control	4.47	2.41	1.85
Treated	1.68	0.96	1.75
% Lost	62	60	

results show that the methanol treatment caused ADP and ATP to be lost to a similar extent. This is in contrast to the effect of illumination where tightly bound ADP but not ATP is released [7].

Discussion

The data presented here show that in addition to increasing ATPase activity, added methanol also causes the release of tightly bound nucleotides from thylakoid-associated CF_1. Release of tightly bound ADP from CF_1 is also observed when thylakoids are either illuminated or incubated with tentoxin, two other conditions which activate the ATPase [4,17]. Similarly, with soluble CF_1, activation by heat [18], octylglucoside [19] or tentoxin [20] all correlate with increases in the exchangability of the bound nucleotides. This correlation over such a diverse set of activation processes has been interpreted to mean that the release of bound nucleotides is closely linked to conditions necessary for an active ATPase.

The release of ADP in methanol was significantly stimulated by ATP. Based on its substrate specificity and apparent K_m this ATP effect ap-

pears to involve a site distinct from the main catalytic site. The failure of the non-hydrolysable ATP analog Ado*PP*[NH]*P* to substitute for ATP may indicate that ATP hydrolysis must occur at this site in order to promote ADP release. On the other hand, the hydrolyzable nucleotide GTP, also fails to promote ADP release. Thus, the lack of effect of Ado*PP*[NH]*P* more likely means that it simply fails to interact with this site. With soluble CF_1, an ATP-binding site which can discriminate between ATP and Ado*PP*[NH]*P* has been characterized [18]. An ATP stimulation of ADP release is also observed with heat-[21] and octylglucoside-[19] activated soluble CF_1 as well as with illuminated thylakoids [22]. The K_m for this effect with soluble CF_1 is 80 μM [18], the same value that we obtained with thylakoids. However, unlike the heat-activated soluble enzyme or light-activated thylakoids, the methanol-induced ADP release is not affected by medium ADP. A similar lack of an ADP effect is also observed with octylglucoside activation [19]. Overall, the optimal conditions for ADP release are quite similar for methanol and octylglucoside. In both cases the release is stimulated by ATP but not by either ADP or Ado[*PP*[NH]*P* while it is inhibited by Mg^{2+}. It is possible that methanol and octylglucoside activate by inducing similar changes in the enzyme. The similarity between octylglucoside and alcohol activation has been noted by others [14].

An important distinction between methanol and other forms of activation, including octylglucoside, is that methanol leads to a net and apparently irreversible loss of the bound nucleotides. With other forms of activation, increased ATPase activity is accompanied by nucleotide exchange. Under such conditions incorporation of medium nucleotides into CF_1 occurs concurrent with the loss of the bound nucleotides to the medium. This incorporation does not occur with methanol. Furthermore, the subsequent capacity of CF_1 to bind nucleotides is greatly reduced following a methanol pretreatment. The nature of the irreversible changes in CF_1 which lead to this loss of nucleotide binding capacity have yet to be determined.

Another difference between illuminated and methanol-treated thylakoids is the effect on the tightly bound ATP. It has been shown that illumination of thylakoids in a nucleotide-free medium leads to the loss of the tightly bound ADP, but causes little or no change in the total ATP content [7]. Methanol, on the other hand, causes an equal loss of both the ADP and ATP. Thus, the effect of light appears to be a change in the nucleotide binding properties of one specific site, while methanol nonspecifically weakens all the nucleotide-CF_1 interactions. This is in agreement with our previous finding [8] that methanol does not induce the same conformational changes in CF_1 as illumination, as evidenced by differential *N*-ethylmaleimide sensitivity.

The exact role of the tightly bound nucleotides is still a matter of controversy. It has been proposed by Boyer and others [6,7] that the tightly bound nucleotides might participate directly in the synthesis of ATP via a 'binding change' mechanism. If the tightly bound ADP does represent a catalytic intermediate then it should be released at a rate similar to that of enzyme turnover. However, as has been shown with both the soluble and thylakoid-bound forms of CF_1 and for both ATP synthesis and ATP hydrolysis, ADP release is always much slower than catalytic turnover [4,18,19,22]. Our results with methanol also show this; the initial rate of ATP hydrolysis is 5000-times greater than the initial rate of ADP release. Additionally, when substrates other than ATP are used there is no correlation between hydrolytic activity and stimulation of ADP release. The tightly bound nucleotides are thus not directly involved in enzyme turnover.

A more recent proposal for the role of the tightly bound ADP is that of regulation. With light-activated thylakoids it has been shown that the time-course for ADP release parallels the time course for activation [4]. This activation can then be reversed by the addition of ADP to the medium. The K_m for ADP in deactivation is the same as that for binding to the tight site [5]. It has thus been proposed that ADP binding to the tight site serves to deactivate the enzyme in the dark. A similar model has been proposed for soluble CF_1 [21]. Our results with methanol are not consistent with this model. The time-course for the onset of this reversible high activity with methanol does not in any way correlate with the release of the bound ADP. Activation occurs much more rapidly than release (Fig. 4). If the presence of bound ADP

were sufficient to prevent catalytic activity, the ATPase rate would have built to its maximum slowly over a 4 min period, instead of achieving the maximal rate within a few seconds.

Conversely, thylakoids which have lost all their bound ADP due to pretreatment with methanol + ATP have also lost 90% of their ATPase activity if assayed (after washing out the methanol) in the absence of methanol (Table I, line 3). Thus, the loss of this nucleotide is not a sufficient condition for permitting the high rate of ATPase to be expressed. Even the smaller irreversible activation which persists after removal of the methanol shows no apparent relationship with the loss of tightly bound ADP. The treatment which causes the smallest ADP loss gives the greatest activation. These results, while not consistent with the model proposed for light activation, do agree quite well with the recent findings of Pick and Bassilian [19] using octylglucoside-activated soluble CF_1. They also could find no correlation between ADP release and ATPase activation in either the kinetics or the optimal incubation conditions for the two processes.

Both our results and those of Pick and Bassilian [19] suggest that release of tightly bound ADP is not an obligatory prerequisite for ATP hydrolysis. This argues against any model in which the tightly bound ADP inhibits by binding directly to the catalytic site [23]. Regulation of the activity presumably occurs via some interaction between the tight binding and the catalytic site with methanol overriding this inhibitory effect. This may also be what happens with trypsin activation, as it has been shown that trypsin treatment also does not cause the release of tightly bound ADP [24].

Several factors exist, however, which allow for alternative interpretations of our results. For instance, since CF_1 contains multiple nucleotide binding sites it is conceivable that the non-physiological methanol-induced ATPase involved a catalytic site different from the one which functions in the light-activated enzyme. The tightly bound ADP could thereby reside at a site which is part of the catalytic sequence of the latter, but not of the methanol-induced activity.

A more significant complication arises from the apparent instability of CF_1 in methanol. It is possible, for example, that ADP release is in fact required for ATPase activation but that the ADP-depleted form of CF_1 is particularly unstable and is rapidly inactivated in methanol. This might results in a balance between activation and inactivation and lead to a relatively constant population of active ATPases. This steady-state population would be obtained after only a small fraction of the total tightly bound ADP had been released and could thus explain why a constant ATPase rate is achieved long before ADP release is complete. This model requires, however, that the true ATPase rates for CF_1 in methanol are significantly higher than the ones calculated based only on observed rates and total amount of enzyme present.

Since the methanol concentration curves for ADP release and ATPase activation are essentially identical, it is clear that the two processes are closely related. While we cannot conclusively rule out that the two effects are directly linked (i.e., that ADP release is required for activation), the simplest interpretation of our results is that they both arise from a common methanol-induced alteration in CF_1 structure. One possiblity is that methanol leads to a disruption of the interactions between the subunits or a partial unfolding of one of the peptide chains. This could expose normally inaccessible portions of the protein and allow the release of the normally non-exchangable nucleotides as well as access to the catalytic site.

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