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CONFORMATION AND ACTIVITY OF CHLOROPLAST COUPLING FACTOR EXPOSED TO LOW CHEMICAL POTENTIAL OF WATER IN CELLS

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Summary

(1) Photophosphorylation, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities of isolated chloroplasts were inhibited 55—65% when the chemical potential of water was decreased by dehydrating leaves to water potentials (Ψ_w) of −25 bars before isolation of the plastids. The inhibition could be reversed in vivo by rehydrating the leaves.

(2) These losses in activity were reflected in coupling factor (CF_1) isolated from the leaves, since CF_1 from leaves with low Ψ_w had less Ca^{2+}-ATPase activity than control CF_1 and did not recouple phosphorylation in CF_1-deficient chloroplasts. In contrast, CF_1 from leaves having high Ψ_w only partially recoupled phosphorylation by CF_1-deficient chloroplasts from leaves having low Ψ_w. This indicated that low Ψ_w affected chloroplast membranes as well as CF_1 itself.

(3) Coupling factor from leaves having low Ψ_w had the same number of subunits, and the same electrophoretic mobility, and could be obtained with the same yields as CF_1 from control leaves. However, direct measurements of fluorescence polarization, ultraviolet absorption, and circular dichroism showed that

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Abbreviations: Ψ_w, water potential CF_1, coupling factor protein (ATP synthetase when attached to the membrane); ϵ-ADP, 1,N^6-ethenoadenosine diphosphate; ϵ-ATP, 1,N^6-ethenoadenosine triphosphate; EDTA, ethylenediaminetetraacetic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine.

CF_1 from leaves having low Ψ_w differed from control CF_1. The CF_1 from leaves having low Ψ_w also had decreased ability to bind fluorescent nucleotides (ϵ-ATP and ϵ-ADP).

(4) Exposure of isolated CF_1 to low Ψ_w in vitro by preincubation in sucrose-containing media inhibited the Ca^{2+}-ATPase activity of the protein in subsequent assays without sucrose. Inclusion of 5 or 10 mM Mg^{2+} in the preincubation medium markedly inhibited Ca^{2+}-ATPase activity.

(5) These results show that CF_1 undergoes changes in cells which alter its phosphorylating ability. Since low cell Ψ_w changed the spectroscopic properties but not other protein properties of CF_1, the changes were most likely caused by altered conformation of the protein. This decreased the binding of nucleotides and, in turn, photophosphorylation. The inhibition of ATPase activity in CF_1 in vitro at low Ψ_w and high ion concentration mimicked the change in activity seen in vivo.

Introduction

The chemical potential of water (hereafter described in terms of the water potential, Ψ_w) * decreases when cell water content decreases [1]. In the chloroplast-containing cells of plants, the decrease is associated with large losses in chloroplast activities, notably electron transport and photophosphorylation [2—5]. The losses occur at Ψ_w that are commonly encountered under natural conditions [2,3] and may account for some of the decrease in rates of photosynthesis observed at low Ψ_w [2,5]. Fellows and Boyer [4] presented evidence that the inhibition was correlated with changes in the thickness of the lamellar membranes of the thylakoids in energized chloroplasts. The changes in thickness could be seen in vivo and persisted in vitro. However, the nature of the membrane structure that accounts for the changes in thickness has not been identified.

In this paper, we present evidence that the molecular basis of these changes is attributable in part to changes in the conformation of chloroplast coupling factor (CF_1). Structural transformations of CF_1 have been demonstrated by the ability of the protein to trap tritiated water [6,7] and to bind *N*-ethylmaleimide [8,9], adenine nucleotides [10—12], and trinitrobenzenesulfonate [13] when the thylakoid membranes are energized in vitro. With the demonstration that CF_1-bound nucleotides undergo a light-dependent and uncoupler-sensitive exchange with free nucleotides [11,14], it has been proposed that the structural transformations are conformational changes in CF_1 that induce changes in the binding affinities for ADP, P_i, and ATP during photophosphorylation [15,16]. Results in the present work indicate that changes in CF_1 conformation also occur within cells, persist after isolation, and lead to altered

* The water potential is related to the chemical potential (μ, ergs · mol^{-1}) of water according to $\Psi_w = (\mu_w - \mu_0)/\overline{V}$, where the subscripts w and 0 refer to the system and the reference, respectively, and $\overline{V}$ is the partial molal volume of liquid water ($cm^3 \cdot mol^{-1}$, virtually a constant over the range of Ψ_w in biological systems). For convenience, we report Ψ_w in pressure units, 1 bar = 10^6 ergs · cm^{-3} = 0.987 atm.

affinities for nucleotides that may account for alterations in phosphorylating activity.

Materials and Methods

Plant material and Ψ_w. Commercial spinach (*Spinacea oleracea* L.) was purchased from a local market. Different leaf Ψ_w were obtained by partial drying of the leaves for 1–3 h in the light in a controlled environment room (temperature = 29°C; relative humidity = 40—60%; irradiance = 150 $W \cdot m^{-2}$ from fluorescent (daylight) bulbs). Leaf Ψ_w was measured with a thermocouple psychrometer by the isopiestic technique described by Boyer and Knipling [17].

Measurement of chloroplast activities. Chloroplasts were isolated from the leaves as described by Lien and Racker [18]. Chloroplast Ca^{2+}-ATPase was activated by heat at 60–64°C for 4 min in the presence of ATP (20 mM) and dithiothreitol (5 mM), and chloroplast Mg^{2+}-ATPase was activated by incubation with dithiothreitol (25 mM) for 15 min. Activities of ATPase were measured as described by Lien and Racker [18] for Ca^{2+}-dependent ATPase, and by replacing Ca^{2+} with 5 mM $MgCl_2$ for Mg^{2+}-dependent ATPase. Phenazine methosulphate-mediated cyclic photophosphorylation was measured potentiometrically with a recording pH meter as described by Dilley [19]. Illumination was with saturating (180 $W \cdot m^{-2}$ between 400 and 700 nm) red light (maximum irradiance at 668 nm), passed through a heat filter [5].

Coupling factor was prepared by extraction with chloroform according to Younis et al. [20]. The protein was stored at 4°C in 2 M $(NH_4)_2SO_4$, 2 mM ATP, 1 mM EDTA, and 20 mM Tricine/OH^-, pH 8, until needed for measurements. Prior to use, the suspension was centrifuged (14 000 × *g*), resuspended in 40 mM Tricine/OH^-, pH 8, recentrifuged (14 000 × *g*), and desalted on a Sephadex G-50 column (20 × 1 cm). This procedure provided a protein of high specific activity relatively free of other proteins (Ref. 20, and see Fig. 3 below). The Ca^{2+}-ATPase of the protein was heat activated at 60–64°C for 4 min and assayed as for the chloroplasts.

Chlorophyll was determined spectrophotometrically in 80% acetone extracts [21]. Protein concentration was measured usually by the method of Lowry et al. [22] and sometimes spectrophotometrically [23]. In the former method, the concentration was multiplied by 1.15 and, in the latter, by a factor of 1.85 to express the protein concentration on a dry weight protein basis [24].

For preparation of CF_1-depleted chloroplasts, the chloroplasts were treated with EDTA as described by McCarty [25]. Phosphorylation was reconstituted in these chloroplasts by adding CF_1, and phenazine methosulfate-mediated cyclic photophosphorylation was assayed as described in Table II.

Polyacrylamide gel electrophoresis of the protein was performed as described by Weber and Osborn [26].

Fluorescence measurements. Fluorescence polarization of CF_1 was measured with a photon-counting polarization photometer in the laboratory of G. Weber. This instrument (see Weber and Bablouzian [27] and Jameson et al. [28]) allows simultaneous monitoring of the parallel and perpendicular components of the polarized fluorescence by using two photomultipliers at right angles to

the exciting beam. It also permits convenient subtraction of background scattering at low signal-to-noise values. The excitation wavelength was 280 nm and the emitted fluorescence was filtered through Corning * glass C.S. 0-54 filters.

The binding of fluorescent nucleotides (ϵ-ADP and ϵ-ATP) to CF_1 was followed by measuring the polarization of the average free and bound ϵ-nucleotide fluorescence as a function of ϵ-nucleotide concentration [29]. The excitation wavelength was 310 nm (passed through a Corning glass C.S. 7--54 filter) and the emission at 405 nm (filtered through a Corning glass C.S. 3-75 filter) was measured.

Circular dichroism measurements. The circular dichroism spectrum of CF_1 in 20 mM Tricine/OH^-, pH 8.0, was measured between 200 and 250 nm with a JASCO model J40A Automatic Recording Spectropolarimeter. The spectropolarimeter was calibrated with D-(+)-camphorsulfonic acid; the pathlength was 1 mm; the protein concentration was 0.5 mg · ml^{-1}, and the temperature was 25°C. The mean residue molecular weight was taken to be 114.

Ultraviolet spectra. Ultraviolet absorption spectra of the protein were measured in an Aminco spectrophotometer DW-2.

Results

The photophosphorylating activity of spinach chloroplasts decreased with decreasing leaf Ψ_w (Fig. 1). In chloroplasts from leaves with a Ψ_w of about --25 bars, the rate of cyclic photophosphorylation was 45% of that in the control chloroplasts. A similar result was found with sunflower leaves, in which photophosphorylation was markedly inhibited and uncoupled from electron flow at low Ψ_w [3]. To determine whether the effect involved the phosphorylating enzyme CF_1 or other aspects of phosphorylation, we assayed for Ca^{2+}-dependent and Mg^{2+}-dependent ATPase activity of the chloroplasts. There was a linear decrease in the Ca^{2+}-ATPase activity of the chloroplasts as leaf Ψ_w decreased (47% of control at Ψ_w of --25 bars, Fig. 2, solid circles). The Mg^{2+}-dependent ATPase activity also decreased with decreasing Ψ_w (Fig. 2, open squares). The Ca^{2+}-ATPase activity recovered fully if the leaves were rehydrated before the chloroplasts were isolated (Fig. 2, open circle).

The above results suggest that some of the effects of low Ψ_w on photophosphorylation were caused by an effect on CF_1 itself. Therefore, we studied CF_1 from leaves with various Ψ_w. Table I shows that CF_1 isolated from chloroplasts of leaves having two different Ψ_w yielded the same amount of CF_1 protein but had different Ca^{2+}-ATPase activities. Activity of the protein from leaves with low Ψ_w was much lower (23% of control) than for protein from leaves with high Ψ_w when the ATPase was not heat activated (Table I). However, the relative difference in activity was reduced after heat activation (Table I). Since the protein was probably closest to the in vivo form before heat activation, we

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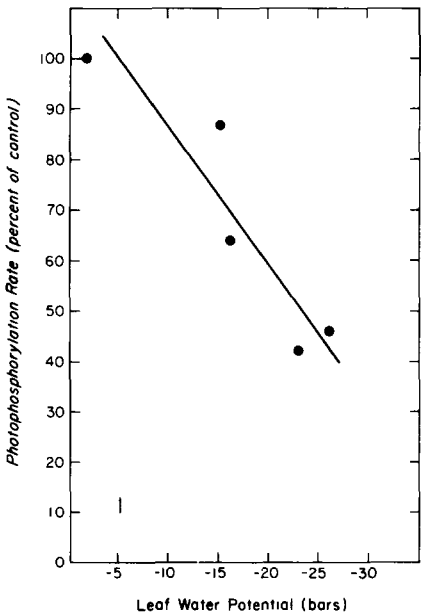

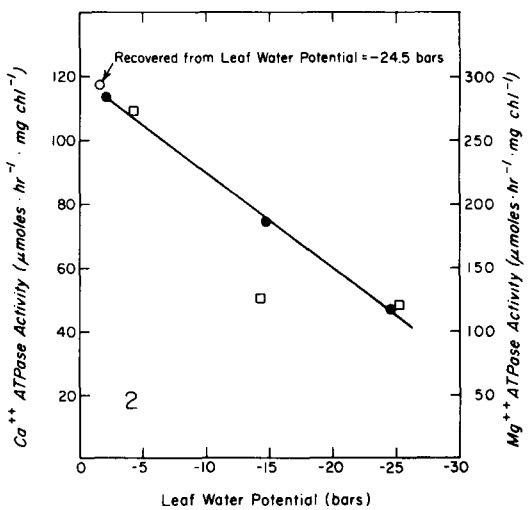

Fig. 1. Photophosphorylating activity of chloroplasts isolated from spinach (*S. oleracea* L.) leaves with various water potentials. Control chloroplasts were isolated from leaves of the same population without desiccation. The control rate was 1060 μmol ATP · h^{-1} · mg^{-1} Chl, phenazinemethosulfate concentration was 15 μM and chlorophyll concentration was 20 μg · ml^{-1}.

Fig. 2. The Ca^{2+}-ATPase (•) and Mg^{2+}-ATPase (□) activities of chloroplasts isolated from spinach leaves with various water potentials. Mg^{2+}-ATPase was activated with dithiothreitol. Recovery from low water potential (○) shows the activity of Ca^{2+}-ATPase after rehydration of the tissue from a water potential of −24.5 bars.

used CF_1 that had not been heat activated during the following measurements, except where stated.

Coupling activity of the isolated proteins

Table II summarizes the results of an experiment in which chloroplasts from leaves with high and low Ψ_w were depleted of CF_1 by EDTA treatment, then

TABLE I

YIELD AND Ca^{2+}-ATPase ACTIVITY OF COUPLING FACTOR ISOLATED FROM CHLOROPLASTS OF LEAVES WITH HIGH (CONTROL) AND LOW WATER POTENTIALS

Experimental conditions as in Material and Methods.

Treatment	Leaf water potential (bars)	Total chlorophyll (mg · extract^{-1})	Total protein (mg · extract^{-1})	Protein released/ chlorophyll (mg · mg^{-1})	Ca^{2+}-ATPase activity (μmol P_i · min^{-1} · mg^{-1} protein)	
					Before heat activation	After heat activation
Control	−3	15.8	8.8	0.55	2	14
Water deficient	−17	15.2	8.9	0.58	0.46	11

TABLE II

RECONSTITUTION OF PHOTOPHOSPHORYLATION IN COUPLING FACTOR-DEFICIENT CHLOROPLASTS BY COUPLING FACTOR FROM LEAVES WITH HIGH (CONTROL) AND LOW WATER POTENTIALS

Phosphorylation was reconstituted by combining the EDTA-treated chloroplasts with 1 ml of their supernatant (containing CF_1) to give a chlorophyll concentration of 0.2 mg/ml. $MgCl_2$ (10 mM) was added after the EDTA-treated chloroplasts had been added and the mixture was incubated for 15 min before assay at room temperature. At the same time, aliquots of the untreated chloroplasts were diluted to the chlorophyll concentration of the treated samples. Phosphorylating activities were measured with 10 μg chlorophyll/ml in a 2 ml reaction mixture containing: 3 mM NaH_2PO_4, 3 mM $MgCl_2$, 17 mM KCl, 1.5 mM ADP and 50 μM phenazinemethosulfate, pH 7.8. Protein concentration in both supernatants was 0.14 mg/ml as determined by the method of Lowry et al. [22].

Treatment	Cyclic photophosphorylation (μmol ATP · h^{-1} · ma^{-1} Chl)
1. Untreated chloroplasts from leaves with a Ψ_w of	
a. −1 bar (controls)	1060
b. −18 bars	440
2. EDTA-treated chloroplasts from leaves with a Ψ_w of	
a. −1 bar (controls)	460
b. −18 bars	160
3. EDTA-treated chloroplasts from leaves with a Ψ_w of −1 bar (controls) plus	
a. CF_1 from leaves with Ψ_w of −1 bar	700
b. CF_1 from leaves with Ψ_w of −18 bars	400
4. EDTA-treated chloroplasts from leaves with Ψ_w of −18 bars plus	
a. CF_1 from leaves with Ψ_w of −1 bar	220
b. CF_1 from leaves with Ψ_w of −18 bars	170

reconstituted by adding back the CF_1-containing supernatant. The CF_1 released from leaves with low Ψ_w did not restore phosphorylation in CF_1-depleted chloroplasts from control leaves (Table II, cf. lines 2a, 3a and b). This result indicates a specific effect of low Ψ_w on CF_1. However, control CF_1 restored phosphorylation only slightly in CF_1-depleted chloroplasts from leaves with low Ψ_w (Table II, cf. lines 2b, 4a and 3a). Therefore, in addition, to a specific effect on CF_1, low Ψ_w also altered the membrane.

Electrophoretic mobility and subunit structure

Coupling factor placed on polyacrylamide gels exhibited a single band with a mobility unaltered by Ψ_w (R_F of 0.43 for CF_1 from leaves with Ψ_w of −2 and −18 bars). When detergent was added to the gels to separate the protein subunits, five subunits were always present (Fig. 3), each with an R_F unaffected by Ψ_w.

Polarization of CF_1 fluorescence

The lifetime of the excited state of fluorescence in molecules is on the order of 10^{-8} s [30,31]. Because the relaxation times for the rotation of fluorescent macromolecules are of the same order of magnitude, their emitted fluorescence is partially polarized, and the degree of polarization affords a convenient means of determining their relaxation time under a variety of circumstances [30,32]. In identical media, and with the assumption that the lifetime of the excited

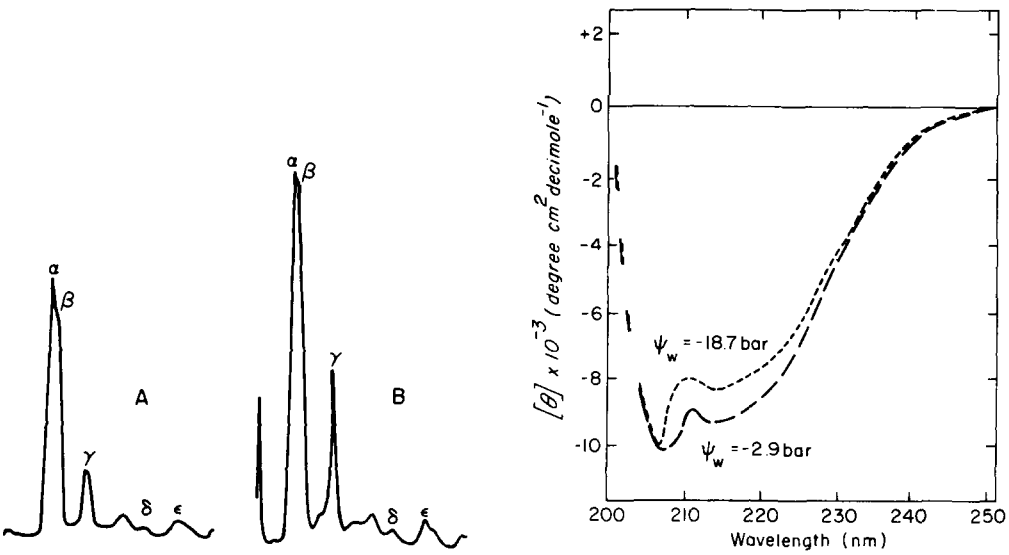

Fig. 3. Densitometric scans along polyacrylamide gels showing the subunits of (a) CF_1 isolated from spinach leaves with a water potential of −2 bars and (B) CF_1 isolated from spinach leaves with a water potential of −18 bars. The protein subunits ($\alpha - \epsilon$) were separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Gels were loaded with 50 μg of protein (A) or 70 μg of protein (B) in 40 mM Tricine/OH^-, pH 8, buffer.

Fig. 4. Circular dichroism spectra of coupling factor isolated from spinach leaves with water potentials of −2.9 bars or −18.7 bars. The pathlength was 1 mm, the protein concentration was 0.5 mg · ml^{-1}, and the buffer was 20 mM Tricine/OH^-, pH 8.0.

state is constant, differences in polarization of a particular substance are caused by differences in conformation. Table III shows that the degree of polarization of fluorescence in CF_1 from leaves with low Ψ_w (−16 bars) was less by about 0.036 (±0.003) than in CF_1 from the control leaves (−1 bar).

Circular dichroism spectra

In the ultraviolet region between 200 and 250 nm, circular dichroism spectra are uniquely determined by protein conformation. Although a number of properties of the protein contribute to the spectra in this region, the quantity

TABLE III

FLUORESCENCE POLARIZATION OF COUPLING FACTOR PROTEIN ISOLATED FROM SPINACH LEAVES WITH DIFFERENT WATER POTENTIALS

The protein was desalted on a Sephadex G-50 (20 × 1 cm) column. The buffer was 40 mM tricine/OH, pH 8. Protein in the same buffer at a concentration of 0.42 mg/ml was used for measuring the polarization of fluorescence as described in Materials and Methods. Degree of polarization is $F_\parallel - F_\perp / F_\parallel + F_\perp$, where $F_\parallel$ and $F_\perp$ are the fluorescence intensity parallel and perpendicular, respectively, to the exciting radiation. Instrument fluctuations introduce error of ±0.003 in polarization values.

Water potential of leaves (bars)	Degree of polarization of fluorescence by isolated coupling factor
−1	0.306
−16	0.270

of α-helix, the orientation of secondary (β) structure, and the arrangement of 'randomly' coiled regions of the protein are particularly contributory. For CF_1 from leaves having Ψ_w of −2.9 and −18.7 bars, the molecular ellipticity (θ) differed in the same buffer and at the same protein concentration (Fig. 4). Both proteins exhibited a similar extremum around 208 nm, which indicates that both contained a similar amount of α-helix (21% as calculated from Greenfield and Fasman [33]). However, CF_1 from leaves with Ψ_w of −18.7 bars had a smaller molecular ellipticity between 208 and 230 nm than CF_1 from control leaves, which suggests that β structure and randomly coiled regions had been altered by treatment at low Ψ_w [33].

Ultraviolet absorption spectra

The CF_1 isolated from leaves with Ψ_w of −17 bars absorbed less in the ultraviolet region of the spectrum than did CF_1 from leaves with high Ψ_w (Fig. 5). The differences in the spectra peaked at 230 and 271 nm (Fig. 5, inset). Because these differences occurred in proteins having the same number of subunits each having essentially the same mobility on polyacrylamide gels, the amino acid composition of CF_1 was unlikely to have been substantially altered by Ψ_w treatment. Therefore, the differences in spectra were probably caused

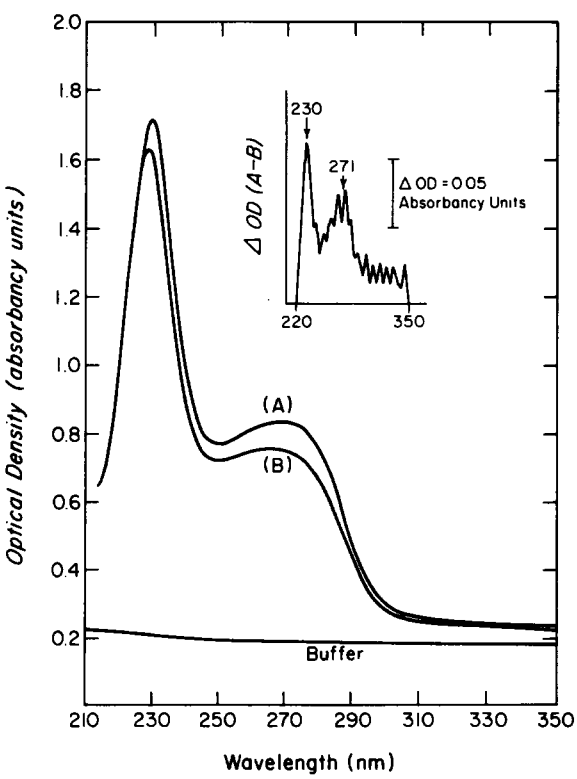

Fig. 5. Ultraviolet absorption spectra of coupling factor from spinach leaves with a water potential of (A) −2 bars and (B) −17 bars. Inset: The spectral difference between (A) and (B). The lowest trace in the main figure is for the buffer. The protein concentration was 1.2 mg/ml in 40 mM Tricine/OH^-, pH 8.

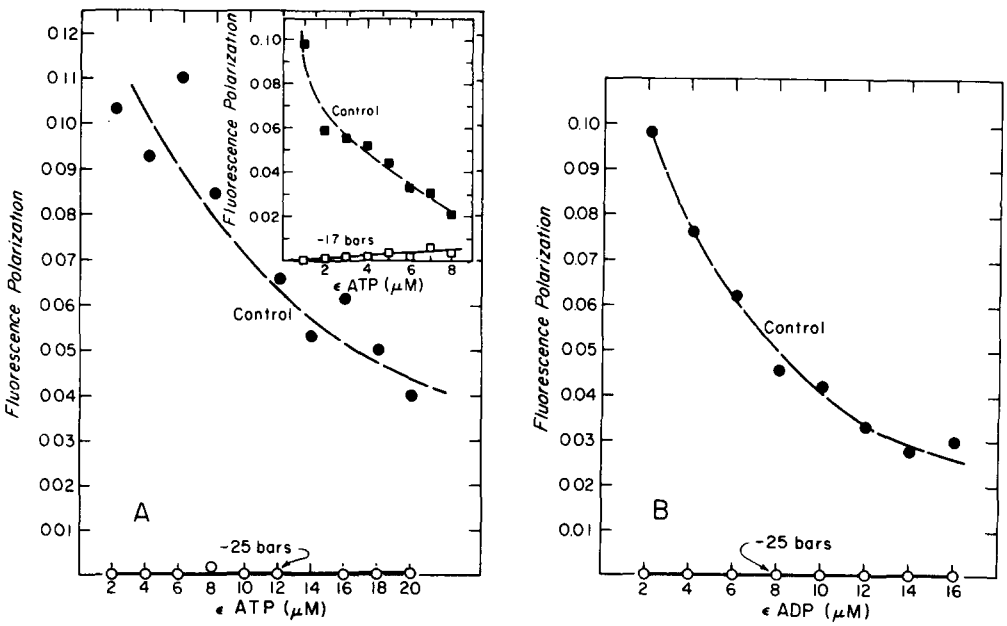

Fig. 6. Polarization of ϵ-ATP and ϵ-ADP fluorescence by coupling factor at various ϵ-ATP and ϵ-ADP concentrations. (A) ϵ-ATP plus coupling factor protein from control leaves with water potential of —2 bars (●) or —25 bars (○). Inset: ϵ-ATP plus coupling factor (a separate preparation) from control leaves with water potential of —2 bars (■) or —17 bars (□). (B) ϵ-ADP plus coupling factor from leaves with water potential of —2 bars (control) (●) or —25 bars (○). The medium contained 1.4 μM protein and 40 mM Tricine/OH^-, pH 8.

TABLE IV

EFFECT OF WATER POTENTIAL AND Mg^{2+} ON HEAT-ACTIVATED Ca^{2+}-ATPase ACTIVITY OF COUPLING FACTOR

The protein from leaves with a high Ψ_w was incubated for 30 min before the assay at room temperature (A) before heat activation or (B) after heat activation. In preparation for incubation, a sample of ammonium sulfate suspension of CF_1 was centrifuged and the pellet was dissolved in 40 mM Tricine/OH^-, pH 8, and recentrifuged again at 14 000 × *g* for 10 min. The clear supernatant was desalted on a 20 × 1 cm Sephadex G-50 column. Sucrose was added as solid sucrose and $MgCl_2$ was added from a 1 M stock solution. After the incubation, Ca^{2+}-ATPase activity was determined after addition of 20 μl of the incubation mixture to 1 ml of the assay medium.

Treatment (component added to protein in 40 mM Tricine/OH^-, pH 8)	Ca^{2+}-ATPase activity (μmol P_i · mg^{-1} protein · min^{-1})	
	(A) Treatment before heat activation	(B) Treatment after heat activation activation
None	20	
0.8 M sucrose	28	14
5 mM $MgCl_2$	21	7
0.8 M sucrose + 5 mM $MgCl_2$	13	13
10 mM $MgCl_2$	13	5
0.8 M sucrose + 10 mM $MgCl_2$	—	3

by a difference in orientation of ultraviolet-absorbing groups on, or light scattering by, the proteins.

Binding of ϵ-ADP and ϵ-ATP

Both ϵ-ADP and ϵ-ATP are fluorescent and bind to CF_1 [29,34]. They can substitute to a considerable degree for ADP and ATP in many reactions in chloroplasts and isolated CF_1 [29,34–36]. We tested the ability of CF_1 from leaves with low Ψ_w to bind ϵ-ATP and ϵ-ADP by the technique of fluorescence polarization. The fluorescence of these analogs in aqueous solutions, as with many small fluorescent molecules, is not polarized because their rotational relaxation times are considerably shorter than their excited lifetimes (approx. 20 ns). The relaxation time is increased by placing the fluorescent molecule in a viscous medium or by attaching the molecule to a macromolecule such as a protein [30,32,37]. As a result, the fluorescence of the adsorbed molecule is partially polarized. Therefore, in any mixture of small fluorescent molecules with a protein, the fraction of the total fluorescence that is polarized will be proportional to the fraction of fluorescent molecules bound to the protein. Under otherwise constant conditions, differences in the binding properties of proteins saturated with the fluorescent molecule represent differences in relaxation times attributable to differences in protein conformation.

In the case of ϵ-ADP and ϵ-ATP, differences in binding properties of CF_1 should reflect differences around the active or regulatory sites for photophosphorylation. Fig. 6 shows the saturation binding of ϵ-ATP and ϵ-ADP to CF_1 from leaves having various Ψ_w in the same buffer and at the same protein concentration. Unlike CF_1 from leaves with a Ψ_w of −2 bars, which bound large amounts of ϵ-ATP (Fig. 6A), CF_1 from leaves with a Ψ_w of −17 bars bound less ϵ-ATP (Fig. 6A, inset). The dramatic decrease in fluorescence polarization could not be attributed to fluorescence quenching by CF_1, since the total fluorescence of the control and treated CF_1 was the same. At Ψ_w of −25 bars, CF_1 appeared unable to bind significant ϵ-ATP or ϵ-ADP (Fig. 6).

Effects of Mg^{2+} and low Ψ_w in vitro on ATPase activity of isolated CF_1

During water loss from cells, the concentration of ions around CF_1 should increase (at a Ψ_w of −25 bars, the water content of the tissue had decreased to about 1/3 that in the controls). To test whether prior incubation of CF_1 in high concentrations of ions could alter the subsequent biological activity of the protein, we preincubated CF_1 in 5 mM or 10 mM Mg^{2+}. Preincubation in 5 mM Mg^{2+} inhibited the subsequent Ca^{2+}-ATPase activity of CF_1 only if the CF_1 had been heat activated (Table IV). At 10 mM, Ca^{2+}-ATPase activity was inhibited regardless of the timing of preincubation in relation to heat activation (Table IV).

The direct effect on CF_1 of a decrease in the chemical potential of water was tested by preincubating CF_1 in 0.8 M sucrose (Ψ_w = −25 bars). Ca^{2+}-ATPase activity was enhanced if preincubation occurred before heat activation of CF_1 but it was inhibited by preincubation after heat activation (Table IV).

The incubation of CF_1 in 10 mM Mg^{2+} plus 0.8 M sucrose was particularly inhibitory to the Ca^{2+}-ATPase activity of the protein (Table IV). Because the

assays measured Ca^{2+} ATPase activity after the incubation, neither sucrose nor Mg^{2+} was present in significant quantity during assay.

Discussion

These results show that the inhibition of photophosphorylation and ATPase activities of chloroplasts isolated from leaves with low Ψ_w is associated with a change in the spectroscopic properties of coupling factor. Evidence for such a change includes differences in circular dichroism (Fig. 4), polarization of protein fluorescence (Table III), ultraviolet absorption (Fig. 5), and ability to bind nucleotides (Fig. 6). However, there were no significant differences in the yield of protein extractable from the chloroplasts (Table I) or in the number of subunits or the mobility of the protein and its subunits on polyacrylamide gels (Fig. 3). Therefore, the primary structure and molecular weight of the protein were unlikely to have been altered by low Ψ_w, although we cannot exclude the possibility of changes in CF_1 binding of other molecules too small to be detected by our gel techniques. With this possible exception, we therefore attribute the spectroscopic differences to changes in the conformation of CF_1.

Evidence for conformational changes in CF_1 has been presented previously for thylakoids energized either by light [6,7], acid-base transitions in the dark [7,13,38], or external voltage pulses [39]. These findings imply that such energy-dependent alterations are required in some way for photophosphorylation. In the present work, CF_1 was exposed in vivo to low Ψ_w, and the effects on photophosphorylation and conformation persisted after isolation of the protein. Apparently, the conformation of CF_1 changed in the cells, and the altered conformation was quite stable. Because the effects of photophosphorylation were reversed by rehydration of the cells before isolation of CF_1, the altered conformation, although stable, must have been inherently reversible under appropriate conditions in the cells. A similar phenomenon has been seen in chloroplast electron transport; the effect of low Ψ_w was reversed only if cells were rehydrated before isolation of the plastids [5,40,41].

The recoupling experiments (Table II) provided a critical confirmation of the effects of low Ψ_w on CF_1. In these experiments, the protein was unable to restore cyclic photophosphorylation under conditions that readily supported restoration by control CF_1. The failure of CF_1 from low Ψ_w to restore phosphorylation must have been caused by altered biological activity, decreased binding affinity for chloroplast membranes, or both. Clearly, the effect persisted after removal of the protein from the membranes, as had been indicated by the spectroscopic measurements.

It is important that control CF_1 was only slightly able to recouple CF_1-deficient chloroplasts from leaves with low Ψ_w. Therefore, although low Ψ_w caused a specific effect on CF_1, it also altered the chloroplast membranes. Thus, low Ψ_w had multiple effects on chloroplasts, as has been suggested previously by alterations in electron transport and non-cyclic photophosphorylation in chloroplasts from leaves having low Ψ_w [3]. The results (Table II) also add more evidence to what has been discussed by others [42,43] that CF_1 added to CF_1-deficient chloroplasts has coupling activity in addition to its structural role on the membrane.

The most obvious and significant change in CF_1 from leaves with a low Ψ_w was the decrease in binding of ϵ-ADP and ϵ-ATP (Fig. 6). It is generally accepted that ATP synthesis involves the binding of ADP and P_i to CF_1 and the release of ATP into the medium. Consequently, the marked losses in binding could have contributed to losses in phosphorylating activity at low Ψ_w (Fig. 1). Since concomitant changes in protein conformation also occurred at low Ψ_w, decreased nucleotide binding may have been caused by physical changes in or around the nucleotide binding sites. It should be noted, however, that residual phosphorylation and ATPase activity remained at Ψ_w that markedly reduced binding of fluorescent nucleotides (cf. Figs. 1 and 2 with Fig. 6). The analogs probably bind with less affinity than the normal substrates because of the altered structure of the analogs. In support of this idea, Shahak et al. [35] showed that CF_1 phosphorylates ϵ-ADP vigorously but the Michaelis constant is about twice that for ADP.

The persistence of changes in CF_1 after its isolation from cells implies that any attempt to reproduce the effect in vitro must involve a pretreatment of CF_1 but assay under uniform conditions to detect effects of the pretreatment. Lowering the water potential of cells increases the concentration of ions in the cytoplasm but also decreases the activity of water molecules. Both effects could bring about conformational changes in CF_1. The losses in activity of CF_1 after preincubation in high Mg^{2+} and/or low water activity (Table IV) suggest that CF_1 was altered to a different reactive state (Ref. 44 and Younis, H.M., Boyer, J.S. and Govindjee, unpublished results). This new state was stable enough to be subsequently detected by a change in ATPase activity even though the assay was conducted with high water activity and negligible Mg^{2+}. Because the inhibition of ATPase activity in vitro was similar to that in vivo, the in vitro pretreatment seemed to mimic the events occurring within the cells.

This conclusion is generally consistent with the behavior of chloroplasts in cells having low Ψ_w. Photosynthetic electron transport and phosphorylation begin to be inhibited 5–10 min [45] after desiccation begins, and recovery after water is resupplied is almost as rapid (15 min [41]). Chloroplast membranes change thickness at the same time but appear normal in other aspects [4]. The rapidity of the changes makes it unlikely that membrane degradation or synthesis is involved and suggest rapid changes in conformation, interaction of membrane subunits, or both. For CF_1, the simulation of cell dessication involved rapid changes in water activities and Mg^{2+} concentrations over a range likely to occur in cells. The similarity of the simulation and cell dessication results suggest that at least part of the desiccation-induced decrease in the phosphorylating activity of chloroplasts is caused by a simultaneous increase in ion concentration and decrease in chemical potential of water that change the conformation of CF_1 and, in turn, decrease the binding of nucleotide substrates to the coupling site(s).

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