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δ Subunit of Chloroplast Coupling Factor 1 Inhibits Proton Leakage through Coupling Factor O*

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The ATP synthase of chloroplasts consists of a proton-conducting portion, CF_O, and a catalytic portion, CF_1. The smaller subunits of CF_1, in particular δ, may play a key role in the coupling of proton transport to ATP synthesis. Purified subunit δ, when added to partially CF_1-depleted thylakoid membranes, can restore photophosphorylation (Engelbrecht, S., and Junge, W. (1987) *Eur. J. Biochem.* 172, 213–218). We report here that it does so by blocking proton conduction through CF_O.

Thylakoids were CF_1-depleted by incubation in hypoosmolar NaCl/EDTA solutions. Variation of the NaCl concentrations and of the incubation times not only changed the overall degree of CF_1 depletion but also the subunit composition of solubilized CF_1, namely CF_1 containing δ and $CF_1(-\delta)$. This was quantified by immunoelectrophoresis and by fast protein liquid chromatography.

Proton conduction was measured by flash spectrophotometry by using standard electrochromic and pH-indicating absorption changes. The removal of integral CF_1 was correlated with high electric conductance of thylakoid membranes, an increased extent of rapid proton leakage, and loss of ATP synthesis activity, which exceeded the percentual loss of CF_1. The removal of predominantly $CF_1(-\delta)$ resulted in comparatively lesser effects on the loss of ATP synthesis and on the extent and velocity of proton leakage. On the same line, addition of integral CF_1 and of purified δ diminished the electric leak in CF_1-depleted thylakoids. Both approaches, the controlled removal of CF_1 and $CF_1(-\delta)$, respectively, and addition of δ and CF_1 showed that δ can act as a "stopcock" to the exposed proton channel CF_O.

ATP synthases of chloroplasts, mitochondria, and bacteria are composed of two parts: F_1, extrinsic to the membrane, contains the binding sites for nucleotides, and F_O, a membrane spanning complex, acts as a proton-conducting device (for a recent review on F_1, see Ref. 1; for F_O, see Ref. 2). CF_1,[1] the ATPase of chloroplasts, is composed of five different polypeptides, named α, β, γ, δ, and ε in order of decreasing molecular mass. The subunit stoichiometry is 3:3:1:1:1 (3),[2] and the total mass is approximately 410 kDa (4). The large subunits, α and β, contain the nucleotide binding sites. The catalytic sites of ATP synthesis and ATP hydrolysis are located on β or between neighboring α and β subunits (5). Three catalytic sites may operate sequentially and cooperatively as proposed by Boyer *et al.* (*cf.* Ref. 6 and references therein). The γ subunit might regulate proton flow through CF_OCF_1, as suggested by McCarty and others (7, 8). This subunit changes its conformation (9) and concomitantly its accessibility to reductants (10) upon energization of the membrane. The ε subunit of CF_1 binds to γ (9) and acts as an inhibitor of ATP hydrolysis (11).

The role of subunit δ is under debate. While earlier work by Nelson and Karny (12) and Younis *et al.* (13) has led to the conclusion that δ is required for binding of CF_1 to CF_O, McCarty's group has shown that δ is not required for rebinding of CF_1 to NaBr-treated, CF_1-depleted thylakoids, but that it is necessary for blocking proton leaks and for high degrees of restoration of photophosphorylation (14). Junge *et al.* (15) have found the δ content in CF_1 that was extracted by EDTA treatment to vary with the extraction conditions. EDTA-treated membranes were leaky when the extracted CF_1 contained δ; they where tight if δ was lacking. This led to the suggestion of δ acting as a "stopcock" for the proton conduction through CF_O. More recently, we observed a 12% release of membrane-bound CF_1 upon EDTA treatment without any increase of the proton permeability of these membranes (16). Additionally, only a small fraction of exposed CF_O was active in proton translocation in EDTA-treated thylakoids. The addition of purified subunit δ to partially CF_1-depleted thylakoids was recently shown to reconstitute ATP synthesis (17, 18).

In this work, we specify conditions for the preferential extraction of δ-free or δ-containing CF_1 in a more systematic way. The total degree of extraction and relative proportions of $CF_1(-\delta)$ to CF_1 in the extract were determined and correlated to the proton leakiness of thylakoid membranes and to the rate of ATP synthesis under continuous illumination. Under conditions in which the total degree of CF_1-extraction was kept constant, while the subunit composition of solubilized CF_1 was altered, we observed a large and fast proton efflux out of CF_1-depleted thylakoids only after extraction of δ-containing CF_1. This could be reversed by addition of CF_1 or purified subunit δ.

MATERIALS AND METHODS

Broken *pea* chloroplasts were prepared according to the procedure for "stacked thylakoids" in Ref. 19 except that Mg^{2+} was omitted in the final suspending medium (10 mM NaCl, 100 mM Sorbitol, 10 mM

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[1] The abbreviations used are: CF_1, chloroplast ATP synthase, soluble portion; $CF_1(-\delta)$, CF_1 lacking the δ subunit; $CF_1(-\epsilon)$, CF_1 lacking the ε subunit; CF_O, chloroplast ATP synthase, membrane portion; DCCD, *N,N'*-dicyclohexylcarbodiimide; Mes, 4-morpholineethanesulfonic acid; FPLC, fast protein liquid chromatography; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

[2] The stoichiometry of subunit δ recently was determined and found to be 1 δ/CF_OCF_1 (S. Engelbrecht, unpublished result).

Tricine/NaOH, pH 7.8). Thylakoids were stored as concentrated stock suspension (2–3 mg of chlorophyll/ml) on ice for up to 6 h before use. They were stable during this interval. CF_1 depletion was carried out as follows. Thylakoids were diluted to 10 μM chlorophyll in a solution containing 100 μM EDTA, NaCl as indicated, and 1 mM Tricine/NaOH, pH 7.8, at room temperature. The incubation time was varied. The release of CF_1 was stopped by adding 1 M NaCl to yield a final NaCl concentration of 30 mM. Thylakoids were centrifuged 20 min at 30,000 × g (4 °C) and resuspended in the same medium as used for the final resuspension after preparation. The CF_1 content of the membranes was determined by rocket electroimmunodiffusion after Laurell (20) modified as in Ref. 21. The buffer system was 75 mM Tris borate, pH 8.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out according to Laemmli (22), and silver staining was according to Wray *et al.* (23). Western blot analysis was done after Howe and Hershey (24), with rabbit anti-CF_1 antiserum as first antibody and peroxidase-coupled anti-rabbit IgG as second antibody.

After the removal of the CF_1-depleted thylakoids by centrifugation (see above), up to 2500 ml of the supernatants were concentrated to a volume of <50 ml by ultrafiltration through an YM 10 (Amicon, nominal cutoff 10 kDa) membrane under 3.8 bar argon. This volume was applied to a Pharmacia LKB Biotechnology Inc. HR 16/10 column (16 × 100 mm) packed with Merck Fractogel TSK DEAE 650 (S) (25). The column was developed with 50 mM Mes/NaOH, pH 6.0, with a linear gradient of 1 M NaCl in that buffer. Elution of proteins was monitored via absorption at 280 nm. Peaks were tested for Mg^{2+}-ATPase activity in the presence of methanol as in Ref. 26. ATPase-containing peaks were applied to a Pharmacia LKB Biotechnology, Inc. HR 5/5 (5 × 50 mm) Mono Q column. The column was developed with the same buffer as above.

Flash spectrophotometric measurements were performed in the same setup as in Ref. 27. The measuring cuvette contained a suspension of thylakoid vesicles with 10 μM chlorophyll in 10 μM methylviologen, and 10 mM NaCl in a volume of 15 ml. The thylakoid suspension was excited with short (15 μs), saturating flashes of red light (>610 nm) at 5-s repetition intervals. Voltage transients were recorded by electrochromic absorption changes of intrinsic pigments (28) at 522-nm wavelength in the presence of 10 mM Tricine/NaOH, pH 7.5. Transient pH changes in the external suspending medium were monitored by absorption changes of phenol red (13 μM) at 559 nm (29). For these measurements, the buffer was omitted and pH 7.5 was adjusted by titration with NaOH. The "pH-indicating absorption changes" were obtained by subtraction of two transient signals, one of which was measured with 13 μM phenol red added and the other one without the dye. 20 signals each were averaged as described previously (19). The specificity of hydrophilic pH-indicating dyes for pH transients in the medium was established elsewhere (30). In EDTA-treated thylakoids with highly proton-conducting CF_O, the pH transients in the medium were composed of several events, all occurring within the same time domain of 400 ms: the uptake of protons at the quinon-reducing site of photosystem II, which made the suspending medium alkaline, and the liberation of protons into the thylakoid lumen, both at the quinole and at the water-oxidizing site, which caused an acidification of the medium after passage of these protons across the membrane (for a review of the partial reactions of proton pumping, refer to Ref. 31). The proton displacement via CF_O could be extracted from the overall transient by subtracting a transient recorded in the presence of DCCD, which blocked CF_O channels by covalent modification of an essential glutamate in subunit III, from a trace recorded in the absence of DCCD (16). For this, thylakoids were incubated with 20 μM DCCD for 10 min at room temperature in the dark and in the standard assay medium. The pH was readjusted before measurement.

ATP synthesis rates were measured as described by Schmid and Gräber (32). For reconstitution experiments, CF_1 was prepared from market *spinach* as described previously (3, 25). CF_1 and $CF_1(-\delta)$ were obtained from this preparation by the same high performance liquid chromatography procedure as described for purification and analysis of extraction supernatants (see above). The δ subunit of CF_1 was prepared as described (17). To block CF_O channels, EDTA-extracted thylakoid membranes were incubated with CF_1 or δ in a 1-ml measuring cuvette. Thylakoids equivalent to 20 μg of chlorophyll were incubated for 10 min in the dark in 500 μl of 10 mM Tricine/NaOH, pH 7.8, 10 mM NaCl, 8 mM $MgCl_2$, and with the indicated amounts of protein added. Prior to measurement, 500 μl of 10 mM Tricine/NaOH, pH 7.8, 10 mM NaCl, and 40 μM methylviologen were added.

Fractogel TSK DEAE 650 (S) was from Merck and agarose (Standard EEO) from Serva. Peroxidase-coupled goat anti-rabbit IgG was obtained from Cappel. All other chemicals were of the highest grade available.

RESULTS

Junge *et al.* (15) observed CF_1 depletion without concomitant proton leakage at very low extraction degrees ranging between 1 and 5% of total CF_1. Proton leakage was observed only with high degrees of CF_1 extraction (70–90%). In a first approach, we asked how much the extraction degree could be increased without obtaining proton-leaky membranes. Incubation of thylakoids in EDTA buffer and in the presence of NaCl in the range from 10 to 30 mM yielded the desired effect: 17 ± 4% loss of CF_1, a concomitant 13 ± 5% loss of ATP synthesis activity (n = 20), and almost no enhanced proton efflux detectable (see below). This was independent of the NaCl concentration in the indicated range, and it was also independent of the incubation time (2–10 min). By lowering the NaCl concentration to 5 mM and shortening the incubation time to 1.5 min, the result was changed. Now an extraction degree of similar magnitude, 13%, was accompanied by a larger drop of the ATP synthesis rate by 22% and by significantly enhanced proton efflux.

Traces in Fig. 1 represent transient electrochromism measured in samples which were subjected to different extraction protocols. These transients were obtained by exciting the thylakoid's photosynthetic reaction centers with short flashes of light, which generated a transmembrane voltage in the subnanosecond time domain. The signal labeled *o* shows the voltage decay obtained with control thylakoids. The transmembrane voltage relaxed via the ionic (non-protonic) leak conductance of the membrane. For *a*, thylakoids were incubated in EDTA solution for 5 min and in the presence of 20 mM NaCl. Almost no acceleration of the voltage decay was observed. After incubation in EDTA solution in the presence of 5 mM NaCl and for 1.5 min, an enhanced decrease of the flash-induced voltage was recorded (*b*). For *c*, finally, thylakoids were incubated for 10 min with 1 mM NaCl present, and this drastically accelerated the decay of the flash-induced voltage. That the acceleration of the decay was due to CF_O-mediated charge translocation was evident from its reversal by standard inhibitors of CF_O like DCCD, as described previously (28).

Fig. 2 shows CF_O-related proton displacement from aliquots

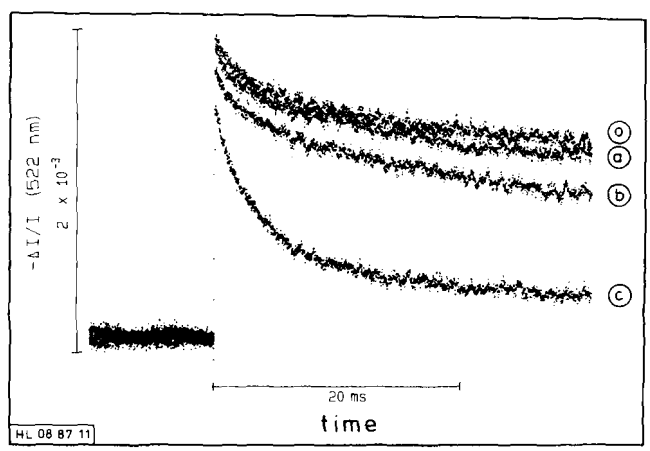

FIG. 1. **Flash-induced voltage transients in thylakoids subjected to different protocols for CF_1 depletion.** Electrochromic absorption changes were monitored at 522 nm as detailed under "Materials and Methods." Thylakoid suspensions were excited with a single flash at time 0. The incubation conditions in EDTA-containing buffer were the same as in Fig. 2 and Table I.

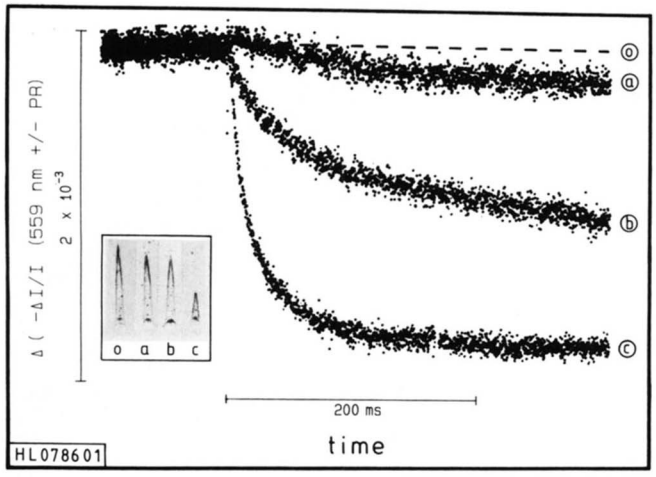

FIG. 2. **CF_O-related proton displacement across thylakoid membranes into the outer suspending medium under different protocols for CF_1-depletion.** The transients shown were obtained by subtraction of traces measured in the presence of 20 μM DCCD from those recorded without DCCD as detailed under "Materials and Methods." Note the 10-fold longer time range of this figure as compared to Fig. 1. *Inset*, contents of membrane-bound CF_1 in EDTA-treated thylakoids. Membranes equivalent to 5 μg of chlorophyll, solubilized in Triton X-100 (3%, v/v), were applied to each well. Immunoelectrophoresis was carried out as detailed in Ref. 21. *o* represents control thylakoids, which were taken as starting material for the EDTA treatments. The degree of CF_1 depletion was: *o*, control no depletion; *a*, 13%; *b*, 13%; *c*, 55%.

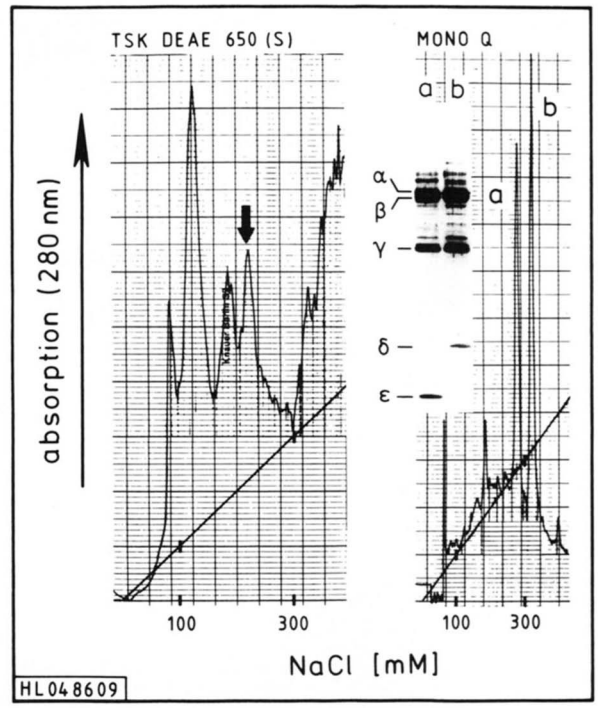

FIG. 3. **Purification of solubilized CF_1 and analysis of subunit composition by FPLC.** The supernatant was concentrated via ultrafiltration as described under "Materials and Methods." The elution profile on the *left* represents the first chromatography on TSK DEAE 650 (S). The peak containing methanol-activated Mg^{2+}-ATPase is marked by an *arrow*. This peak was submitted to a second chromatography on Mono Q (*right*). For both runs, buffer A was 50 mM Mes/NaOH, pH 6.0; buffer B was 50 mM Mes/NaOH, pH 6.0, and 1 M NaCl. The flow rate was 10 ml/min for the TSK column, and 1 ml/min for the Mono Q column. Absorption at 280 nm is given at the *ordinate*. The rising lines give the increasing NaCl concentration of the applied buffer, two concentrations each are marked by *ticks* on the abscissa as well as in the elution profile. A SDS gel electrophoresis of samples from *peaks a* and *b* (12.5% gel) is documented beside the Mono Q elution profile. 5 μg of protein was applied to each slot.

of the same samples as used in Fig. 1. The *inset* shows immunoprecipitation "rockets" which illustrate the residual content of membrane-bound CF_1 after EDTA treatment. With control thylakoids, no CF_O-related or DCCD-sensitive proton efflux was observed as indicated by a *broken line* (*o*). Surprisingly, in the sample (*a*) which had lost 13% of total CF_1, there was almost no CF_O-related proton displacement, in good correlation with the negligible acceleration of the voltage decay shown in Fig. 1. In *b*, the extraction degree was 13% again, but now accompanied by appreciable proton efflux. The extraction conditions for *c* resulted in 55% CF_1-extraction and in greater extent and higher rate of proton efflux, in agreement with the greatly accelerated voltage decay (*cf.* Fig. 1). The extent of this signal was near the expected maximum of one proton per photosystem as documented elsewhere (16). Taken together, the results documented in Figs. 1 and 2 showed that under removal of identical amounts of total CF_1 the membranes could remain proton tight or become leaky to protons, depending on the extraction procedure. Therefore, we asked for differences in the extracted CF_1.

The extraction protocol resulted in large volumes (up to 2500 ml) of supernatant after collection of CF_1-depleted thylakoids by centrifugation. Solubilized CF_1 was highly diluted, and it was accompanied by other proteins. In order to examine the subunit composition of solubilized CF_1, supernatants were concentrated by ultrafiltration to a volume of 50 ml. This volume was subjected to anion exchange FPLC on TSK DEAE 650 (S). Fig. 3 shows the elution profiles of a typical FPLC run with the supernatant of an extraction at 1 mM NaCl, incubation time 10 min (*c* in Figs. 1 and 2). The TSK DEAE 650 (S) elution profile (*left*), showed several peaks, but only one contained ATPase activity (marked by an *arrow*). This peak was further analyzed by FPLC on Mono Q (*right*). The amounts of protein separated by this run were too small for analysis by gel electrophoresis. Therefore, we subjected 1 mg of pea CF_1 from a large scale preparation (25) to Mono Q FPLC. Two peaks were eluted at the same two NaCl concentrations as in Fig. 3. The peak at 278 mM NaCl contained $CF_1(-\delta)$ and the peak at 320 mM NaCl $CF_1(-\epsilon)$. Peak integration of the profile given in Fig. 3 revealed that the peak at 278 mM comprised 48% of total CF_1, *i.e.* the ratio $CF_1(-\delta)$:$CF_1(+\delta)$ was about 1:1 in this particular extraction experiment. The supernatants produced by the two other extraction protocols (*cf. a* and *b* in Figs. 1 and 2) were also subjected to this analysis. Table I summarizes the ATP synthesis rates, the degree of CF_1 extraction (*cf.* Fig. 2, *inset*), and the ratio of δ-free *versus* δ-containing enzyme for control thylakoids and for the three extracted samples. Comparison of *a* and *b* revealed that a higher amount of δ-containing CF_1 in the supernatant (at constant extraction degree) was accompanied by higher proton leakage (*cf.* Fig. 2) and by a greater decrease of the photophosphorylation rate. As the decrease of the latter was higher than the decrease of CF_1 content, it was mainly caused by dissipation of protonmotive force (in agreement with measured proton efflux; *cf.* Fig. 1 and 2) and not by loss of catalytic activity. On the other hand, after extraction of $CF_1(-\delta)$, rapid proton efflux was blocked.

From these results, a function of δ as a stopcock to CF_O was highly probable. In a reverse experiment, we checked whether the leakiness of CF_1-depleted membranes could be mended after incubation with CF_1 or its δ subunit. Spinach CF_1 was prepared via Mono Q FPLC as in Fig. 3, and the δ subunit was purified as described (17). In Fig. 4, voltage

TABLE I

ATP synthesis at different protocols for CF_1-depletion of thylakoids

ATP synthesis rates were measured with 50 μM phenazinemethosulfate as mediator of cyclic electron transport. CF_1-content after extraction was taken from the electroimmunodiffusion assay documented in the insert of Fig. 2. The relative amounts of δ-containing CF_1 *versus* $CF_1(-\delta)$ in the extraction supernatants were determined by peak integration of the respective elution profiles on Mono Q anion exchange chromatography.

Incubation in EDTA-buffer	Transients in Figs. 1 and 2	Rate of ATP synthesis		Relative amount of CF_1 remaining on thylakoids	Composition of extracted CF_1
		μmol ATP / mg chlorophyll·h	%	%	% δ-containing
Control thylakoids	*o*	688	100	100	
5 min, 20 mM NaCl	*a*	608	88	87	18
1.5 min, 5 mM NaCl	*b*	528	78	87	36
10 min, 1 mM NaCl	*c*	128	19	45	54

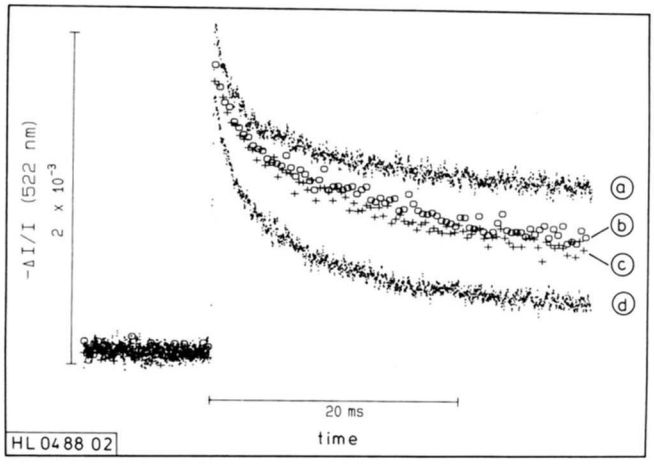

FIG. 4. **Voltage transients from extracted thylakoids after incubation with CF_1 and δ.** Voltage transients were measured as in Fig. 1, but in a 1-ml measuring cuvette and at 20 μM chlorophyll. Thylakoids were extracted as in *c* of Table I. Samples were incubated for 10 min in the dark with: *a*, 20 μM DCCD; *b*, *open circles*, 100 μg of CF_1; *c*, *crosses*, 43 μg of δ; *d*, 50 μg of ovalbumin.

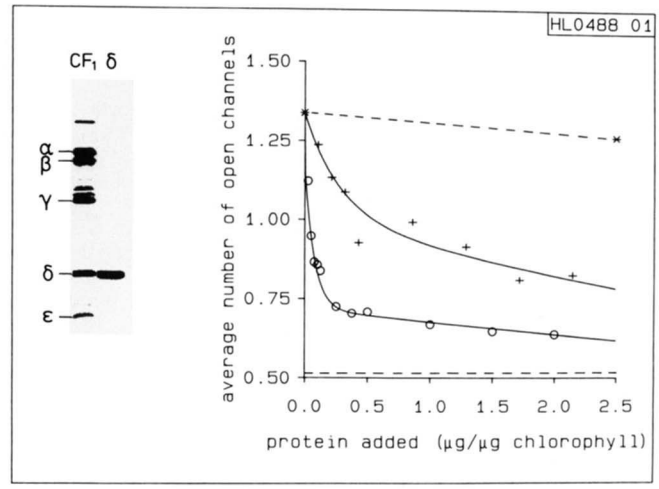

FIG. 5. **Resealing of CF_1-extracted thylakoid membranes by CF_1 and δ.** The protein samples used in these experiments were characterized by Western blot analysis (*left*). 0.5 μg δ and 10 μg of CF_1 have been run in the initial electrophoresis. Both samples were incubated with the same anti-CF_1 antiserum. From traces as shown in Fig. 4, the average number of open channels per vesicle was evaluated by a Poisson statistical fit as detailed in Ref. 33. The average number of open channels in DCCD-inhibited vesicles after a 10-min incubation in the reconstitution medium, but with 50 μg of ovalbumin present, was 0.52 as indicated by a *broken line*. The *star* on the *abscissa* depicts extracted thylakoids without additions, the *star* at 50 μg of protein shows the effect of the presence of 50 μg of ovalbumin in the reconstitution experiment. *Open circles* stand for CF_1, *crosses* for δ.

transients as in Fig. 1 are shown. Obviously, purified subunit δ was effective in blocking open channels as was integral CF_1. In Fig. 5, we determined the number of active CF_O-channels by fitting curves like those documented in Fig. 4 by the Poisson statistical approach which we introduced previously (33). The signals were recorded from thylakoids which had been EDTA-treated for 10 min (*sample c*), followed by a 10-min incubation with the indicated amounts of CF_1 or δ. Clearly, CF_1 and purified subunit δ both inhibited CF_O-mediated charge translocation. *Left* of the figure shows Western blots of the protein samples employed in this experiment, carried out with an anti-CF_1 antiserum and demonstrating that no other subunits of CF_1 were present in the δ-preparation.

DISCUSSION

We investigated the role of the δ subunit of chloroplast ATP synthase (CF_OCF_1). An extraction protocol was established which allowed to vary the δ content of solubilized CF_1 under constant total degree of extraction of the bulk enzyme. With the same extraction degree of 13% of total CF_1 (*a* and *b* in Figs. 1 and 2 and Table I), proton leakage through CF_O was 5-fold higher after extraction of $CF_1(-\delta)$. Increased proton leakage was paralleled by a decreased rate of ATP synthesis, as expected. It was reasonable to assume that subunit δ remained bound to CF_O in thylakoid membranes which were depleted from $CF_1(-\delta)$. Proton leakage through those $CF_O(+\delta)$ was prevented.

A loss of δ during the collection, concentration, and analysis of the solubilisate was unlikely, since δ-containing CF_1 in considerable amounts was detected by the same protocol in other samples. Moreover, we purified the δ-containing fraction of CF_1 out of a large scale preparation of the enzyme by the same protocol involving Mono Q and the same buffer, Mes/NaOH, pH 6.0, in order to obtain samples for gel electrophoresis. Rechromatography of this fraction under the same conditions did not produce any δ-free CF_1, but it yielded one peak at the NaCl concentration typical for the δ containing enzyme (not shown). This excluded artifactual production of $CF_1(-\delta)$ during purification. The attribution of the band denominated δ in the *inset* of Fig. 2 to subunit δ was checked by Western blot analysis (not shown).

The Poisson statistical approach which we used in this study to determine the number of highly active CF_O has been introduced (33). This analysis allows determination of the

average number of fast conducting channels per thylakoid vesicle and their time-averaged single-channel conductance. This was calibrated against gramicidin (33). It resulted in a time averaged single channel conductance of 1 pS for active CF_O. A second state of exposed CF_O, much less active and no more inhibitable by CF_1 or δ is currently under investigation in our laboratory.

Addition of CF_1 to CF_1-depleted thylakoids could restore higher synthesis rates in two ways, namely by blocking proton leaks and by adding catalytic capacity (17, 18). On the other hand, δ alone could act only by plugging proton leaks. This action was not due to residual CF_1 in our δ preparation as documented by the Western blot shown in Fig. 5. This was in line with a recently published report of Joshi *et al.* (34), who showed that the mitochondrial subunit oligomycin sensitivity-conferring protein, which bears appreciable homology to the chloroplast δ (18, 35), remained bound to MF_O upon extraction of MF_1. During stepwise degradation of the oligomycin sensitivity-conferring protein by trypsin they observed a concomitantly increased leakiness of submitochondrial vesicles, a finding which could be interpreted in terms of oligomycin sensitivity-conferring protein acting as a stopcock to MF_O.

In contrast to the findings of Richter *et al.* (36, 37) with NaBr-treated thylakoids, our study did not indicate any influence of subunit ε on proton efflux through CF_O. Extraction of $CF_1(-\delta)$ removed all subunits but δ from CF_O and the proton leakage through CF_O was still low, whereas extraction of $CF_1(-\epsilon)$ (*cf.* Fig. 3, *inset*) resulted in high proton leakage. Addition of either δ alone (18) or $CF_1(-\epsilon)$ (25) restored photophosphorylation. The reasons for these discrepancies between our labs at present are unclear and require further experimentation.

Although subunit δ of CF_1 obviously acted as an inhibitor of proton flow through exposed CF_O, its function in phosphorylating CF_OCF_1 must go beyond this merely passive role. If direct participation of protons in ATP synthesis is assumed after their passage through CF_O into CF_1 during the catalytic cycle (38, 39), the block of proton flow by δ must be relieved. A possible candidate for this control function on δ is the γ subunit of CF_1, which was proposed to be a proton gate (7, 8). On the other hand, if we assume a conformational coupling between proton flow and ATP synthesis (40, 41), δ could transmit conformational changes toward the larger α and β units as a result of proton flow through CF_O. The rod and the piston in a combustion engine may serve as an illustration. Transmission of information from F_O (binding of inhibitors) into F_1 (altered affinities of nucleotide binding sites, altered conformational response on ligand binding) has been demonstrated recently for the mitochondrial F_OF_1 (42, 43).

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