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Chloroplast ATP synthase contains one single copy of subunit δ that is indispensable for photophosphorylation

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F_0F_1 ATP synthases synthesize ATP in their F_1 portion at the expense of free energy supplied by proton flow which enters the enzyme through their channel portion F_0. The smaller subunits of F_1, especially subunit δ, may act as energy transducers between these rather distant functional units. We have previously shown that chloroplast δ, when added to thylakoids partially depleted of the coupling factor CF_1, can reconstitute photophosphorylation by inhibiting proton leakage through exposed coupling factor CF_0. In view of controversies in the literature, we reinvestigated two further aspects related to subunit δ, namely (a) its stoichiometry in CF_0CF_1 and (b) whether or not δ is required for photophosphorylation.

By rocket immunoelectrophoresis of thylakoid membranes and calibration against purified δ, we confirmed a stoichiometry of one δ per CF_0CF_1. In CF_1-depleted thylakoids photophosphorylation could be reconstituted not only by adding CF_1 and subunit δ but, surprisingly, also by $CF_1(-\delta)$. We found that the latter was attributable to a contamination of $CF_1(-\delta)$ preparations with integral CF_1. To lesser extent $CF_1(-\delta)$ acted by complementary rebinding to CF_0 channels that were closed because they contained δ [$CF_0(+\delta)$]. This added catalytic capacity to proton-tight thylakoid vesicles.

The ability of subunit δ to control proton flow through CF_0 and the absolute requirement for δ in restoration of photophosphorylation suggest an essential role of this small subunit at the interface between the large portions of ATP synthase: δ may be part of the coupling site between electrochemical, conformational and chemical events in this enzyme.

ATP synthesis in thylakoid membranes, in the inner mitochondrial membrane and in the plasma membrane of microorganisms is performed by F_0F_1 ATP synthases which couple ATP synthesis to proton translocation. ADP and P_i bind to the water-soluble F_1 part of the enzyme. According to current concepts, ATP is formed spontaneously but remains firmly bound to F_1 [1]. Its energy-requiring release is driven by proton flow through the membrane-embedded F_0 portion which causes conformational changes in F_1 that promote the release of bound ATP [1, 2]. For recent reviews see [1–7]. The smaller subunits of CF_1, namely γ and δ, probably link F_1 to F_0. As possible transducing elements between electrochemical, conformational and chemical events they are of particular interest. We focussed on the role of subunit δ.

CF_1 consists of five different subunits (α to ε) with a stoichiometry of $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ [8–10] (and this paper). Only one group has reported a stoichiometry of δ/CF_0CF_1 greater than one [11]. For the analogous subunit to chloroplast δ in mitochondria, the oligomycin-sensitivity-conferring protein (OSCP), a stoichiometry of two OSCP per MF_1 has been shown by Penin et al. [12, 13]. In chloroplasts we found that a stoichiometry greater than one was not compatible with two observations. (a) In coreconstitution experiments with $CF_1(-\delta)$ and δ, which were added to partially CF_1-depleted thylakoids, restoration of photophosphorylation was maximal with about one molecule of δ per molecule of $CF_1(-\delta)$ (see Fig. 3 in [14]). (b) The δ content of solubilized CF_1 in our hands never exceeded one δ per CF_1. Therefore we reassessed the δ content of membrane-bound CF_0CF_1.

By several independent lines of evidence we showed that subunit δ can inhibit proton flow through exposed CF_0 [14–19]. This blocking action on exposed CF_0 implies an essential valve or energy-transducing function of δ in integral CF_0CF_1. An absolute requirement of δ for photophosphorylation however seemed debatable [20, 21] in the light of the ability of purified $CF_1(-\delta)$ partially to restore photophosphorylation in CF_1-depleted thylakoids [17,21]. Therefore we reinvestigated this issue with emphasis on possible contaminations of $CF_1(-\delta)$ preparations with CF_1. In this context a brief review of the properties of exposed CF_0 in CF_1-depleted thylakoid vesicles is helpful. As detailed in [16] and [22], EDTA treatment of thylakoids results in small vesicles with about 10^5 chlorophyll molecules. On average, this is equivalent to a total of about 100 molecules of CF_0 per vesicle and, in typical CF_1-depletion experiments, about 50 CF_1, i.e. about half of the CF_0 are 'exposed' and the remaining 50 still carry their CF_1 counterpart. Among the exposed CF_0 molecules only a very small fraction, of the order of one molecule per vesicle, is in

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Abbreviations and definitions. CF_0 = chloroplast coupling factor 0 (proton channel); open CF_0 = CF_1-depleted CF_0, active in proton conduction upon membrane energization; exposed CF_0 = CF_0 without CF_1 counterpart, not necessarily active in proton conduction; CF_1 = chloroplast coupling factor 1 (integral ATPase); $CF_1(-\delta)$ = CF_1 lacking the δ subunit; $CF_1(-\varepsilon)$ = CF_1 lacking the ε subunit; Mega 9 = *N*-(D-gluco-2,3,4,5,6-pentahydroxyl)-*N*-methylnonanamide.

Enzyme. Chloroplast ATP synthase (EC 3.6.1.34).

a high-conductance form. One single CF_0 molecule of this type is sufficient to dissipate the proton motive force in a vesicle even in competition with a high rate of proton pumping (under phenazinemethosulfate-mediated cyclic electron transfer). The residual photophosphorylating activity of the vesicle population depends on the product of two factors, namely the proportion of vesicles without any high-conductance channel times and the proportion of CF_0CF_1 remaining on 'leaky' vesicles. It is also important to note that CF_0 molecules may be nonconducting because they are blocked either by CF_1 or because they retained bound subunit δ [16, 22].

MATERIALS AND METHODS

Preparation of coupling factors: reconstitution assay

CF_1, $CF_1(-\delta)$ and EDTA vesicles were prepared essentially as described [14, 17, 19, 23]. The degree of CF_1 depletion in EDTA-treated spinach thylakoids was higher than previously observed [14], the difference probably being due to different batches of commercially available spinach at different times of the year. The δ and $CF_1(-\delta)$ preparation was simplified in the following way. CF_1 prepurified on DEAE-cellulose (Whatman DE 52) [17, 23] was applied to an HR 16/10 (16 × 100 mm) column packed with Merck Fractogel TSK DEAE 650 (S), followed by elution with the usual Mes/NaOH buffer, pH 6.0 [17] until all ribulose bisphosphate carboxylase was eluted (usually at about 200 mM NaCl). After washing with a few millilitres of the same buffer containing no NaCl but 0.5 mM Mg^{2+}/ADP, the FPLC system was reequilibrated by making use of the built-in wash program. Subunit δ was eluted then by a linear gradient of NaCl in 50 mM Tris/HCl, 22 mM Mega 9, 1 mM EDTA, pH 7.8. Immediately after elution of δ (usually at about 170 mM NaCl) the FPLC system again was reequilibrated and $CF_1(-\delta)$ was eluted by a linear gradient of NaCl in 25 mM Tris/HCl, pH 7.8. The modified procedure is much faster than the original one [19], because the tedious removal of ATP and Mega 9 by pressure dialysis can be skipped (except removal of detergent from δ). The increase in Mega 9 concentration was necessitated because of slight variations in the commercially available preparations.

Reconstitution conditions were rechecked and set to the following. CF_1 and $CF_1(-\delta)$ were prepared and used within two weeks. During this time the preparations were stored at 4 °C in 25 mM Tris/HCl, 1 mM K-ADP, pH 7.8 with no significant loss of ATPase activity which was about 25 U/mg for both preparations (measured as Mg-ATPase in the presence of 40 mM Tris/HCl, pH 7.8, 4 mM ATP, 1.5 mM $MgCl_2$, 30%, by vol., methanol, 10 mM Na_2SO_3 [24, 25]. For reconstitution the desired amount of protein was pipetted into 25 mM P_i, pH 7.8 containing 6 mM $MgCl_2$. The volume at this point was always 300 µl. The mixture was cooled on ice and 10 µl 1 mM Chl (either thylakoids or EDTA vesicles) were added. After incubation for 10 min on ice in the dark, the mixture was removed from the ice bath and supplemented with 665 µl of a medium containing 100 mM sorbitol, 50 mM Tricine/NaOH, 10 mM NaCl, 5 mM $MgCl_2$, 5 mM K_2HPO_4, pH 8.0, 30 µl 100 mM K-ADP and 5 µl 10 mM phenazine methosulfate. Sometimes P^1,P^5-di(adenosine-5′)pentaphosphate (10 µM) was added in order to inhibit endogenous kinases (e.g. contained in the anti-δ antisera). The mixture was illuminated for 1 min with heat-filtered, saturating white light, then 500 µl 0.5 M trichloroacetic acid was added and synthesized ATP was measured via the LKB luciferin/luciferase assay [17].

Electrophoresis

Electrophoresis was performed with the Pharmacia PhastSystem in 10–15% polyacrylamide gels and commercially available SDS-buffer strips according to the protocol provided by Pharmacia. Staining was by a combined $AgNO_3$/tungstosilicic acid stain [26, 27] which was slightly modified in the following manner. Immediately after the electrophoretic separation had finished, the Phastgel was transferred into the developing chamber of PhastSystem and fixed with 20% (mass/vol.) trichloroacetic acid, 6 min, 25 °C, followed by three washes in 10% (by vol.) EtOH/5% (by vol.) acetic acid at 50 °C for 2 min, 3 min, 3 min respectively. The gel was further washed with Milli Q water at 50 °C (2 × 2 min) and finally incubated in 75% (by vol.) glycerol. The gel was removed now from the developing chamber and dried under warm air until the surface of the separating gel was no longer sticky. For development, the gel was placed in a small beaker containing 5 ml 5% (mass vol.) Na_2CO_3 and 9 ml of a solution containing 0.2% (mass vol.) NH_4NO_3, 0.2% (mass vol.) $AgNO_3$, 1% (mass vol.) $H_4[Si(W_3O_{10})_4]$aq, 1.47% (by vol.) of 37% HCHO. Protein bands developed within about 6–10 min. After completion of the stain, the by now rather black suspension was discarded, the gel washed briefly with water and then incubated for a few minutes in 10% (by vol.) glycerol, 5% (by vol.) acetic acid, followed by drying as above. In order to obtain a good signal/noise ratio, CF_1 samples were set to a concentration of 200 ng/µl, δ samples to 20 ng/µl. With the 12-sample combination, 0.3-µl samples were separated.

Immunological techniques

Immunization of rabbits was as follows. After bleeding the animals once in order to obtain preimmune serum, they were injected initially with 160 µg purified δ in complete Freund's adjuvant, followed by biweekly bleeding with intermittent boosts of 150 µg δ in incomplete adjuvant. Western blots were essentially as described [17], Triton X-100 was present in the bovine serum albumin blocking solution and modifications as described by Dunn [28] were included. Bands were visualized by reaction of diaminobenzidine with peroxidase-coupled goat anti-(rabbit IgG) IgG obtained from Cappel/Dynatech, Denkendorf, FRG.

Rocket immunoelectrophoresis was performed as previously described [17] but, as subunit δ in thylakoids did not form precipitin lines without preceding denaturation by SDS, the protocol was slightly modified according to Plumley and Schmidt [29], i.e. samples were heated in the presence of SDS, excess of SDS was complexed by Triton X-100 and precipitation in the agarose was enhanced by inclusion of 4% (mass vol.) poly(ethylene glycol) 4000.

Other methods

Amino acid analysis and protein determination were carried out according to standard procedures [17]. Mega 9 was purchased from Oxyl Chemie, Bobingen, FRG, all other chemicals were of the highest grade available.

RESULTS

The stoichiometry of subunit δ in CF_0CF_1

This was measured by electroimmunodiffusion. To this end δ was prepared [19] and its purity checked by SDS

Table 1. *Comparison of an amino acid analysis of purified subunit δ with predicted data from its cDNA sequence [30]*
nd, not determined

Amino acid	Number of residues	
	predicted	found
Ala	16	15.99
Cys	0	0
Gly	8	9.18
Ile	20	16.71
Leu	15	15.57
Met	2	1.77
Phe	7	7.56
Trp	0	nd
Tyr	4	3.36
Val	22	19.08
Pro	4	4.08
Ser	13	11.13
Thr	12	12.84
Arg	6	6.42
His	2	3.84
Lys	12	11.52
Asx	24	22.74
Glx	20	20.16

electrophoresis, by N-terminal sequencing [14] and by amino acid analysis. After these checks the protein was injected into rabbits as described in Materials and Methods. The results of an amino acid analysis are shown in Table 1 in comparison with the recently published cDNA sequence of spinach δ [30]. It is clear that the sample was at least 90% pure. It must be noted that the amino acid analysis revealed a marked overestimation of δ concentrations in protein determinations by the Coomassie binding assay (up to 30%), which, if not taken into consideration, easily shifts stoichiometric data e.g. from 1 to about 1.5 which may be misinterpreted as 'at least more than one' copy of δ per CF_0CF_1.

The quality of the polyclonal antiserum was checked by Western blot, shown in Fig. 1. The left part of the figure shows a silver-stained phastgel with integral CF_1 (lane 1), δ (lane 2), $CF_1(-\delta)$ (lane 3), the right part shows a Western blot with anti-CF_1 serum (lane 4) and anti-δ serum (lane 5).

The protocol for determination of the δ content of CF_0CF_1 was as follows. (a) Determine by rocket immunoelectrophoresis the content of CF_1 in thylakoids by running thylakoid samples together with standard CF_1 (the precise concentration of which was determined via amino acid analysis) against anti-CF_1 antibodies. (b) Do the same for subunit δ with anti-δ antibodies. As δ did not form precipitin lines without prior treatment with SDS, the protocols for electroimmunodiffusion were changed accordingly [29] for both runs, CF_1 and δ. (c) Check the validity of results in view of the fact that thylakoid membranes with 1 mg Chl roughly contain a background of 7 mg total protein. The detection limit for subunit δ was around 100 ng. If one assumes one δ per CF_0CF_1 and 0.5 mg CF_1/mg Chl [4], 100 ng antigen must be recognized against a background of 28 μg total protein.

Fig. 2 shows the result of an immunoelectrophoresis with δ and antiserum against δ. In order to improve the statistics, double-sized plates were used, allowing for simultaneous run of 16 samples. Similar runs, where purified δ was run against a background of bovine serum albumin as a substitute for thylakoid proteins, gave identical results. We suspected a somewhat different effect of membrane proteins and ran standard δ in the presence of fixed amounts of thylakoids. This suppressed the reaction of δ in a way that the standard curve was shifted in parallel towards about 15% smaller absolute values, i.e. the deviation was not sufficient to shift the overall result significantly (data not shown).

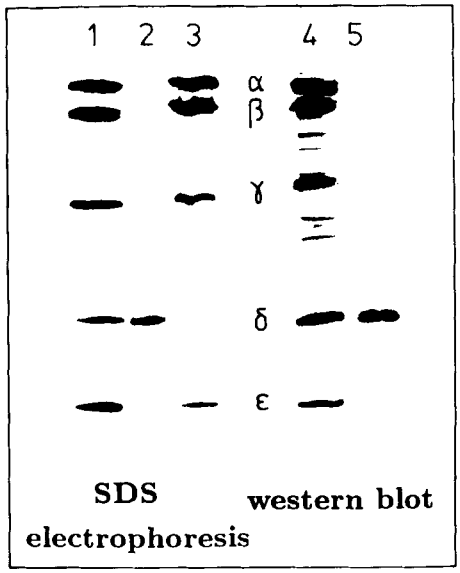

Fig. 1. *SDS electrophoresis and Western blot of CF_1 and δ.* Lanes 1–3, SDS electrophoresis (silver/silicotungstic acid stain) of 70 ng CF_1, 7 ng δ and 70 ng $CF_1(-\delta)$, respectively; migration from top (–) to bottom (+). Lanes 4 and 5, Western blot (peroxidase/diaminobenzidine stain) of antisera directed against CF_1 (dilution 1:10000) and against δ (dilution 1:4000). 20 μg Chl each of spinach thylakoids were electrophoresed and blotted; migration from top (–) to bottom (+). The antiserum against subunit δ showed a weak spot in the region of subunit β, which was lost in photographic processing

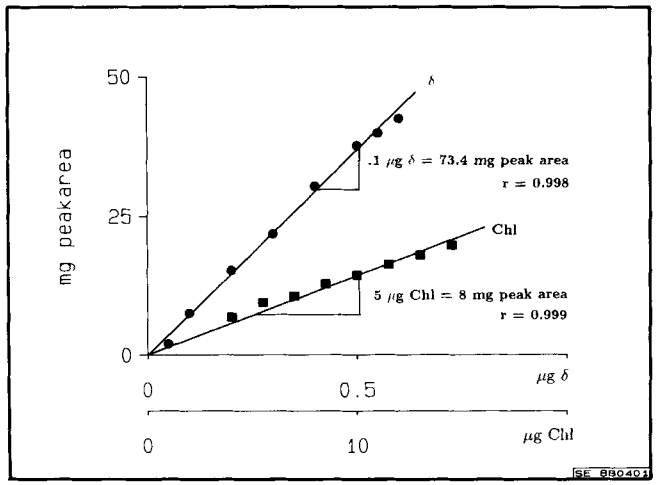

Fig. 2. *Evaluation of data obtained from electroimmunodiffusion of purified spinach δ and spinach thylakoids against δ-specific polyclonal antiserum.* Per plate (9.5 × 16.5 cm), 2 ml antiserum was contained in 1% agarose (standard eeo), 4% poly(ethylene glycol) 4000, Tris/borate buffer pH 8.6

Table 2 summarizes the data that were obtained in a number of similar experiments. It is evident that the CF_1 content of spinach thylakoids fell well into the known range [4]. We found that δ accounted for 5.1% (by mass) of the total molecular mass of CF_1. When the molecular masses of the CF_1

Table 2. *Summary of data concerning the relative amounts of subunit δ contained in solubilized CF_1 and in spinach thylakoids and the relative amount of CF_1 in thylakoids*

Ratio	Value	*n*
	(mass/mass)	
δ/solubilized CF_1	0.046 ± 0.10	15
CF_1/Chl	0.47 ± 0.04	8
δ/Chl	0.024 ± 0.003	11

Table 3. *Restoration of photophosphorylation in dependence of the order of addition of CF_1 and $CF_1(-\delta)$*

Sample	ATP synthesis	*n*
	μmol h^{-1} mg Chl^{-1}	
Thylakoids	1393	
EDTA vesicles	229	
Vesicles after addition of 50 μg protein each of:		
CF_1 + $CF_1(-\delta)$	617 ± 29	12
(1) CF_1, (2) $CF_1(-\delta)$	646 ± 57	11
(1) $CF_1(-\delta)$, (2) CF_1	547 ± 43	12

subunits were taken into account (α 55462 Da [31], β 53874 Da [32], γ 38000 Da (SDS electrophoresis), δ 20468 Da [30], ε 14702 Da [32]), this implied a stoichiometry of one δ per CF_0CF_1. Based on these data the calculated molecular mass of spinach CF_1 was ≈ 401200 Da if the subunit composition was $\alpha_3\beta_3\gamma\delta\varepsilon$ with δ accounting for 5.1% (by mass), as observed.

Reconstitution of photophosphorylation in CF_1-depleted thylakoids

We next addressed the question of why addition of $CF_1(-\delta)$ reconstitutes photophosphorylation in CF_1-depleted thylakoids. Tentatively we have explained this finding as an indication of complementary rebinding of $CF_1(-\delta)$ to $CF_0(+\delta)$ [17]. If this was true, simultaneous addition of both CF_1 and $CF_1(-\delta)$ should yield higher photophosphorylation rates than addition of CF_1 alone. This was expected because CF_1 could bind to open CF_0 but, due to steric hindrance, not to $CF_0(+\delta)$, which in turn should react with $CF_1(-\delta)$. Table 3 shows that this expectation was not met: the highest photophosphorylation rates were obtained if CF_1 was added first, followed by addition of $CF_1(-\delta)$. If CF_1 and $CF_1(-\delta)$ were added simultaneously, there was some competition between CF_1 and $CF_1(-\delta)$ indicating that $CF_1(-\delta)$ does not bind exclusively to $CF_0(+\delta)$. If the vesicles were reacted first with $CF_1(-\delta)$ and then with CF_1, the restoration of photophosphorylation was lowest, probably because some open CF_0 had bound $CF_1(-\delta)$. This did not close the proton leak and, in addition, prevented CF_1 from binding. This finding also corroborates that subunit δ of chloroplasts is not required for binding of CF_1 to CF_0, as shown earlier by Andreo et al. [20].

Again, the rather high efficiency of $CF_1(-\delta)$ was surprising: given that one single open CF_0 (high-conductance form) per vesicle is sufficient to dissipate the proton motive force required for ATP synthesis [16, 22], one should expect that

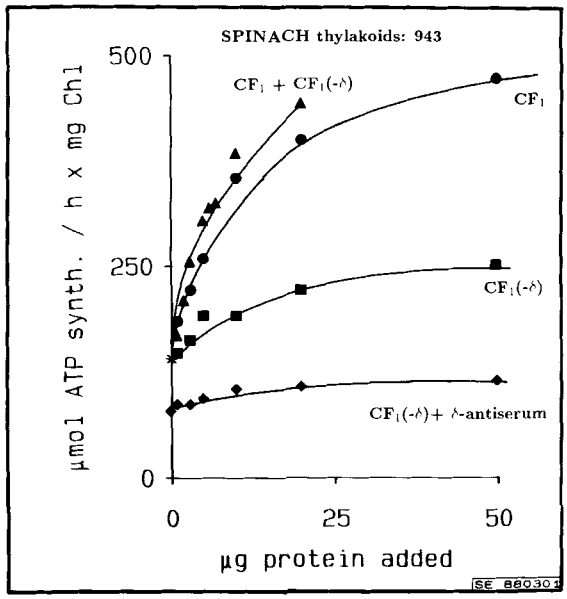

Fig. 3. *The effect of added protein mass on the rate of phenazine-methosulfate-mediated cyclic photophosphorylation in partially CF_1-depleted spinach vesicles.* The vesicles (equivalent to 10 μg chlorophyll) were derived from spinach thylakoids by EDTA treatment. The added proteins were CF_1 (●), $CF_1(-\delta)$ (■), $CF_1(-\delta)$ that was preincubated with anti-δ antiserum (◆), and CF_1 together with $CF_1(-\delta)$ (▲). The latter data were corrected for the amount of CF_1 that was contained in the $CF_1(-\delta)$ sample. The activity of control thylakoids is shown in the insert; the asterisk gives the intial activity of the partially CF_1-depleted EDTA vesicles

only a small fraction of vesicles remained proton-tight, despite EDTA treatment. Only this fraction was susceptible to reconstitution of photophosphorylation after rebinding of $CF_1(-\delta)$ to $CF_0(+\delta)$. Alternatively, a mixed mechanism might have been at work: trace contaminants of CF_1 contained within the $CF_1(-\delta)$ preparation plugged the leaks and $CF_1(-\delta)$ added catalytic capacity to such vesicles which became proton-tight. We considered the extent of CF_1 contamination within $CF_1(-\delta)$ preparations (see below). Finally, if $CF_1(-\delta)$ was indeed effective without subunit δ being involved at all, this would put in serious doubt the proposed essential role of δ in photophosphorylation [14, 17]; a similar finding obviously has led McCarty and coworkers to their revised point of view, stating that δ is not an absolute requirement for ATP synthesis (cf. [20] vs [21]).

Fig. 3 shows the rate of ATP synthesis as function of the added amount of CF_1 (●) and of $CF_1(-\delta)$ (■) to CF_1-depleted thylakoids. If one assumes saturation at 50 μg $CF_1(-\delta)$ added to 10 μg Chl (vesicles) and ascribes the restoration of photophosphorylation by $CF_1(-\delta)$ to contamination by CF_1, the $CF_1(-\delta)$ sample should have contained about 5 μg CF_1 in 50 μg $CF_1(-\delta)$, i.e. 10% (by mass). This value matched the 13% contamination that was detected by rocket immunoelectrophoresis [0.055 μgδ/CF_1; 0.007 μg δ/$CF_1(-\delta)$]. If the reconstitutive effect of $CF_1(-\delta)$ indeed was due to trace contamination by CF_1, then pretreatment of the $CF_1(-\delta)$ sample with antiserum directed against subunit δ should prevent binding of CF_1 and therefore should abolish the reconstitutive activity of $CF_1(-\delta)$.

This was observed. After preincubation of $CF_1(-\delta)$ with anti-δ antiserum (100 μl/sample) the restoration of photophosphorylation was nearly inhibited (◆). The absolute rates

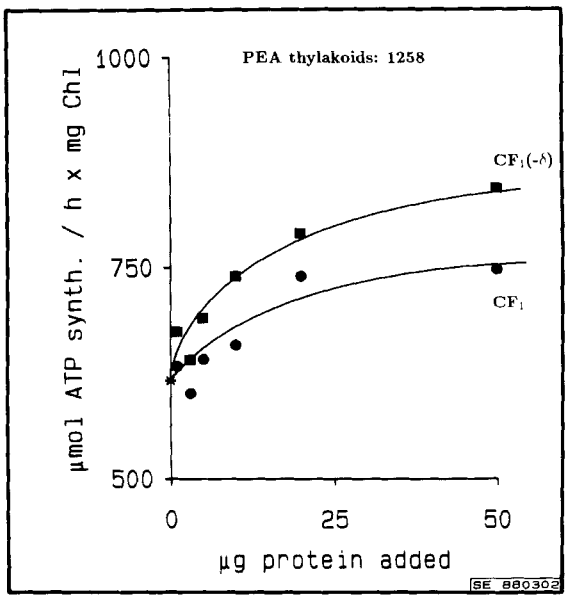

Fig. 4. *The effect of added protein mass on the rate of phenazine-methosulfate-mediated cyclic photophosphorylation in partially CF_1-depleted pea vesicles.* The vesicles (equivalent to 10 µg chlorophyll) were derived from pea thylakoids by EDTA treatment. The added proteins were CF_1 (●) and $CF_1(-\delta)$ (■). The activity of control thylakoids is shown in the insert; the asterisk gives the initial activity of the partially CF_1-depleted EDTA vesicles

of ATP synthesis however were even lower, possibly because unreacted antibodies removed CF_1 from the membrane as observed earlier with *Escherichia coli* by Smith and Sternweis [33].

The very small amount of reconstitutive activity which remained was also detectable in coreconstitution of both CF_1 and $CF_1(-\delta)$ (▲). Despite a correction for the amount of CF_1 contained within $CF_1(-\delta)$, the restoration of photophosphorylation was significantly higher than with CF_1 alone. We considered next the precise degree of depletion in both CF_1 and $CF_1(-\delta)$, measured by rocket immunoelectrophoresis as above. We found residual amounts of 47 ± 6% CF_1 and 44 ± 9% δ ($n = 9$) on the membrane. Within experimental error, the removal of CF_1 matched that of subunit δ. It was thus not surprising to obtain only a very small effect by coreconstitution of CF_1 together with $CF_1(-\delta)$.

Following unpublished results of H. Lill, we reinvestigated this issue by using pea vesicles. If pea thylakoids are treated with 100 µM EDTA (10 µM Chl, 1 mM NaCl, 10 min) at 0 °C they remain essentially proton-tight but show diminished rates of photophosphorylation which closely match the loss of $CF_1(-\delta)$ ($\approx 35\%$) i.e. these vesicles are predominantly $CF_1(-\delta)$-depleted. Fig. 4 shows that with such a vesicle preparation, $CF_1(-\delta)$ indeed was more effective in restoration of photophosphorylation than CF_1. This demonstrated the existence of a complementary rebinding of $CF_1(-\delta)$ to $CF_0(+\delta)$.

DISCUSSION

We have investigated the stoichiometry and function of subunit δ in chloroplast ATP synthase. We have corroborated evidence that CF_0CF_1 contains only one copy of δ and we have shown that restoration of photophosphorylation is strictly dependent on the presence of subunit δ.

It has been shown earlier that δ can inhibit rapid proton flow through exposed CF_0 [14]. This inhibition of proton flow through the CF_0 portion must be relieved, however, in intact ATP synthase. If one assumes conformational coupling between proton flow through CF_0 and ATP liberation in CF_1 [1, 5], subunit δ must resume a different conformational state in CF_0CF_1 which allows for the transduction of conformational energy from CF_0 into the $(\alpha\beta)_3\gamma$ entity with strict coupling to the translocation of a stoichiometric number of protons. It is compatible with this transducing role that δ, which is not necessary for the rebinding of CF_1 to CF_0 [20] (and this paper), not only attaches to CF_0 [14, 18, 19] but also binds with $K_d = 100$ nM to one particular site on the $\alpha_3\beta_3\gamma\varepsilon$ aggregate [34]. If conformational changes are required for product release [1, 2], there must be at least two elements between F_0 and F_1: tightly bound ATP can be liberated only upon conformational strain which is enforced by proton flow and built up between a mobile part (possibly subunit δ) and an immobile bearing (possibly subunits I and II of CF_0 or, in *E. coli*, the two b subunits.

The stoichiometry of one δ per CF_0CF_1 puts CF_1 next to microbial ATP synthases which also contain only one copy of δ [35] in contrast to mitochondria which seem to contain two $OSCP/MF_0MF_1$ [12, 13]. Is this difference in stoichiometry compatible with the essential role in photophosphorylation that subunit δ is proposed to play?

When assuming a merely conformational role of δ it is not so disturbing that the stalk which connects F_1 to F_0 [36, 37] in several synthases is composed of different elements: *E. coli* F_0F_1 contains two copies of subunit b and one δ, chloroplasts contain one or more subunits I and II and one δ, while mitochondria contain two OSCP plus a so-far ill-defined set of further subunits [38]. Moreover, there are only weak sequence similarities between δ from *E. coli* [39, 40] and from spinach chloroplasts [30] and OSCP from beef-heart mitochondria [41]. It is thus conceivable that the stalk does not function by several strictly conserved amino acid residues which might participate in any kind of hydrogen-bonded chain [30]. This is in line with a critical argument [36] that for electrical insulation problems a stalk like the one seen in electron microscopy cannot conduct protons into the F_1 part of ATP synthases. Given that it is not an electronmicroscopial artifact, it is conceivable that the stalk which includes δ acts through its conserved three-dimensional structure as a conformational coupler between proton movement through F_0 and ATP liberation in F_1 [1, 5, 36]. This would be compatible with the elongated shape of δ with dimensions of $10 \times 2.4 \times 2.4$ nm as determined by its rotational diffusion in solution [34]. These ideas are in line with those discussed about ten years ago by Sternweis and Smith [35, 42].

The proposed role for subunit δ acting as a conformational transducer fits into the concept of allosterically linked catalytic cooperativity between the three $\alpha\beta$ couples as suggested by Boyer in his binding-change mechanism which, despite continuing controversy, still is the most reasonable explanation for the $\alpha_3\beta_3$ stoichiometry and the existence of multiple nucleotide-binding sites in F_1 [2].

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