Evidence for solvent-induced conformational changes of the soluble *Dunaliella* chloroplast coupling factor 1 (CF_1)

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The ATPase activity of the chloroplast coupling factor 1 (CF_1) isolated from the green alga *Dunaliella* is completely latent. A brief heat treatment irreversibly induces a Ca^{2+}-dependent activity. The Ca^{2+}-dependent ATPase activity can be reversibly inhibited by ethanol, which changes the divalent cation dependency from Ca^{2+} to Mg^{2+}. Both the Ca^{2+}-dependent and Mg^{2+}-dependent ATPase activities of heat-treated *Dunaliella* CF_1 are inhibited by monospecific antisera directed against *Chlamydomonas reinhardi* CF_1. However, when assayed under identical conditions, the Ca^{2+}-dependent ATPase activity is significantly more sensitive to inhibition by the antisera than is the Mg^{2+}-dependent activity. These data are interpreted as indicating that soluble *Dunaliella* CF_1 can exist in a variety of conformations, at least one of which catalyzes a Ca^{2+}-dependent ATPase and two or more of which catalyze an Mg^{2+}-dependent ATPase.

Coupling factor 1 *ATPase* Dunaliella *Antiserum* *Heat-activation*

1. INTRODUCTION

The reversible energy-transducing ATP synthase complex associated with chloroplast thylakoid membranes can be functionally divided into two parts: an integral membrane sector (CF_0), whose function is to conduct protons across the membrane, and an extrinsic membrane sector (CF_1), which contains the active site(s) for ATP synthesis. A number of treatments can be employed to dissociate the CF_1 portion of the complex from the thylakoid membrane, but in most cases the solubilized protein is devoid of catalytic activity [1,2]. The ATPase activity of the isolated protein can usually be markedly enhanced by proteolytic digestion [3], incubation of the enzyme in relatively high concentrations of a low potential reductant [4] or detergent [5], or brief heat treatment [6]. These treatments invariably induce a Ca^{2+}-dependent ATPase activity. An Mg^{2+}-dependent ATPase activity can sometimes be observed by choosing appropriate buffers [7] or the inclusion of either detergents [5] or selected organic solvents [8] directly in the reaction mixture. The effect of solvents on the latent Mg^{2+}-dependent ATPase activity of CF_1 has been shown to be completely reversible [9].

Recently, we described the preparation and properties of a 4-subunit CF_1 isolated from the halotolerant green alga *Dunaliella bardawil* [10]. Similar to higher (vascular) plant coupling factors, the ATPase activity of *Dunaliella* CF_1 was completely latent. The ATPase activity could, however, be expressed either by a short heat treatment, which irreversibly induced a calcium-dependent ATPase, or selected organic solvents (most effectively ethanol), which reversibly induc-

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Abbreviation: CF_1, chloroplast coupling factor 1

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ed a magnesium-dependent ATPase. Although both activities were equally sensitive to most inhibitors tested [11], one notable exception was glycerol. (Glycerol is the osmoticum accumulated by *Dunaliella* when cultured in high concentrations of NaCl [12].) Glycerol, at concentrations up to 25% (v/v), had virtually no effect on the solvent-induced, Mg^{2+}-dependent *Dunaliella* CF_1 ATPase activity, whereas the heat-induced, Ca^{2+}-dependent activity was inhibited 50% by about 7% (v/v) glycerol. From these results and solvent titration curves, we concluded that both the calcium- and magnesium-dependent ATPase activities of *Dunaliella* CF_1 were mutually exclusive, and we suggested that these activities were an expression of different conformational states of the enzyme [10].

Here, we present immunological evidence which supports the suggestion that the Mg^{2+}-dependent and Ca^{2+}-dependent ATPase activities of *Dunaliella* CF_1 indeed represent different conformational states of the enzyme. We also demonstrate that, even though heat-treated *Dunaliella* CF_1 is irreversibly activated as a Ca^{2+}-dependent ATPase, ethanol inhibits the heat-activated, Ca^{2+}-dependent activity but concomitantly induces a reversible Mg^{2+}-dependent ATPase. These results will be discussed in terms of a simple model which attempts to rationalize the changes in metal dependency with changes in enzyme conformation.

2. MATERIALS AND METHODS

The 4-subunit (δ-less) CF_1 was isolated and purified from either *D. bardawil* or *D. salina* and assayed for either Ca^{2+}-dependent or Mg^{2+}-dependent ATPase activity as in [10]. Note that one unit of activity is defined as the amount of ATP hydrolyzed (μmol) per min at 37°C.

Rabbit non-immune sera and monospecific antisera directed against the 4-subunit CF_1 from *Chlamydomonas reinhardi* were generous gifts from Dr Sabeeha Merchant (Department of Biochemistry, University of Wisconsin, Madison, WI). *Dunaliella* strains were generously supplied by Professor Mordhay Avron, Weizmann Institute of Science, Rehovot, Israel. All other reagents were of analytical grade or better.

3. RESULTS

3.1. *The relationship between the Ca^{2+}-dependent and Mg^{2+}-dependent ATPase activities of heat-treated* Dunaliella *CF_1*

Heating a solution of *Dunaliella* CF_1 (0.5–2.5 mg protein/ml) for a short time (3 min) at 60°C in the presence of both ATP (5 mM) and dithiothreitol (5 mM) induces a Ca^{2+}-dependent ATPase activity, as described [10]. This activity, however, is very sensitive to the presence of organic solvents in the reaction mixture and, as summarized in table 1, 50% of the Ca^{2+}-dependent ATPase activity is inhibited by less than 15% (v/v) ethanol. By 20% (v/v) ethanol, the Ca^{2+}-dependent activity is completely inhibited. On the other hand, as the concentration of ethanol in the reaction mixture increases, an Mg^{2+}-dependent ATPase activity of heat-treated *Dunaliella* CF_1 is induced. The induced activity is half maximal at about 15% (v/v) ethanol and maximal at 23% (v/v). Above 23% (v/v) ethanol, the activity of the enzyme rapidly declines, probably because of thermal inactivation at the reaction temperature (not shown). The ethanol titration curve for the induc-

Table 1

The ethanol sensitivity of the calcium and magnesium ATPase activities of heat-treated *Dunaliella* CF_1

% Ethanol (v/v)	ATPase activity (% of maximum)	
	Ca^{2+}	Mg^{2+}
0	100[a]	4
5	100	4
10	100	9
15	33	48
20	7	97
23	0	100[b]

[a] Control rate, 3.5 units/mg protein
[b] Control rate, 12.8 units/mg protein

Dunaliella bardawil CF_1 was isolated and heat-treated as in [10]. Reaction mixtures contained, in 0.1 ml, 40 mM Tricine–NaOH (pH 8.0), 1.0 mM EDTA, 5 mM [γ-^{32}P]ATP, 0.5 μg protein, either 6.0 mM $MgCl_2$ or $CaCl_2$, and the indicated amount of ethanol. Reaction mixtures were incubated at 37°C for 2 min. Thereafter, the reactions were stopped, and the mixtures were analyzed for $^{32}P_i$ as in [9]

tion of an Mg^{2+}-dependent ATPase activity with heat-treated *Dunaliella* CF_1 is virtually identical to the curve obtained for the latent enzyme (not shown). Thus, the heat-treated enzyme either has Ca^{2+}-dependent (in the absence of solvents) or Mg^{2+}-dependent (in the presence of solvents) ATPase activity, but not both.

Table 2 demonstrates that the effect of ethanol on the induction of an Mg^{2+}-dependent ATPase activity with heat-treated *Dunaliella* CF_1 is completely reversible (cf. lines 2 and 3; rows 2 and 3). Removal of ethanol from the enzyme (by dilution) returns the rate of the Mg^{2+}-dependent ATPase back to its basal level, which can again be stimulated by the addition of ethanol to the reaction mixture. In contrast, the Ca^{2+}-dependent ATPase activity of the heat-treated enzyme which is inhibited by ethanol, is completely restored upon the removal of the solvent (cf. lines 2 and 3; row 1). Thus, the effects of solvents on the *Dunaliella* CF_1 are completely reversible, regardless of whether the latent or heat-treated proteins are exposed.

3.2. *Sensitivity of the Ca^{2+}- and Mg^{2+}-dependent ATPase activities of* Dunaliella *CF_1 to antisera directed against CF_1*

Fig.1 shows antiserum titration curves for the inhibition of the ethanol-induced, Mg^{2+}-dependent ATPase activity of the latent CF_1 and the Ca^{2+}-dependent ATPase activity of the heat-treated enzyme. Although both activities of *Dunaliella* CF_1 are inhibited by the anti (*C. reinhardi*) CF_1 antiserum, clearly the Ca^{2+}-dependent ATPase activity of the heat-treated enzyme is more sensitive to the antiserum than is the Mg^{2+}-dependent ATPase activity of the latent enzyme. Similar results have been obtained using several different monospecific antisera directed against the *C. reinhardi* CF_1 (not shown).

Table 2

Contrasting effects of heat and ethanol on the induction of *Dunaliella* CF_1 ATPase activities

Treatment	Units/mg CF_1		
	Ca^{2+}	Mg^{2+}	
		− Ethanol	+ Ethanol (25%)
None	0.3	0.2	18.2
Heat	8.8	0.8	27.0
Heat + ethanol	6.1	0.6	18.6

Dunaliella bardawil CF_1 was isolated, purified and, where indicated, heat-treated as in [10]. Assay conditions were as in table 1. After both heat treatment (60°C for 3 min) and ethanol treatment (37°C for 5 min; 25%, v/v) (line 3) the ethanol was removed by dilution. When present, ethanol was added to a final concentration of 25% (v/v)

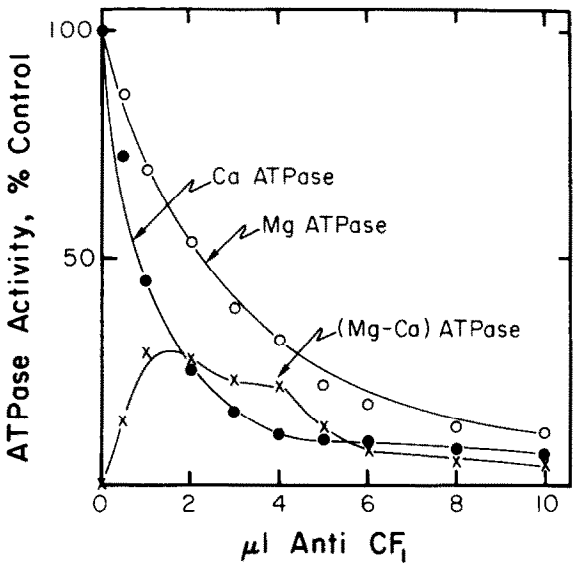

Fig.1. Differential inhibition by an anti (*C. reinhardi*) CF_1 antiserum of the *Dunaliella bardawil* CF_1 Ca^{2+}- and Mg^{2+}-dependent ATPase activities. The *D. bardawil* CF_1 was isolated, purified, and assayed as in [10]. The Ca^{2+}-dependent activity was measured after heat activation of the protein whereas the Mg^{2+}-dependent activity was determined by the addition of ethanol (25%, v/v) to reaction mixtures containing the latent enzyme. Where indicated, addition of antiserum was made prior to the start of the ATPase assay. Assay conditions were as in table 1. Rates have been corrected by the addition of non-immune sera. Control rates were 25.9 and 9.7 units/mg protein for the Mg^{2+}- and Ca^{2+}-dependent activities, respectively.

Although the above results might suggest that the antisera recognize different conformational states of the *Dunaliella* CF_1 as influenced by the presence or absence of ethanol, the reaction conditions used to assay the different metal-dependent activities are quite different, and it might be

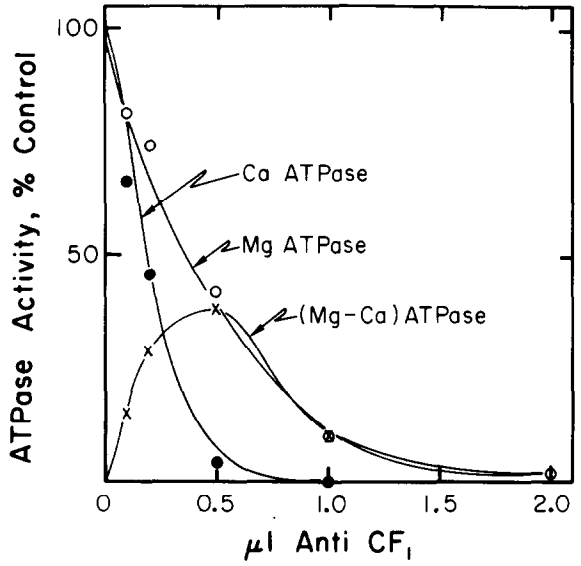

Fig.2. Differential inhibition by an anti (*C. reinhardi*) CF_1 antiserum of the heat-treated *Dunaliella salina* CF_1 Ca^{2+}- and Mg^{2+}-dependent ATPase activities. Assay conditions were as in fig.1 except that prior to both assays the enzyme was neat-treated. Note that the antiserum preparation used for the experiment in fig.2 was not the same as that used for fig.1. Control rates were 31.9 and 2.8 units/mg protein for the Mg^{2+}- and Ca^{2+}-dependent ATPase activities, respectively.

argued that these conditions could influence the interaction between the antigen and antisera. To circumvent this problem, the heat-treated enzyme was assayed under conditions where neither the Ca^{2+}-dependent activity was completely inhibited by ethanol nor the Mg^{2+}-dependent activity fully induced, i.e., in the presence of 15% (v/v) ethanol (see table 1). Thus, the effect of the antisera on the different metal-dependent ATPase activities could be directly compared under identical, albeit suboptimal, reaction conditions. These results are shown in fig.2, and are qualitatively identical to those shown in fig.1; i.e., the Ca^{2+}-dependent ATPase activity is more sensitive to inhibition by the anti (*C. reinhardi*) CF_1 antiserum than is the Mg^{2+}-dependent activity. Similar results have been obtained with other antisera preparations (not shown).

4. DISCUSSION

We have shown that the solvent-induced, Mg^{2+}-dependent ATPase activity of both the latent *C. reinhardi* CF_1 [9] and *D. bardawil* CF_1 [10] is completely reversible. In the case of *C. reinhardi* CF_1, however, we have as yet been unable to induce any substantial Ca^{2+}-dependent ATPase activity. The *D. bardawil* enzyme does have a Ca^{2+}-dependent ATPase activity if it is heated for a short time in the presence of both ATP and dithiothreitol. In this respect, the activation of the Ca^{2+}-dependent ATPase activity of the *Dunaliella* enzyme is similar to the activation of higher (vascular) plant coupling factors. Over a relatively short period of time (several hours), the heat-induced Ca^{2+}-dependent ATPase activity of the *Dunaliella* enzyme is irreversible. The Ca^{2+}-dependent activity is, however, reversibly inhibited by ethanol, the presence of which changes the divalent cation specificity of the enzyme from Ca^{2+} to Mg^{2+}.

The effect of solvents on the activity of CF_1 can be rationalized by changes in the conformation of the enzyme, perhaps due to changes in the hydrophobicity of the solvent as in [8]. A simplistic model for this is shown in fig.3. The addition of solvents to the latent enzyme alters the conformation of the enzyme such that it can hydrolyze Mg-ATP (corresponding to a transition from state I to II). This is a reversible process as has been shown not only for the soluble *C. reinhardi* [9], *D. bar-*

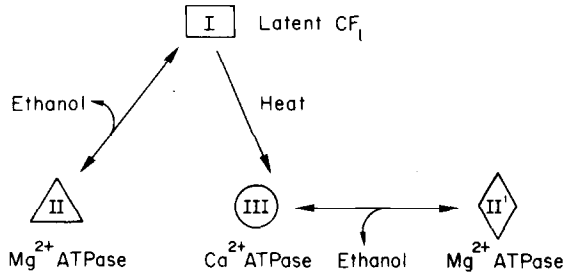

Fig.3. A model for the heat- and solvent-induced *Dunaliella* CF_1 Ca^{2+}- and Mg^{2+}-dependent ATPase activities. I, II, II′, and III represent different conformational states of the *Dunaliella* CF_1. The solvent-induced interconversion between states I and II and III and II′ is reversible whereas the heat-induced conversion of state I to state III is irreversible. In state I, the enzyme is in an inactive conformation whereas states II and II′ represent conformations of the enzyme that have Mg^{2+}-dependent ATPase activity. In state III, the enzyme has Ca^{2+}-dependent activity. For more details, see section 4.

dawil [10] and spinach [8] enzymes, but also for the spinach thylakoid membrane-bound CF_1 [13].

The heat treatment typically employed to activate a Ca^{2+}-dependent ATPase activity with other coupling factors undoubtedly alters the conformation of the enzyme (see, e.g. [14]) (which corresponds to the irreversible transition from state I to III, fig.3). It is not clear what is occurring at the molecular level during the heat activation of the enzyme although it has been suggested that a rearrangement of disulfide bonds may be taking place [10,15]. Nevertheless, this conformational state of the enzyme can also be perturbed by the addition of solvents, the result being a change in the divalent metal cation specificity (corresponding to a transition from state III to II′, fig.3).

We have unsuccessfully attempted to detect reversible, solvent-induced, conformational changes in CF_1 by measuring intrinsic fluorescence depolarization and the reactivity of CF_1 to a variety of covalent chemical modifiers. In many other studies, antisera directed against specific protein conformations have been used to detect protein conformational changes [16], and the results shown in fig.1 and confirmed in fig.2 strongly suggest that the various monospecific antisera directed against *C. reinhardi* CF_1 employed here do indeed detect solvent-induced conformational changes in *Dunaliella* CF_1.

An interesting question is the relationship between the conformations of CF_1 corresponding to states II and II′ (fig.3). Further work will be necessary to clarify this point.

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