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## Activation of Magnesium Ion Specific Adenosinetriphosphatase in Chloroplast Coupling Factor 1 by Octyl Glucoside<sup>†</sup>

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**ABSTRACT:** The effects of the neutral detergent octyl glucoside on ATPase activity of chloroplast coupling factor 1 (CF<sub>1</sub>) have been studied. (1) The presence of octyl glucoside above its critical micellar concentration activates Mg-ATPase but not Ca-ATPase of CF<sub>1</sub>. The optimal detergent concentration for activation at 37 °C is 30 mM and the maximal rate of ATP hydrolysis is 20-40 μmol (mg of protein)<sup>-1</sup> min<sup>-1</sup>. Conversely, a brief preincubation of CF<sub>1</sub> with 30 mM octyl glucoside in the presence of ATP, followed by a dilution of the detergent, activates Ca-ATPase but not Mg-ATPase of CF<sub>1</sub>. (2) Mg-ATPase activation is highly cooperative with respect to octyl glucoside concentration. A similar Hill *n* value of about 7.5 was calculated for both the octyl glucoside monomers and for the detergent micelles. (3) The incubation of CF<sub>1</sub> with the detergent at 37 °C in a buffer solution causes an irreversible inactivation of the ATPase activity. ATP, ADP, and high concentrations of salt effectively protect against inactivation, but inorganic phosphate, dithiothreitol (DTT), and 5'-adenylyl imidodiphosphate [AMP-P(NH)P] are completely ineffective. (4) The addition of 30 mM octyl glucoside to CF<sub>1</sub> preparations that had been preactivated by trypsin, heat, DTT, or octyl glucoside stimulates Mg-ATPase and inhibits Ca-ATPase of

CF<sub>1</sub>, suggesting a reversible modification of the catalytical properties of CF<sub>1</sub> by the detergent. (5) Of the detergents tested only two classes, the bile detergents and saturated fatty acids containing an 8-12 carbon chain, slightly activate CF<sub>1</sub>-Mg-ATPase at above their critical micellar concentration. These results suggest that stimulation of CF<sub>1</sub>-Mg-ATPase is obtained by its interaction with small detergent micelles. (6) In the presence of octyl glucoside, free Mg<sup>2+</sup> appears to be a partial noncompetitive inhibitor with respect to the substrate and only partly inhibits ATPase activity even at 10-100-fold excess over the substrate, Mg-ATP.  $K_{is}(Mg^{2+}) = 0.9$  mM,  $K_{ii}(Mg^{2+}) = 10$  mM,  $K_s(Mg-ATP) = 150$  μM, and  $V = 40$  μmol of ATP hydrolyzed (mg of protein)<sup>-1</sup> min<sup>-1</sup>. Free ATP is a competitive inhibitor with respect to the substrate.  $K_i(ATP) = 2.6$  mM,  $K_i(Mg-ATP) = 60$  μM, and  $V = 31$  μmol of ATP hydrolyzed (mg of protein)<sup>-1</sup> min<sup>-1</sup>. The results suggest that the high apparent Mg-ATPase activity that is obtained in the presence of octyl glucoside is mainly due to the decreased inhibition by free Mg<sup>2+</sup> ions and to the increase in the affinity for the substrate Mg-ATP. (7) The mechanism of CF<sub>1</sub>-ATPase activation by octyl glucoside and its possible physiological implications are discussed.

The chloroplast coupling factor (CF<sub>1</sub>)<sup>1</sup> is part of the DCCD-sensitive ATPase complex that phosphorylates ADP in the process of photophosphorylation (Avron, 1963; Vambutas & Racker, 1965). In broken thylakoid preparations the enzyme does not hydrolyze ATP unless it is preactivated by illumination in the presence of dithiol reagents (Petrack & Lipmann, 1961). The activated enzyme hydrolyzes ATP in the presence of Mg<sup>2+</sup> but not Ca<sup>2+</sup> ions. ATP hydrolysis is coupled to proton uptake into the thylakoid membrane and probably reflects the reversal of ATP synthesis (Carmeli, 1970). It has also been recently demonstrated that illumination

of intact chloroplasts can activate the CF<sub>1</sub>-ATPase in the absence of exogenous dithiol reagents (Mills & Hind, 1979). It appears, therefore, that light activation of the ATPase occurs also *in vivo*.

ATPase activity of the solubilized CF<sub>1</sub> is also very poor, but it can be activated by trypsin (Vambutas & Racker, 1965;

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<sup>1</sup> Abbreviations: CF<sub>1</sub>, chloroplast coupling factor 1; OG, octyl β-D-glucopyranoside; tricine, N-[tris(hydroxymethyl)methyl]glycine; AMP-P(NH)P, 5'-adenylyl imidodiphosphate; quercetin, 3,3',4',5,7-penta-hydroxyflavone; phloridzin, 1-[2-(β-D-glucopyranosyloxy)-4,6-di-hydroxyphenyl]-3-(4-hydroxyphenyl)-1-propanone; cmc, critical micellar concentration; DCCD, dicyclohexylcarbodiimide; NBD-Cl, 7-chloro-4-nitro-2,1,3-benzoxadiazole; FITC, fluorescein 5'-isothiocyanate; PMS, N-methylphenazonium methosulfate; ANS, 8-anilino-1-naphthalene-sulfonate; DTT, dithiothreitol; CHAPS, 3-[(3-cholamidopropyl)di-methylammonio]-1-propanesulfonate; EDTA, ethylenediaminetetraacetic acid.

Deters et al., 1975), heat (Farron & Racker, 1970), or DTT (McCarty & Racker, 1968). However, unlike the thylakoid-bound CF<sub>1</sub>, the purified CF<sub>1</sub> catalyzes ATP hydrolysis in the presence of Ca<sup>2+</sup> rather than Mg<sup>2+</sup> ions. Moreover, Ca-ATPase activity of purified CF<sub>1</sub> appears to be extremely sensitive to free Mg<sup>2+</sup> ions. Hochmann et al. (1976) have suggested that the apparent low Mg-ATPase activity of heat-activated CF<sub>1</sub> is mainly due to an inhibition by free Mg<sup>2+</sup> ions that compete with the substrate Mg-ATP (Hochmann et al., 1976; Hochmann & Carmeli, 1981).

Several treatments that reversibly stimulate a Mg-ATPase activity of purified CF<sub>1</sub> have also been described. They include certain carboxylic acids (Nelson et al., 1972), some organic solvents, notably alcohols and ketones (Sakurai et al., 1981; Selman-Reimer et al., 1981a,b), lipophilic pH indicators (Soe et al., 1978), and a high concentration of tentoxin (Conrad et al., 1981).

We have recently demonstrated that the detergent octyl glucoside may activate either a Mg-specific ATPase or a Ca-specific ATPase of CF<sub>1</sub> (Pick & Bassilian, 1981). The presence of octyl glucoside in the assay mixture reversibly activated Mg-ATPase of CF<sub>1</sub>. Conversely, preincubation of CF<sub>1</sub> with the detergent followed by a dilution step activated Ca-ATPase of CF<sub>1</sub>. We have demonstrated that octyl glucoside stimulated Mg-ATPase activity also in a trypsinized CF<sub>1</sub> preparation composed of  $\alpha$  and  $\beta$  subunits and suggested that the Mg-ATPase activation involves binding of the detergent micelles to the  $\alpha$  and  $\beta$  subunits of CF<sub>1</sub>. This paper describes in detail the characteristics of OG activation and the kinetic properties of ATP hydrolysis in the presence of OG.

#### Materials and Methods

Coupling factor (CF<sub>1</sub>) was prepared either from lettuce by the EDTA extraction procedure (Lien & Racker, 1971) or from spinach (*Spinaca oleracea*, hybrid 424, Ferry Morse Seed Co., Mountain View, CA) according to Strotmann et al. (1973). The two enzyme preparations were slightly different in the rates of Mg-dependent ATP hydrolysis in the presence of octyl glucoside [20–25 or 30–40  $\mu$ mol of ATP hydrolyzed (mg of protein)<sup>-1</sup> min<sup>-1</sup> in lettuce or spinach CF<sub>1</sub>, respectively] but were very similar with respect to ATPase activation by octyl glucoside, the M<sup>2+</sup> ion specificity, and the interactions with other ligands.

Activation of Ca<sup>2+</sup>-ATPase was done by incubation of CF<sub>1</sub> (1 mg of protein/mL) for 10 min at 37 °C in a solution containing 30 or 40 mM octyl glucoside, 30 mM Na-tricine, pH 8, 0.1 mM EDTA, and 5 mM ATP. The activation was terminated by a 4-fold dilution with 30 mM Na-tricine, pH 8–0.1 mM EDTA buffer. Ca<sup>2+</sup>-ATPase activity was measured by the release of <sup>32</sup>P from [ $\gamma$ -<sup>32</sup>P]ATP in the presence of 80 mM Na-tricine, pH 8, 1 mM NaEDTA, 8 mM CaCl<sub>2</sub>, 4 mM ATP containing 15–25  $\mu$ Ci/mmol of [ $\gamma$ -<sup>32</sup>P]ATP, and 4  $\mu$ g of activated CF<sub>1</sub> in a 0.5-mL reaction mixture. Incubation was terminated after 10 min at 37 °C by 5% trichloroacetic acid. Extraction of <sup>32</sup>P was done as described before (Avron, 1961).

OG-dependent Mg-ATPase was assayed similarly in the presence of 30 or 40 mM OG, 2 mM MgCl<sub>2</sub>, 4 mM ATP, [ $\gamma$ -<sup>32</sup>P]ATP, and 1  $\mu$ g of CF<sub>1</sub> in 0.5 mL for 3–5 min at 37 °C. Heat activation of CF<sub>1</sub>-ATPase was done according to Farron & Racker (1970). For activation by DTT, CF<sub>1</sub> (1 mg/mL) was incubated for 90 min at 23 °C with 50 mM DTT, 5 mM ATP, 30 mM Na-tricine, pH 8, and 0.1 mM EDTA. Activation by trypsin or tentoxin was done by including trypsin (12  $\mu$ g of chymotrypsin-free trypsin) or tentoxin (300  $\mu$ M) in the reaction mixture. Activation of ATP hydrolysis in

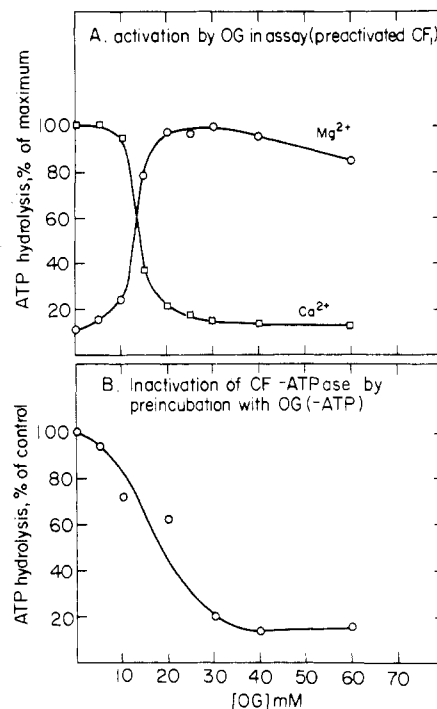


FIGURE 1: OG concentration dependence of activation of CF<sub>1</sub>-ATPase. ATPase activity of lettuce CF<sub>1</sub> was measured in the presence of 4 mM ATP and either 8 mM CaCl<sub>2</sub> or 2 mM MgCl<sub>2</sub> at 37 °C. (A) CF<sub>1</sub> was preactivated by preincubation with 30 mM OG and 5 mM ATP and then diluted 1:100 into the assay medium, which contained the indicated OG concentration. The maximal rates of Ca-ATPase (in the absence of OG) and of Mg-ATPase (in the presence of 30 mM OG) were 6.7 and 22  $\mu$ mol (mg of protein)<sup>-1</sup> min<sup>-1</sup>, respectively. (B) CF<sub>1</sub> was preincubated with OG at the indicated concentration as in (A) but in the absence of ATP. ATPase activity was measured in the presence of Mg<sup>2+</sup> and 30 mM OG. The control activity was 24  $\mu$ mol (mg of protein)<sup>-1</sup> min<sup>-1</sup>. Other details are described under Materials and Methods.

chloroplast thylakoids was done by 2-min illumination in the presence of 5 mM DTT and 30  $\mu$ M PMS as described by McCarty & Racker (1968).

8-Anilino-1-naphthalenesulfonate (ANS) fluorescence was measured in a Perkin-Elmer MPF-44 spectrofluorometer (excitation 365 nm, emission 520 nm). Octyl glucoside was obtained from Calbiochem, FITC and NBD-Cl were obtained from Molecular Probes, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate was obtained from Polyscience, and tentoxin was a gift from Professor B. Selman. Other chemicals and detergents were obtained from Sigma Chemical Co. [ $\gamma$ -<sup>32</sup>P]ATP was prepared by illumination of chloroplast thylakoids with ADP and <sup>32</sup>P, followed by separation of [ $\gamma$ -<sup>32</sup>P]ATP on a poly(ethylenimine)-cellulose according to Magnusson et al. (1976).

#### Results

**Octyl Glucoside Activation of CF<sub>1</sub>-ATPase.** The interactions of CF<sub>1</sub> with the detergent octyl glucoside can lead to a stimulation of Mg-ATPase activity or Ca-ATPase activity or to an inactivation of the enzyme as is demonstrated in Figure 1. The presence of 30 mM OG in the assay medium activated the Mg<sup>2+</sup>-specific ATPase of CF<sub>1</sub> but not Ca-ATPase activity (Figure 1A, Pick & Bassilian, 1981). The activation of Mg-ATPase by OG at 37 °C under our assay conditions appeared to be almost instantaneous since a linear rate of <sup>32</sup>P<sub>i</sub> production was observed starting from the first few seconds of incubation. However, the linearity was preserved for no more than 5 min. Longer incubations resulted in a significant decrease in the rate of ATP hydrolysis due to an

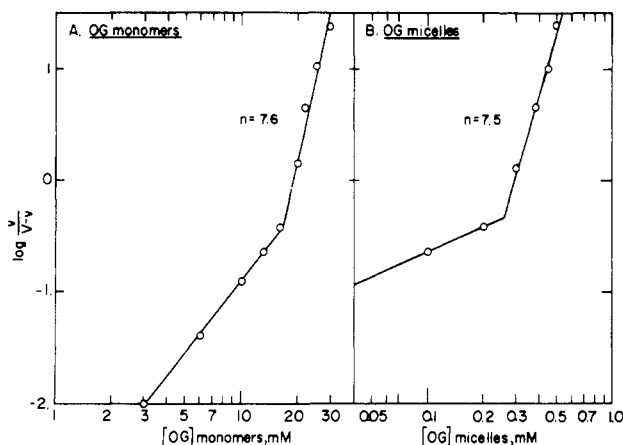


FIGURE 2: Cooperative activation of Mg-ATPase by OG. The initial rates of Mg-ATPase in the presence of different OG concentrations are analyzed according to the Hill equation: (A) dependence on OG monomer concentration; (B) dependence on OG micelle concentration. The micelle concentration (mM) was calculated from the following equation:  $[OG]_{micelle} = ([OG]_{monomer} - 10)/30$ , on the assumption of a cmc value of 10 mM and an aggregation number of 30.  $v$ , rate of Mg-ATPase;  $V$ , maximal rate of Mg-ATPase.

irreversible inactivation of the enzyme (not shown). The dependence of the activation on OG concentration was sigmoidal with very little activation below 10 mM OG; 50% of the maximal activation obtained at about 20 mM OG and maximal activation at 30 mM OG. The high cooperativity with respect to OG concentration is clearly demonstrated by the Hill-plot analysis presented in Figure 2a. Only the activating OG concentrations (0–30 mM) were considered in the analysis. The obtained  $n$  value was 7.6, and the apparent dissociation constant was 19.5 mM. This activity will be referred to as OG-dependent Mg-ATPase. Conversely, a 10-min preincubation of concentrated CF<sub>1</sub> (1 mg/mL) with 30–40 mM OG and ATP at 37 °C, followed by removal of the detergent, activated a Ca<sup>2+</sup>- but not a Mg<sup>2+</sup>-dependent ATP hydrolysis as described before (Pick & Bassilian, 1981). The dependence of Ca-ATPase activation on OG concentration in the preincubation medium was also sigmoidal and notably similar to Mg-ATPase activation by OG when present in the assay (Pick & Bassilian, 1981). It appears, however, that the hydrolysis of ATP in the presence of Ca<sup>2+</sup> following the preincubation with OG does not require the presence of OG in the assay medium since it was diluted 100-fold into the assay medium. We also observed that the activated enzyme retained its full activity for at least 24 h at 23 °C. It appears, therefore, that the preincubation with OG induced an irreversible activation of the enzyme. This activity will be referred to as OG-preactivated Ca-ATPase. The presence of OG in the assay medium not only increased the Mg-ATPase activity but also decreased Ca-ATPase activity as is shown in Figure 1A. In this experiment CF<sub>1</sub> was first preactivated by an incubation with 30 mM OG before the assay. The activation of Mg-ATPase and the inhibition of Ca-ATPase activity occurred at very similar OG concentrations (50% of the maximal effect at about 14 mM OG). It may be noted that even though the rates of OG-dependent Mg-ATPase obtained with the preactivated (Figure 1A) and control (Pick & Bassilian, 1981) CF<sub>1</sub> were very similar, there seems to be a decrease of about 5 mM in the threshold OG concentration required for the activation of CF<sub>1</sub> after the preincubation treatment relative to that of the nontreated enzyme. This might indicate that the preincubation with OG sensitized the enzyme to OG activation during the assay. In contrast to the activation of Ca-ATPase obtained by preincubation of CF<sub>1</sub> with OG in the

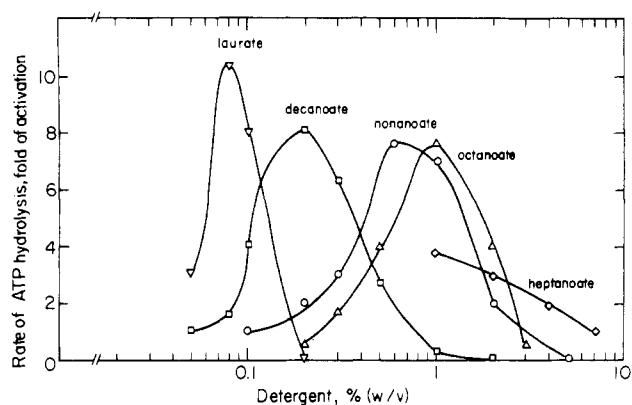


FIGURE 3: Activation of Mg-ATPase in spinach CF<sub>1</sub> by short saturated fatty acids. Mg-ATPase activity was measured in the presence of the sodium salts of the fatty acids. The concentrations of the detergents are expressed in weight per volume with respect to the unneutralized acid form of the detergent.

presence of ATP, preincubation of CF<sub>1</sub> with OG in the absence of ATP led to an irreversible inactivation of CF<sub>1</sub>-ATPase as shown in Figure 1B. The enzyme was inactive in ATP hydrolysis in the presence of OG and Mg<sup>2+</sup> and could not be reactivated by any other procedure after removal of the detergent. Also, in this case the OG concentration dependence was sigmoidal with very little inactivation below 10 mM OG and maximal inactivation at 30 mM OG.

The similar sigmoidal dependence on OG concentration for the activations of Mg-ATPase and Ca-ATPase and for the inactivation of CF<sub>1</sub>-ATPase may suggest an interaction of CF<sub>1</sub> with micelles of the detergent. The cmc of OG has previously been reported to range between 13–23 mM, depending on the ionic strength of the medium (De Grip & Bovee-Geurts, 1979). Measurements of the cmc for OG under our experimental conditions by the ANS fluorescence enhancement technique (Horowitz, 1977) yielded a value of 12.5 mM (Pick & Bassilian, 1981). This correlates very nicely with the threshold in OG concentration required for the activation and inactivation (about 10 mM). The dependence of Mg-ATPase activation on OG micelle concentration was analyzed by assuming a cmc of 10 mM and an aggregation number of 30 for OG. The apparent affinity for OG micelles was 0.25 mM, and the  $n$  value was 7.5. This might indicate a cooperative activation of the enzyme by multiple OG micelles.

**Detergent Specificity.** In order to find out to what extent Mg-ATPase activation of CF<sub>1</sub> is specific for octyl glucoside, we have tested the effects of several different detergents on ATP hydrolysis in the presence of Mg, and the results are summarized in Table I. None of the detergents tested including neutral, anionic, cationic, and sugar containing detergents was nearly as effective as OG. Slight stimulations of Mg-ATPase activity were obtained by the bile detergents cholate, deoxycholate, and CHAPS (a zwitterionic cholate analogue) and by saturated fatty acids with 8–12 carbon atom chains. Sodium heptanoate gave only a poor activation. Fatty acids containing more than 12 carbon atoms could not be tested because of insolubility. Figure 3 demonstrates the concentration dependence for activation by the short-chain fatty acids. For each detergent the activity sharply rose above a threshold concentration and was strongly inhibited by high concentrations of the detergents. The optimal concentrations for activation shifted from 0.08% (4 mM) for sodium laurate to 1% (70 mM) for sodium octanoate. The cmc of sodium nonanoate was determined by ANS fluorescence enhancement and was found to be 0.2% (12 mM) (not shown)—quite similar to the threshold concentration for activation of ATP hydrolysis.

Table I: Effects of Detergents on Mg-ATPase Activity of CF<sub>1</sub><sup>a</sup>

detergent	cmc (mM)	detergent concn tested (mM)	max stimulation of ATP hydrolysis (x-fold activation)	optimal concn (mM)
experiment I				
OG	15	5-40	35.0	30-40
digitonin		0.085-8.5	1.2	0.25
Triton X-100	0.23	0.15-15	1.0	
Tween-80	0.013	0.1-10	1.0	
sodium dodecyl sulfate	5	0.3-30	1.5	0.3
octyltrimethylammonium bromide		0.3-30	1.3	30
sodium cholate	14	2.2-44	3.4	20-40
sodium deoxycholate	5	0.25-25	2.5	7.5
CHAPS	8	0.2-20	3.3	20
experiment II				
OG	15	5-80	61.0	30
sodium <i>n</i> -octanoate		7-350	8.5	70
sodium <i>n</i> -nonanoate	12	3-170	7.0	33
sodium <i>n</i> -decanoate		3-170	8.4	15
sodium <i>n</i> -laurate		1-25	10.9	4
<i>n</i> -octylamine		7-700	1.0	

<sup>a</sup> ATPase activity of lettuce CF<sub>1</sub> (I) and of spinach CF<sub>1</sub> (II) was measured in the presence of 4 mM ATP, and 2 mM MgCl<sub>2</sub>, and the indicated detergent for 5 min at 37 °C. The latent and the maximal rates of ATP hydrolysis with 30 mM OG were 0.6 and 22 μmol (mg of protein)<sup>-1</sup> min<sup>-1</sup> for lettuce CF<sub>1</sub> (I) and 0.5 and 30 μmol (mg of protein)<sup>-1</sup> min<sup>-1</sup> for spinach CF<sub>1</sub> (II).

Table II: Effect of Octyl Glucoside on ATPase Activity of CF<sub>1</sub> Activated by Different Procedures<sup>a</sup>

activation	μmol of ATP hydrolyzed (mg of protein) <sup>-1</sup> min <sup>-1</sup>			
	Ca-ATPase		Mg-ATPase	
	control	+OG	control	+OG
none	0.2	1.5	0	18.5
OG + ATP + DTT	8.2	2.0	1.3	23.8
heat	7.4	2.4	1.0	24.4
50 mM DTT	3.6	2.3	0.2	24.8
trypsin	4.2	0.12	0	18.6
1 mM tentoxin	0.3	1.9	2.8	36.8

<sup>a</sup> Lettuce CF<sub>1</sub> was preactivated by 10-min preincubation at 37 °C with 30 mM OG and 5 mM DTT (OG + ATP + DTT), by 4-min incubation at 64 °C with 20 mM ATP and 10 mM DTT (heat), or by 90-min incubation at 23 °C with 50 mM DTT and 5 mM ATP (50 mM DTT). The concentration of CF<sub>1</sub> in the preincubation medium was 1 mg/mL. Trypsin (12 μg/0.5 mL) and tentoxin (0.3 mM) were included in the assay medium where indicated. ATPase activity was measured in the presence or absence of 30 mM OG, with 4 mM ATP and either 8 mM CaCl<sub>2</sub> or 2 mM MgCl<sub>2</sub>.

Since the short-chain fatty acids and the bile detergents like octyl glucoside form relatively small micelles and have high cmc values and since the optimal stimulation was obtained by a concentration just above the cmc, it seems that the detergent activation of CF<sub>1</sub>-ATPase is induced by interaction with small detergent micelles.

**Interrelations between OG Activation and Other CF<sub>1</sub>-ATPase Activation Procedures.** The interrelations between OG activation and other CF<sub>1</sub>-ATPase activation procedures are summarized in Table II. The presence of OG in the assay medium inhibited Ca-ATPase activity that had been induced by treatments with heat, DTT or trypsin or preincubation with OG plus ATP, parallel with the stimulation of Mg-ATPase activity. These results suggest that OG, when present in the assay mixture, stimulates CF<sub>1</sub>-Mg-ATPase irrespective of the mode of activation. An exception to this is the synergistic activation of Mg-ATPase by high tentoxin concentrations (1 mM) and OG, which will be described in detail elsewhere (Pick et al., 1982).

Table III: Effect of CF<sub>1</sub> Ligands on Activation and Inactivation by Octyl Glucoside<sup>a</sup>

additions to preincubation medium	μmol of ATP hydrolyzed (mg of protein) <sup>-1</sup> min <sup>-1</sup>	
	Ca-ATPase	OG-dependent Mg-ATPase
control without OG	1.2	22.0
none	0.4	1.5
5 mM ATP	7.2	21.2
5 mM ADP	6.2	
5 mM AMPP(NH)P	0.2	0.8
5 mM GTP	2.0	
5 mM P <sub>i</sub>	0.2	1.2
10 mM DTT	0.4	1.4
10 mM DTT + 5 mM ATP	12.5	22.8
5 mM Mg	5.4	11.6
5 mM Ca	2.2	5.4
100 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	5.9	22.3
heat-activated control	11.2	22.4

<sup>a</sup> Lettuce CF<sub>1</sub> (1 mg/mL) was preincubated for 10 min at 37 °C in the presence of 40 mM OG and the indicated additions. The incubation was stopped by 5-fold dilution with a solution containing 30 mM Na-tricine, pH 8, and 0.1 mM EDTA at 23 °C. ATPase activity was measured either in the presence of CaCl<sub>2</sub> (8 mM) or in the presence of 30 mM OG and 2 mM MgCl<sub>2</sub> as described under Materials and Methods.

**Effect of CF<sub>1</sub> Ligands on OG Activation and Inactivation.** The effect of ligands of CF<sub>1</sub> on its activation and inactivation by preincubation with OG at 37 °C is summarized in Table III. Activation was estimated from the increase of Ca-ATPase, and inactivation was measured by the decrease in OG-dependent Mg-ATPase. The table demonstrates a good correlation between the effectiveness of different ligands to activate the Ca-ATPase and to protect against the inactivation of OG-dependent Mg-ATPase. Of the nucleotides tested, ATP and ADP but not GTP and AMP-P(NH)P were effective in the activation and in the protection against inactivation. The apparent affinity for ATP in the activation of Ca-ATPase and in the protection against inactivation of OG-dependent Mg-ATPase was very similar (50% effect obtained with 30 and 40 μM ATP, respectively, not shown). DTT alone was inef-

Table IV: Inhibitors of ATP Hydrolysis by CF<sub>1</sub><sup>a</sup>

inhibitor	concn	% of control		
		OG-dependent Mg-ATPase	OG-preactivated ATPase	trypsin-activated Ca-ATPase
anti-CF <sub>1</sub> serum	2 $\mu$ L	69	27	84
	10 $\mu$ L	0	0	0
	20 $\mu$ M	86	55	93
quercetin	100 $\mu$ M	35	0	15
	1 mM	74	59	74
	5 mM	48	30	
DCCD	30 $\mu$ M	40	33	
	200 $\mu$ M	3	5	
NBD-Cl	5 $\mu$ M	20	21	38
	20 $\mu$ M	0		4
	100 $\mu$ M	12		12
FITC	100 $\mu$ M	2		2
FITC + 5 mM Ca <sup>2+</sup>	100 $\mu$ M	37	0	6
tentoxin	1 $\mu$ M	52	0	9

<sup>a</sup> Modification of lettuce CF<sub>1</sub> was done with DCCD by 30-min preincubation at pH 7 according to Shoshan & Selman (1980), with NBD-Cl by 20-h incubation at 23 °C according to Deters et al. (1975), and with FITC by 1-h incubation at 37 °C according to Pick (1981). The excess of reagents was removed by passing the modified enzyme through Sephadex G-50 columns. The control rates of ATP hydrolysis in the absence of inhibition (lettuce CF<sub>1</sub>) were 22, 6.8, and 9.1  $\mu$ mol of ATP hydrolyzed (mg of protein)<sup>-1</sup> min<sup>-1</sup> for OG-dependent Mg-ATPase, OG-preactivated Ca-ATPase, and trypsin-activated Ca-ATPase, respectively.

fective, but in the presence of ATP it further increased the Ca-ATPase activity to rates of hydrolysis similar to those obtained by heat activation. Mg<sup>2+</sup> and Ca<sup>2+</sup> in the presence of OG were only partially effective in Ca-ATPase activation and in the protection against inactivation. The apparent affinity for Mg<sup>2+</sup> for both effects (50% obtained by 60  $\mu$ M in the activation and 100  $\mu$ M in the protection against inactivation) was found to be much higher than that for Ca<sup>2+</sup> ions (50% of maximal activation by 2 mM Ca<sup>2+</sup>, 50% of maximal protection by 5 mM Ca<sup>2+</sup>, data not shown). The presence of 100 mM ammonium sulfate provided complete protection against inactivation of OG-dependent Mg-ATPase and activated Ca-ATPase with OG just like ATP. Similar effects were obtained with KCl, indicating that this is probably a general salt effect rather than a specific one. The good correlations between activation of CF<sub>1</sub>-Ca-ATPase and protection against inactivation by OG strongly indicate that both phenomena are triggered by the same sensitization of the enzyme induced by OG binding.

**Effect of OG on Susceptibility of CF<sub>1</sub>-ATPase to High Ionic Strength.** Solubilized CF<sub>1</sub> is very sensitive to a high ionic strength, particularly at low temperature (McCarty & Racker, 1966; Posorske & Jagendorf, 1976). Figure 4 demonstrates that in the presence of OG, CF<sub>1</sub>-ATPase becomes much more resistant to inhibition by a high ionic strength. OG-preactivated Ca-ATPase was extremely sensitive to ammonium sulfate. Similarly, we observed that heat-activated Ca-ATPase was completely inhibited by 100 mM ammonium sulfate (not shown). In contrast, OG-dependent Mg-ATPase was markedly less sensitive to ammonium sulfate. Similar results were obtained with KCl, indicating that the effects are not specific to ammonium sulfate. The results suggest that the presence of the detergent lowers the sensitivity of the activated enzyme to salt.

**Effect of CF<sub>1</sub> Inhibitors on OG-Activated ATP Hydrolysis.** A comparison of the effects of specific CF<sub>1</sub> inhibitors on OG-dependent Mg-ATPase, on OG-preactivated Ca-ATPase, and on trypsin-activated Ca-ATPase is summarized in Table IV. Similar inhibitions were obtained by CF<sub>1</sub> antiserum, by DCCD, by NBD-Cl, by FITC, and by phloridzin and quercetin. Tentoxin (1 and 10  $\mu$ M) inhibited OG-dependent Mg-ATPase by about 50% in contrast to complete inhibition of trypsin-activated and OG-preactivated Ca-ATPase. The

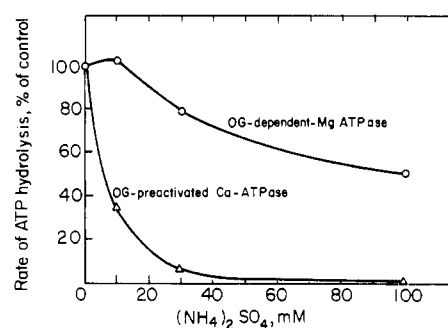


FIGURE 4: Effect of ammonium sulfate on CF<sub>1</sub>-ATPase. OG-preactivated Ca-ATPase and OG-dependent Mg-ATPase activities were measured in the presence of the indicated concentrations of ammonium sulfate in the assay medium. The control rates of Ca-ATPase and Mg-ATPase were 5.9 and 21.8  $\mu$ mol (mg of protein)<sup>-1</sup> min<sup>-1</sup>, respectively.

lower sensitivity of the OG-dependent Mg-ATPase activity to tentoxin may be due either to partial neutralization of the hydrophobic peptide by the detergent or to a partial masking of the tentoxin binding site of CF<sub>1</sub> by OG. This may be also the reason for the slightly lower sensitivity of the OG-dependent Mg-ATPase to anti-CF<sub>1</sub> and to quercetin in comparison to that of the OG-preactivated Ca-ATPase.

**Specificity of CF<sub>1</sub> OG-Activated ATP Hydrolysis for Divalent Metal Ions.** ATP hydrolysis by activated CF<sub>1</sub> at a constant ATP concentration is markedly influenced by the overall M<sup>2+</sup> ion concentration (Hochmann et al., 1976). Therefore, in order to gain information about the specificity of CF<sub>1</sub> ATPase for M<sup>2+</sup> ions, it is necessary to test different M<sup>2+</sup> concentrations. Figure 5 demonstrates the specificity of CF<sub>1</sub>-ATP hydrolysis for M<sup>2+</sup> ions in the presence of OG (A), following preactivation by OG (B) or by a heat treatment (C) and in light-activated chloroplast thylakoids. The ATP concentration in all the measurements was 4 mM and the pH was 8.

The figure demonstrates general similarities between ATP hydrolysis by CF<sub>1</sub> in the presence of OG (A) and by light-activated thylakoids (D)—in both measurements the highest apparent rates of hydrolysis were obtained with Mg<sup>2+</sup> and Mn<sup>2+</sup> ions, and very low rates of hydrolysis were obtained in the presence of Ca<sup>2+</sup> ions. The optimal concentrations of Mg<sup>2+</sup> and Mn<sup>2+</sup> were about 2–5 mM, and an excess of Mg<sup>2+</sup> over

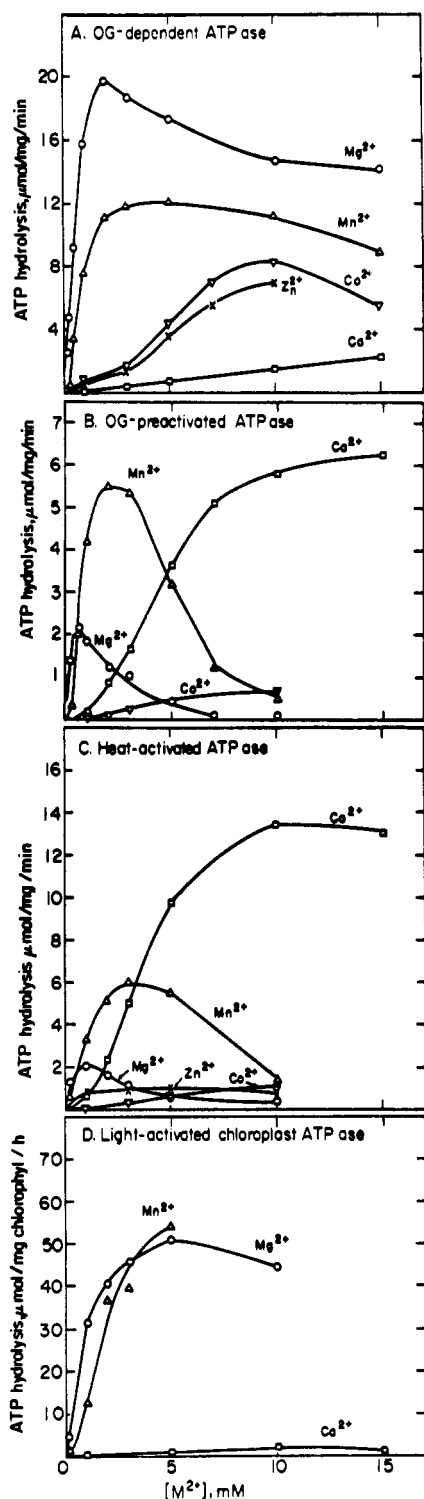


FIGURE 5: Effect of  $M^{2+}$  ion concentration on ATPase activity. ATPase activity of lettuce CF<sub>1</sub> was measured in the presence of 4 mM ATP and the indicated  $M^{2+}$  ion concentrations at 37 °C. (A) The activity was measured in the presence of 30 mM OG. (B and C) CF<sub>1</sub> was activated by preincubation with OG, ATP, and DTT or by a heat treatment as in Table II. Light activation of chloroplast thylakoids (D) was done by 3-min preillumination of chloroplasts (54  $\mu\text{g}$  of chlorophyll/mL) in the presence of 10 mM DTT, 10  $\mu\text{M}$  PMS, 1 mM inorganic phosphate, 30 mM KCl, and 30 mM Na-tricine, pH 8.0. ATP hydrolysis was measured for 10 min in the dark in the presence of ATP and the metal ions, which were added immediately after illumination.

ATP resulted in only a partial inhibition.

In contrast, with OG-preactivated (B) or heat-treated CF<sub>1</sub> (C), the highest rates of ATP hydrolysis were obtained in the

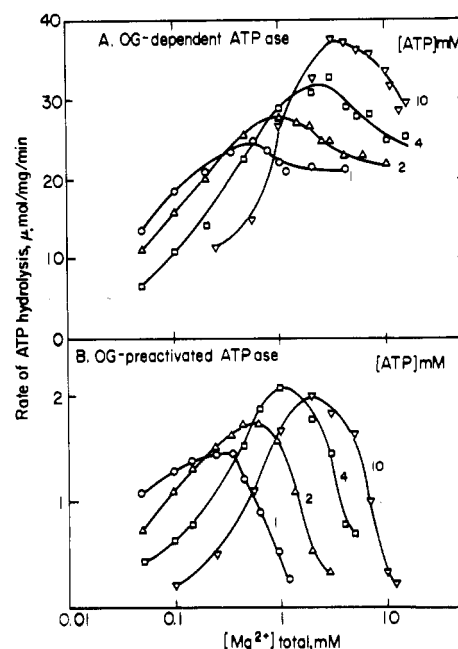


FIGURE 6: Dependence of ATPase activity in OG-activated CF<sub>1</sub> on  $[Mg^{2+}]$ . The initial rate of ATP hydrolysis by spinach CF<sub>1</sub> was measured in the presence of 1, 2, 4, or 10 mM ATP and different concentrations of  $Mg^{2+}$ . (A) 30 mM OG was included in the assay medium. (B) CF<sub>1</sub> was preactivated by preincubation with 30 mM OG, 5 mM DTT, and 5 mM ATP as in Table II, and OG was not included in the assay medium.

presence of 10–15 mM  $\text{Ca}^{2+}$  (in the presence of 4 mM ATP). Low rates of hydrolysis were obtained in the presence of 0.7–1 mM  $\text{Mg}^{2+}$ , whereas 2–3 mM  $\text{Mn}^{2+}$  ions catalyzed much higher rates of ATP hydrolysis. However, excess of either  $\text{Mg}^{2+}$  or  $\text{Mn}^{2+}$  completely inhibited the activity. We have also observed that free  $\text{Mg}^{2+}$  ions strongly inhibited ATP hydrolysis by OG-preactivated CF<sub>1</sub> in the presence of either  $\text{Ca}^{2+}$  or  $\text{Mn}^{2+}$ . A 50% inhibition of ATP hydrolysis was obtained by 100  $\mu\text{M}$  and 1 mM  $\text{Mg}^{2+}$  ions with 8 mM  $\text{Ca}^{2+}$  or with 2 mM  $\text{Mn}^{2+}$ , respectively (with 4 mM ATP, not shown). A similar  $M^{2+}$  ion dependence of ATP hydrolysis by heat-activated CF<sub>1</sub> was described before by Hochmann & Carmeli, (1981). The only notable difference in our studies is the higher optimal  $\text{Mn}^{2+}$  ion concentration for ATPase stimulation. This difference may be due to the source or to the method of preparation of CF<sub>1</sub>. It may be noted also that  $\text{Co}^{2+}$  and  $\text{Zn}^{2+}$  ions were quite effective in catalyzing ATP hydrolysis in the presence of OG (A) but rather ineffective in OG-preactivated (B) and in heat-treated (C) CF<sub>1</sub> preparations.

The biphasic  $[M^{2+}]$  dependence of ATP hydrolysis of activated CF<sub>1</sub> was studied before by Hochmann et al. (1976). It was suggested that this phenomenon is due to a competition by both the free  $M^{2+}$  and the free nucleotide with the substrate M-ATP. Their kinetic analysis of ATP hydrolysis by heat-activated CF<sub>1</sub> at different  $[M^{2+}]$  was consistent with this hypothesis. Figure 6 demonstrates the dependence of the rate of ATP hydrolysis on  $[Mg^{2+}]$  at four different ATP concentrations for OG-dependent ATPase (A) and for OG-preactivated ATPase (B) of CF<sub>1</sub>. In spite of the prominent difference in the absolute rates, the general pattern of biphasic  $[M^{2+}]$  dependence and the shift observed in the optimal  $[Mg^{2+}]$  with increasing ATP concentrations appear to be similar in the two experiments. However, the inhibition by excess of  $\text{Mg}^{2+}$  was notably smaller in the OG-dependent ATPase (A).

A kinetic analysis of the competition between  $\text{Mg}$ -ATP and free ATP was made from the data presented in Figure 6A,

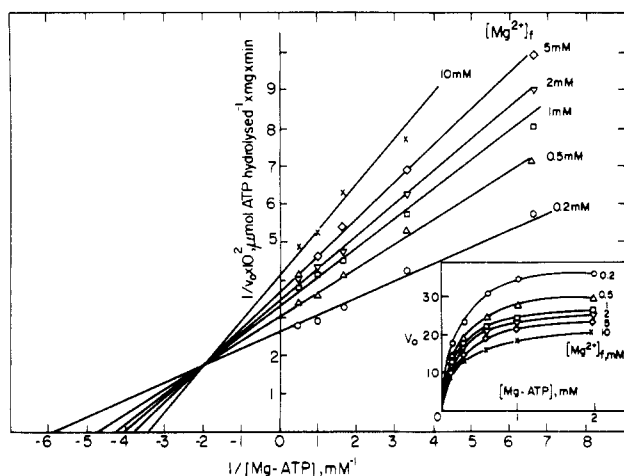


FIGURE 7: Kinetic analysis of  $\text{Mg}^{2+}$  inhibition of  $\text{CF}_1$ -ATPase in the presence of OG. The initial rate of ATP hydrolysis at different calculated  $\text{Mg}$ -ATP concentration and constant free  $\text{Mg}^{2+}$  concentration was measured (inset) and plotted as  $1/v$  vs.  $1/[\text{Mg-ATP}]$ .  $\text{Mg}$ -ATP concentration was calculated by assuming a  $\text{Mg}$ -ATP association constant of 10800 (Walaas, 1958).

the points left of the peaks of activity (excess free ATP, low free  $\text{Mg}^{2+}$ ) being taken. Competition by free  $\text{Mg}^{2+}$  ions was neglected. This assumption is justified only as long as the affinity of the enzyme for inhibition by free  $\text{Mg}^{2+}$  ions is significantly lower than the affinity for the substrate  $\text{Mg}$ -ATP, which is indeed the case in the presence of OG in the assay (Figure 7). The  $1/v$  over  $1/s$  plots of the data suggested a competition between free ATP and  $\text{Mg}$ -ATP (not shown). The apparent inhibition constant of  $K_i(\text{ATP}) = 2.6 \text{ mM}$  is in good agreement with Hochmann & Carmeli, (1981) for heat-activated  $\text{CF}_1$  (3 mM). The apparent  $V$  is  $31 \mu\text{mol}$  of ATP hydrolyzed  $(\text{mg of protein})^{-1} \text{ min}^{-1}$ , and the apparent dissociation constant for the substrate  $K_s(\text{Mg-ATP})$  is  $60 \mu\text{M}$ . This  $K_s$  value is markedly lower than the value calculated for heat-activated  $\text{CF}_1$  that was 2 mM (Hochmann & Carmeli, 1981).

The kinetic analysis of the inhibition of ATP hydrolysis by free  $\text{Mg}^{2+}$  in the presence of OG is shown in Figure 7. In this experiment an excess of free  $\text{Mg}^{2+}$  ions over the substrate ( $\text{Mg}$ -ATP) was kept in order to minimize inhibition by free ATP. The analysis shows that  $\text{Mg}^{2+}$  acts as a partially non-competitive inhibitor with respect to the substrate  $\text{Mg}$ -ATP and that it both lowers  $V$  and increases  $K_s$ . The calculated kinetic parameters are  $K_s(\text{Mg-ATP}) = 150 \mu\text{M}$ ,  $K_{is}(\text{Mg}^{2+}) = 0.9 \text{ mM}$ ,  $K_{ii}(\text{Mg}^{2+}) = 10 \text{ mM}$ , and  $V = 40 \mu\text{mol}$  of ATP  $(\text{mg of protein})^{-1} \text{ min}^{-1}$ . ( $K_{is}$  and  $K_{ii}$  are the inhibitor dissociation constants calculated from the slope and from the  $x$  axis intercept points, respectively). In contrast, the analysis of  $\text{Mg}^{2+}$  inhibition of  $\text{CF}_1$ -preactivated ATPase (Figure 6B) indicates a competition between free  $\text{Mg}^{2+}$  and  $\text{Mg}$ -ATP, with  $K_i(\text{Mg}^{2+}) = 30 \mu\text{M}$  (not shown). These results suggest that the major reasons for the high apparent rates of ATP hydrolysis by  $\text{CF}_1$  in the presence of OG and  $\text{Mg}^{2+}$  are (a) the low affinity for inhibition by free  $\text{Mg}^{2+}$ , (b) the high affinity for the substrate  $\text{Mg}$ -ATP, and (c) the high  $V$ .

## Discussion

The procedures that activate ATP hydrolysis in purified  $\text{CF}_1$  may be divided into two general categories: The first is treatments that irreversibly activate a  $\text{Ca}$ -ATPase such as trypsin activation (Deters et al., 1975), heat (Farron & Racker, 1970), high [DTT] (McCarty & Racker, 1968), and the preincubation with OG, which is described here and before (Pick & Bassilian, 1981). These activation procedures are

irreversible in the sense that ATPase activity is retained in the absence of the "activator". The second is reversible activation procedures that depend on the presence of the effector in the medium. This class includes organic solvents (mainly alcohols) in spinach (Sakurai et al., 1981) and *Chlamydomonas reinhardtii* (Selman-Reimer et al., 1981a,b), tentoxin ( $10^{-4}$ – $10^{-3} \text{ M}$ ) in tentoxin-sensitive strains (Conrad et al., 1981), some lipophilic pH indicators and some short-chain ionic detergents (below their cmc) in *Rhodospirillum rubrum*  $\text{EF}_1$  (Soe et al., 1978), and octyl glucoside. Of these, octyl glucoside, some organic solvents (*tert*-butyl alcohol; Sakurai et al., 1981), the pH indicators, and the ionic detergents in *R. rubrum* enhance  $\text{Mg}$ -ATPase activity and simultaneously inhibit  $\text{Ca}$ -ATPase activity. The most effective activators of this class are octyl glucoside and the organic solvents that activate  $\text{Mg}$ -ATPase of  $\text{CF}_1$  by 30–100-fold (Selman-Reimer et al., 1981a,b; Pick & Bassilian, 1981).

The marked similarity between the irreversible activation of  $\text{Ca}$ -ATPase and the reversible activation of  $\text{Mg}$ -ATPase by OG with respect to the detergent concentration (Pick & Bassilian, 1981) and to the temperature dependence of the activation (not shown) and the close correlation between the effect of ligands on the activation of  $\text{Ca}$ -ATPase and protection against inactivation of OG-dependent  $\text{Mg}$ -ATPase (Table II) suggest that the same OG-binding site is involved in both activations. It may be concluded that OG reversibly binds to  $\text{CF}_1$  and induces a conformational change in the enzyme that may lead to (a) a reversible activation of  $\text{Mg}$ -ATPase activity, (b) an inactivation (in the absence of protecting ligands), or (c) an irreversible activation of  $\text{Ca}$ -ATPase, which becomes apparent only after dilution of the detergent to below the cmc (10–15 mM).

The properties of OG-preactivated  $\text{CF}_1$  appear to be very similar to those of trypsin-activated and to heat-activated  $\text{CF}_1$  preparations with respect to the  $\text{M}^{2+}$  ion specificity (Figure 5), the sensitivity to high-ionic strength (McEvoy & Lynn, 1973; McCarty & Racker, 1966; Posorske & Jagendorf, 1976), and the reversible inhibition by OG (Table II). DTT seems to have an additive effect due to the following: (a) it enhances OG-preactivated  $\text{Ca}$ -ATPase but does not affect the OG-dependent  $\text{Mg}$ -ATPase (Table II); (b) the rate of preactivation of  $\text{Ca}$ -ATPase by OG alone is fast (completed within 3–5 min at  $37^\circ\text{C}$ ), while with OG plus DTT the activation appears to be biphasic and contains also a slow component (Pick & Bassilian, 1981); (c) the inhibition of  $\text{Ca}$ -ATPase activity by the presence of OG after high DTT activation is rather small (Table II) as compared with that of the other  $\text{Ca}$ -ATPase activation procedures. After activation with trypsin (Table II) or with OG in the absence of DTT (Figure 1A) the suppression of  $\text{Ca}$ -ATPase activity is almost complete. Therefore, the effect of DTT on  $\text{CF}_1$  may be different from OG preactivation and from trypsin activation. The observation that preincubation of  $\text{CF}_1$  with DTT and with OG has an additive effect on the activation of  $\text{CF}_1$ -ATPase may explain why the optimal conditions for solubilization of an activated  $\text{CF}_1$ - $\text{CF}_0$ -ATPase complex from chloroplast thylakoid membranes are in the presence of 30 mM OG and 50 mM DTT (Pick & Racker, 1979).

The observation that activation of  $\text{Mg}$ -ATPase was obtained only by detergents that form relatively small micelles and always above the critical micellar concentration (Table I, Figure 3) strongly suggests that the activation is a result of specific interactions with small detergent micelles. The fact that fatty acids with an equivalent chain length, which presumably form micelles of a similar size as those of octyl glu-

coside, are far less effective activators may suggest that OG has a specific effect on the enzyme that may involve a combination of hydrophobic interactions and the formation of hydrogen bonds. Also, fatty acids at high concentrations strongly inhibited the activity (Figure 3) while inhibition by superoptimal OG concentrations was very mild (Figure 1). This may be another reason for the higher activation obtained by OG. The highly cooperative nature of OG activation (Figure 2) is consistent with a cooperative interaction of detergent micelles with multiple binding sites, which induces conformational changes in the enzyme. This conclusion is consistent with our previous observation that OG probably interacts with the  $\alpha$  and  $\beta$  subunits (Pick & Bassilian, 1981), which are present in two or three copies in the enzyme complex.

The activation of ATP hydrolysis in CF<sub>1</sub> by organic solvents (Sakurai et al., 1981; Selman-Reimer et al., 1981a,b) resembles the reversible activation of Mg-ATPase by OG in the specificity for M<sup>2+</sup> ions in ATP hydrolysis (Selman-Reimer et al., 1981b), in the protection against ATPase inactivation by high ionic strength (Sakurai et al., 1981; Figure 4), and in the increased sensitivity to heat inactivation (Selman-Reimer et al., 1981a,b; Pick & Bassilian, 1981). The activation of CF<sub>1</sub>-ATPase by organic solvents appears to be limited to certain types of solvents (alcohols and ketones) that are capable of hydrogen bonding, and it also appears to be related to a relative lipophilic character of the solvent (Sakurai et al., 1981; Selman-Reimer et al., 1981a,b). We tentatively suggest that the activation of CF<sub>1</sub>-Mg-ATPase by octyl glucoside micelles, by organic solvents, and perhaps also the activation by the lipophilic pH indicators and by the ionic detergents in *R. rubrum* EF<sub>1</sub> occur by a common mechanism that involves hydrophobic and hydrophilic (mainly hydrogen bonding) interactions of the effector with a special site in CF<sub>1</sub>. This induces a reversible conformational change that converts the enzyme from a latent or a Ca-ATPase to an active Mg-ATPase.

The kinetic analysis of ATP hydrolysis by activated CF<sub>1</sub> is complex and characterized by a biphasic dependence on M<sup>2+</sup> ion concentration at a constant ATP concentration (Hochmann et al., 1976). This phenomenon was suggested to reflect an inhibition by free ATP or free M<sup>2+</sup> ions, which compete with the substrate, M-ATP, complex (Hochmann et al., 1976; Hochmann & Carmeli, 1981). Their kinetic analysis showed that free Mg<sup>2+</sup> is a potent competitive inhibitor ( $K_i = 20 \mu\text{M}$ ) of the substrate, Mg-ATP ( $K_s = 2 \text{ mM}$ ), and therefore, in spite of the high  $V$  value the apparent activity is low (Hochmann et al., 1976; Hochmann & Carmeli, 1981). In agreement with this conclusion is the strong inhibition of Ca-ATPase activity by Mg<sup>2+</sup> ions in trypsin-, heat-, and OG-preactivated CF<sub>1</sub>. Similarly, Gepstein & Carmeli (1974) attributed the inhibition of Mg-ATPase in trypsin-treated *Chromatium* chromatophores by high MgCl<sub>2</sub> concentrations to competition with the substrate, Mg-ATP, at the active site. In contrast, Edwards & Jackson (1976) demonstrated that the inhibition of Mg-ATPase by Mg<sup>2+</sup> ions in uncoupled *R. rubrum* chromatophores is noncompetitive with respect to the substrate. Similarly, Mal'yan (1981) recently demonstrated a noncompetitive inhibition of Mg-ATPase in soluble CF<sub>1</sub> by free Mg<sup>2+</sup> ions that was dependent on the presence of low ADP concentrations and was relieved by organic solvents. In this work we have shown that in the presence of OG there is a large stimulation of ATP hydrolysis in the presence of Mg<sup>2+</sup> that seems to be due to (a) a marked decrease in the inhibition by free Mg<sup>2+</sup> ions due to a decreased affinity and probably also

a conversion from a competitive to a partially noncompetitive inhibition with respect to the substrate (Figures 6 and 7) and (b) an increase in the affinity for the substrate and in  $V$ . Our calculated  $K_s$  (Mg-ATP) values vary from 60 to 150  $\mu\text{M}$ . The variation in the calculated  $K_s$  and  $V$  values may reflect the fact that inhibition by free Mg<sup>2+</sup> was neglected in the analysis of ATP inhibition and vice versa. However, even within this range of variability, the calculated  $K_s$  is at least 1 order of magnitude lower than the  $K_s$  calculated for heat-activated CF<sub>1</sub> reported by Hochmann & Carmeli (1981). These seem to be the two major reasons for the apparent stimulation of Mg-ATPase activity by OG. A simple kinetic mechanism that is consistent with the data presented here is to assume two different binding sites for Mg-ATP (catalytic site) and for free Mg (regulatory site). The incomplete inhibition by an excess of free Mg<sup>2+</sup> ions would suggest that the quaternary complex Mg-CF<sub>1</sub>-Mg-ATP also hydrolyzes ATP at considerable rates. This model differs from the model of Hochmann et al. (1976), which suggested a competition in the binding between Mg-ATP and free Mg<sup>2+</sup> at the catalytic site, but it is consistent with the results of Edwards & Jackson (1976) and of Mal'yan (1981), which suggested that Mg<sup>2+</sup> acts as an allosteric regulator of CF<sub>1</sub>-ATPase via a binding site that is distinct from the substrate site.

The possible physiological implications of CF<sub>1</sub>-Mg-ATPase activation by OG are still obscure. However, the similarities between light-activated thylakoid-bound CF<sub>1</sub> and OG-activated soluble CF<sub>1</sub> with respect to the M<sup>2+</sup> ion specificity, the low inhibition by free Mg<sup>2+</sup>, and the resistance to high ionic strength all seem to suggest that in the presence of OG micelles, soluble CF<sub>1</sub> resembles the membrane-bound enzyme in its structure and in its catalytic properties. We tentatively suggest that detachment of CF<sub>1</sub> from the membrane exposes a hydrophobic site on the enzyme that is normally blocked in the membrane-bound CF<sub>1</sub>. The exposure of this site induces a conformational change in the enzyme that leads to the changes in the catalytic properties of the enzyme. Upon the binding of OG micelles to this site, CF<sub>1</sub> resumes its native conformation. It could be expected that this site may be involved in the binding of CF<sub>1</sub> to the thylakoid membrane and in the light-triggered activation of ATP hydrolysis in vivo.

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## Purification and Characterization of Spermidine/Spermine $N^1$ -Acetyltransferase from Rat Liver<sup>†</sup>

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**ABSTRACT:** An enzyme catalyzing the acetylation of the polyamines, spermidine or spermine, has been purified 112 000-fold to homogeneity from livers of rats treated with carbon tetrachloride. Major purification steps involved affinity chromatography on *sym*-norspermidine-Sepharose, from which the enzyme was eluted by spermidine, and affinity chromatography on Cibacron blue agarose, from which the enzyme was released by coenzyme A. Similar final specific activities were obtained by using either method as a final purification step, providing strong evidence for homogeneity. The enzyme preparation gave a single band on polyacrylamide gel electrophoresis carried out under native or denaturing conditions. It had an apparent molecular weight of about 115 000 made up of two subunits of 60 000. The acetyltransferase acted on spermidine to form only  $N^1$ -acetylspermidine. Spermine was also a substrate, giving  $N^1$ -acetylspermine, and  $N^1$ -acetylspermine could be acetylated

to form  $N^1,N^{12}$ -diacetylspermine. The  $V_{\max}$  values for spermidine, spermine, and  $N^1$ -acetylspermine were 8, 1.8, and 1.2  $\mu\text{mol of product min}^{-1} \text{mg}^{-1}$ , respectively, when assays were carried out in the presence of saturating concentrations of acetyl-CoA. The  $K_m$  for acetyl-CoA was 1.5  $\mu\text{M}$  and the  $K_m$ s for spermidine, spermine, and  $N^1$ -acetylspermine were 130  $\mu\text{M}$ , 35  $\mu\text{M}$ , and 30  $\mu\text{M}$ , respectively. These results suggest that both spermidine and spermine would be physiological substrates for this enzyme. Coenzyme A was quite strongly inhibitory, having a  $K_i$  of 40  $\mu\text{M}$ , but an even more powerful inhibitor could be produced by reacting coenzyme A with methyl methanethiosulfate to form coenzyme A methyl disulfide. The purified enzyme had no deacetylase activity and the reaction was effectively irreversible. The name spermidine/spermine  $N^1$ -acetyltransferase is suggested for this enzyme, which may play an important role in the interconversion of polyamines.

**T**he degradation of spermine into spermidine and of spermidine into putrescine, which reverses the normal biosynthetic pathway for polyamines (Jänne et al., 1978; Williams-Ashman

& Canellakis, 1979), has now been shown to involve the sequential activity of two enzymes, an acetylase and polyamine oxidase (Pegg et al., 1981; Seiler et al., 1981a). Polyamine oxidase cleaves  $N^1$ -acetylspermine or  $N^1$ -acetylspermidine at the secondary nitrogen to yield 3-acetamidopropanal and spermidine or putrescine, respectively (Hölttä, 1977; Bolkenius & Seiler, 1981). An enzyme catalyzing the acetylation of these polyamines has been found to be induced by treatment with hepatotoxins, which are known to enhance the conversion of

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