Eur. J. Biochem. *189*, 193–197 (1990)

Reconstitution of photophosphorylation in EDTA-treated thylakoids by added chloroplast coupling factor 1 (ATPase) and chloroplast coupling factor 1 lacking the δ subunit

Structural or functional?

Siegfried ENGELBRECHT, Gerd ALTHOFF and Wolfgang JUNGE

Fachbereich Biologie/Chemie, Abteilung Biophysik, Universität Osnabrück, Osnabrück, Federal Republic of Germany

(Received August 30/November 20, 1989) – EJB 891068

Upon EDTA treatment thylakoids lose the chloroplast coupling factor 1 (CF_1) part of their ATP synthase, CF_0CF_1, this exposes the proton channel, CF_0. The previously established ability of the CF_1 subunit δ to block the proton leak through CF_0 prompted us to study (a) the ability of complete CF_1 and, for comparison, CF_1 lacking the δ subunit to block proton leakage and thereby to reconstitute structurally some photophosphorylation activity of the remaining CF_0CF_1 molecules and (b) their ability to form functional enzymes (functional reconstitution). In order to discriminate between activities caused by added CF_1 or $CF_1(-\delta)$ and remaining CF_0CF_1, the former were inhibited by chemical modification of subunit β by *N,N'*-dicyclohexyl carbodiimide (DCCD) and the latter by tentoxin. We found that added CF_1 acted both structurally and functionally while added DCCD-treated CF_1 (DCCD-CF_1) acted only structurally. In contrast to previous observations, $CF_1(-\delta)$ and DCCD-$CF_1(-\delta)$ also acted structurally although the reduction of proton leakage was smaller than with DCCD-CF_1. Hence there was no functional reconstitution without subunit δ present. Previous studies indicated that only a small fraction of exposed CF_0 is highly conducting and that this small fraction is distinguished by its high affinity for added CF_1. The results of this study point rather to a wider distribution of CF_0 conductance states and binding affinities.

ATP synthesis at the expense of a transmembrane protonmotive force is catalysed by F_0F_1 ATP synthases which exist in thylakoids, mitochondria and a wide range of microorganisms. These enzymes comprise a membrane-embedded proton-conducting part (F_0) and a water-soluble, extrinsic part which contains the nucleotide binding sites (F_1). Solubilized F_1 functions as an ATPase. CF_1 contains five different subunits with the stoichiometry of $(\alpha,\beta)_3\gamma\delta\varepsilon$. For recent reviews on the enzyme, see Senior [1] and Schneider and Altendorf [2].

By various approaches we have demonstrated the essential role in photophosphorylation of subunit δ, one of the smaller subunits of CF_1. It was shown that subunit δ can stay on the membrane despite $CF_1(-\delta)$ removal by EDTA treatment. It probably remains attached to CF_0 where it acts as a plug in the open proton channel thus preventing dissipating proton efflux [3–5]. When purified subunit δ is added to EDTA-treated and therefore leaky membranes, it enhances the rate of photophosphorylation [6, 7]. As subunit δ does not carry catalytic activity by itself, this enhancement is further evidence for a plugging action on open channels: added subunit δ restored the protonmotive force which is necessary for ATP synthesis by those coupling factors which remained on the membrane after EDTA treatment [4, 5, 7]. The ability of purified subunit δ to plug open CF_0 cannot fully reflect the role of the subunit in intact and phosphorylating CF_0CF_1, which conducts protons in a coupled reaction [8]. As it has also been shown that subunit δ is indispensable for an active coupling factor [9], it is necessary to postulate a more involved role for subunit δ as a valve or even as part of the proton-to-conformation transducer. At first sight, the reconstitutive activity of $CF_1(-\delta)$ on EDTA-treated thylakoids seemed to argue against such an essential role for subunit δ in phosphorylation, but this could be attributed to traces of residual subunit δ which are difficult to remove from $CF_1(-\delta)$ preparations and, to a small extent, to a complementary binding of $CF_1(-\delta)$ to $(+\delta)$ [9].

In this context we attempted a differentiation between structural and functional reconstitution in EDTA-treated thylakoids (vesicles) by CF_1 and $CF_1(-\delta)$. The terms functional and structural reconstitution relate to two ways in which the phosphorylation ability of EDTA vesicles are impaired: (a) proton leakage through exposed CF_0 channels prevents the generation of a sufficiently large protonmotive force by light-driven proton-pumping; (b) even with unimpaired proton efflux the partial loss of functional coupling factors would decrease the catalytic capacity of the vesicles. In partially CF_1-depleted vesicles, addition of CF_1 or of certain fragments thereof may reconstitute photophosphorylation in either way: structurally by plugging proton leaks, i.e. without increasing the catalytic capacity, or functionally by increasing catalytic

Correspondence to S. Engelbrecht, Universität Osnabrück, Abteilung Biophysik, Barbarastrasse 11, D-4500 Osnabrück, Federal Republic of Germany

Abbreviations. CF_0 chloroplast coupling factor 0 (proton channel); CF_1 chloroplast coupling factor 1 (ATPase); $CF_1(-\delta)$ chloroplast coupling factor 1 lacking the δ subunit; DCCD, *N,N'*-dicyclohexyl carbodiimide.

Enzyme. ATPase (EC 3.6.1.34).

capacity. The latter can be accompanied by the former. It is noteworthy that these two different kinds of reconstitution are relevant only in vesicle preparations which are not completely depleted of CF_1, a situation, however, which is common for all known CF_1-depleted thylakoid vesicles: even NaBr-treated thylakoids are not completely depleted of CF_1 [10, 11]. This is further complicated by the finding that EDTA treatment liberates both CF_1 and $CF_1(-\delta)$ from the membrane, leaving behind the respective complement, CF_0 or $(+\delta)$ [3–5, 9, 12]. Functional reconstitution theoretically can then occur in two ways, by complementary rebinding of CF_1 to CF_0, and of $CF_1(-\delta)$ to $(+\delta)$. The latter possibility, however, cannot be shown directly, because of the unavoidable concomitant proton leaks generated through open CF_0 channels.

Experimental investigations have previously followed different approaches: Selman and Durbin [13] used the tentoxin-insensitve CF_1 from *Nicotiana tabacum* and *Nicotiana knightiana* for reconstitution of tentoxin-sensitive thylakoid vesicles obtained by EDTA treatment of spinach thylakoids. They observed functional reconstitution. Shoshan and Selman [14] and Bar-Zvi, Yoshida and Shavit [15] compared the efficacity of CF_1 inhibited by *N,N'*-dicyclohexyl carbodiimide (structural reconstitution) and CF_1 alone (functional reconstitution). The latter authors concluded that mainly structural reconstitution occurred. This approach was also used by us some years ago [12]. Berzborn and Schröer [16] reported that, depending on the specific activity of added CF_1, the extent of photophosphorylation after reconstitution exceeded the number of residual coupling factors that remained on the membrane. Hesse et al. also concluded that functional reconstitution took place [17] based upon the finding that [^{14}C]ADP-labelled CF_1 after reconstitution and membrane energization exchanged its adenylate moiety.

We combined and extended these previous approaches [13–15] placing emphasis on the role of subunit δ: CF_1 and $CF_1(-\delta)$ were added to EDTA vesicles with selective poisoning of either the added or the remaining CF_1.

MATERIALS AND METHODS

CF_1 was prepared from spinach thykaloids by EDTA treatment followed by anion-exchange chromatography on Whatman cellulose DE 52 and Merck fractogel TSK DEAE-650 (S) as described [12, 18]. Prior to use, it was supplemented with a slight excess of purified chloroplast subunit δ (1.3 mol δ/mol CF_1) in order to get a homogeneous population of five-subunit CF_1 ($K_d \approx 100$ nM) [19]. $CF_1(-\delta)$ was prepared by anion-exchange chromatography in the presence of 25 mM *N*-(D-gluco-2,3,4,5,6-pentahydroxyl)-*N*-methylnonanamide [6, 9, 12]; the procedure was carried out twice in order to remove remaining subunit δ quantitatively. This preparation of $CF_1(-\delta)$ did not contain any subunit δ as determined by rocket immunoelectrophoresis [9] with rabbit anti-(δ subunit) antibodies. Immunoelectrophoresis was also used for the determination of the relative levels of extraction of CF_1 and subunit δ after EDTA treatment of thylakoids.

Subunit δ, which was obtained in crude form with the anion-exchange chromatography in the presence of *N*-D (gluco-2,3,4,5,6-pentahydroxyl)-*N*-methylnonanamide (Oxyl Chemie, Bobingen, FRG) was further purified to apparent homogeneity by twofold dilution with water (to bring the detergent concentration below its critical micellar concentration), addition of 125 mM ammonium sulphate, and hydrophobic interaction chromatography on a self-prepared column [12] of fractogel TSK Butyl-650 (S), Merck. Subunit β of CF_1, nucleotides, the detergent and other contaminants passed through the column, leaving subunit δ behind. Elution with 25 mM Tris/HCl, pH 7.8, then liberated pure subunit δ from the column. This procedure circumvented the lengthy pressure dialysis steps and rechromatography that were formerly used for preparation of chloroplast subunit δ [6, 9, 12]. To date, however, chloroplast subunit δ which was prepared according to this procedure was not active in restoring photophosphorylation. Subunit δ was added to CF_1 at 1.5% (by mass) and to $CF_1(-\delta)$ at 6.5% (by mass) in order to get subunit δ-saturated CF_1 (see above) or $CF_1(-\delta)$ with added δ subunit.

N,N'-Dicyclohexyl carbodiimide modifications were carried out according to Bar-Zvi et al. [15]: after gel filtration against 50 mM Mops/NaOH, 1 mM EDTA, 2 mM K^+-ADP, pH 7, the samples [CF_1 and $CF_1(-\delta)$ at ≈ 1 mg/ml] were incubated with 0.5 mM DCCD for 1 h at 37°C, followed by gel filtration against 25 mM Tris/HCl, pH 7.8. This treatment decreased the Mg^{2+}-ATPase activities of CF_1 and $CF_1(-\delta)$ (≈ 17 U/mg) to 0.3–1.5 U/mg for DCCD-CF_1 and DCCD-$CF_1(-\delta)$ (measured in the presence of 30% (by vol.) methanol and 10 mM Na_2SO_3) [9].

EDTA vesicles were prepared by a 20-min incubation of thylakoids (400 μM chlorophyll) with 1 mM EDTA on ice, followed by centrifugation, washing and repeated centrifugation as first described by Shoshan and Shavit [20]. Tentoxin vesicles were prepared by the same procedure, 5 μM tentoxin (Sigma) were present during EDTA treatment and 2 μM tentoxin were included in the wash with 0.4 M sorbitol, 10 mM Tricine/NaOH, pH 7.8. After the second centrifugation, the vesicles were suspended in 100 mM sorbitol, 10 mM Tricine/NaOH, 10 mM NaCl, pH 7.8 at ≈ 3 mM chlorophyll.

For reconstitution, the chlorophyll concentration was lowered to 1 mM, 10 μg chlorophyll were incubated with 100 μg of sample in 25 mM phosphate, 6 mM $MgSO_4$, pH 7.8 in a volume of 300 μl on ice in the dark for 10 min, followed by illumination for 1 min in the presence of 50 μM phenazine methosulphate and 3 mM K^+-ADP. The mixture was quenched by the addition of 0.5 ml 0.6 M trichloroacetic acid, and ATP was determined via the luciferin/luciferase assay (LKB) as described before [7, 9, 12].

Oxygen measurements were performed with a Clark-type electrode in 0.2 M sucrose, 30 mM Tricine/NaOH, 20 mM NaCl, 10 mM $MgCl_2$, 2 mM $K_3Fe(CN)_6$, pH 7.5; 15 μM chlorophyll, 300 μg sample. Electrochromism was measured as described [21].

RESULTS AND DISCUSSION

Restoration of photophosphorylation in EDTA vesicles and in tentoxin vesicles was assayed as a function of added CF_1, DCCD-treated CF_1, $CF_1(-\delta)$, DCCD-$CF_1(-\delta)$, and $CF_1(-\delta)$ supplemented with an excess of purified subunit δ.

Fig. 1 shows the result of one representative set of experiments with EDTA-treated spinach thylakoids, the data were obtained at very high excess of CF_1/chlorophyll (10:1). In addition, the binding curves shown in Fig. 2 revealed very similar shapes with all four samples [CF_1, DCCD-CF_1, $CF_1(-\delta)$, DCCD-$CF_1(-\delta)$]. Saturation of reconstitution occurred between 2 μg and 3 μg CF_1/μg chlorphyll. Although the absolute rates of photophosphorylation varied from preparation to preparation, the relative extents of reconstitution were very reproducible, e.g. DCCD-CF_1 was always approximately half

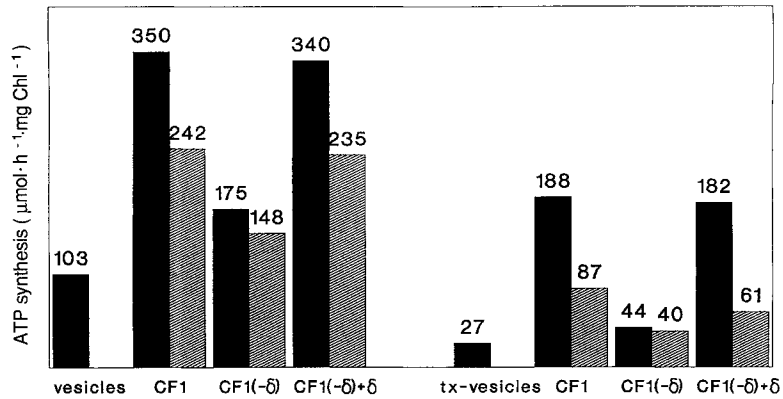

Fig. 1. *Restoration of phenazine-methosulphate-mediated photophosphorylation in EDTA-treated spinach thylakoids or EDTA-treated spinach thylakoids that were treated in addition with tentoxin (tx).* 100-µg samples were incubated for 10 min on ice in the dark with 10 µg chlorophyll (Chl), followed by 1-min illumination with saturating, heat-filtered, white light. Rocket immunoelectrophoresis indicated losses of 52% CF_1 and of 45% subunit δ in both EDTA-treated thylakoids and EDTA/tentoxin-treated thylakoids. (■) Native samples; (▨) DCCD-treated samples. $CF_1(-\delta)$ $(+\delta)$, $CF_1(-\delta)$ supplemented with subunit δ. Control thylakoid activity was 830 µmol ATP · h^{-1} · mg chlorophyll^{-1}

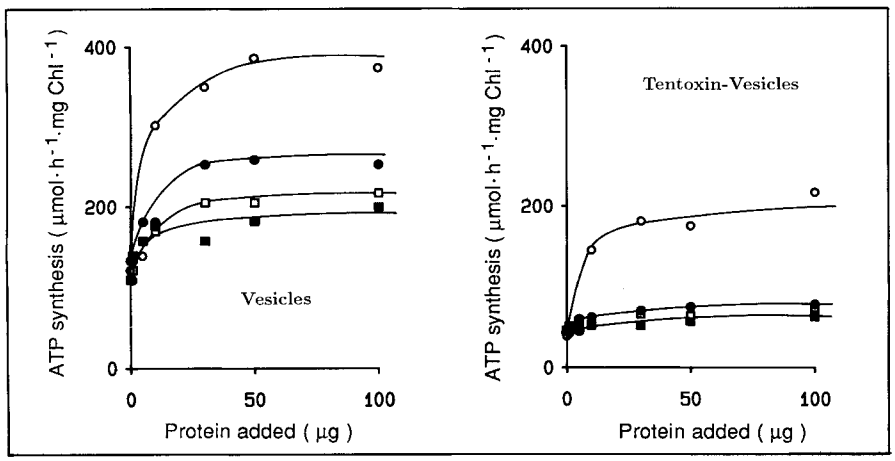

Fig. 2. *Restoration of phenazine-methosulphate-mediated photophosphorylation in EDTA-treated spinach thylakoids (vesicles) or EDTA-treated spinach thylakoids that were treated in addition with tentoxin ('tentoxin vesicles').* The indicated amounts of CF_1 (○) DCCD-CF_1 (●) $CF_1(-\delta)$ (□) and DCCD-$CF_1(-\delta)$ (■) were incubated for 10 min on ice in the dark with 10 µg chlorophyll (Chl) followed by 1 min illumination with saturating, heat-filtered, white light and luminometric ATP determination. Control thylakoids activity was 868 µmol ATP · h^{-1} · mg chlorophyll^{-1}

as effective in reconstitution as CF_1 ($55 \pm 4\%$, $n = 6$). Very similar results were also obtained with pea thylakoids and with variation of the extraction protocol (duration of EDTA treatment, temperature during extraction, concentrations of chlorophyll and EDTA, data not shown).

CF_1 restored photophosphorylation to a certain extent. DCCD-CF_1 was also effective, but to a lesser extent. Either it bound less avidly to CF_0, or it was only structurally active. In view of the similar binding curves of CF_1 and DCCD-CF_1 (Fig. 2) the first possibility could be excluded (see also below). DCCD-CF_1 only plugged leaks, thereby enabling the remaining CF_1 to resume ATP synthesis. We interpreted the clear difference in the degree of restoration of photophosphorylation by CF_1 and DCCD-CF_1 as functional reconstitution by added CF_1, in complete accordance with previously published data [13–17].

$CF_1(-\delta)$ restored photophosphorylation, but to an even smaller extent than DCCD-CF_1. DCCD-$CF_1(-\delta)$ was only slightly less effective than $CF_1(-\delta)$ alone. According to earlier work [9, 12] no reconstitution at all was expected neither through plugging of leaks nor through addition of catalytic capacity. Taken together, these results had to be explained by the assumption that both, $CF_1(-\delta)$ and DCCD-$CF_1(-\delta)$, after rebinding to CF_0 impaired proton efflux slightly, thereby enlarging the protonmotive force a little.

$CF_1(-\delta)$ supplemented with subunit δ was nearly indistinguishable from CF_1. This suggested highly intact preparations of $CF_1(-\delta)$ and subunit δ which were fully competent to recombine to form native CF_1.

In the tentoxin vesicle approach EDTA vesicles were treated with tentoxin in order to inhibit the remaining CF_1, a subsequent wash removed excess tentoxin. The tentoxin vesicles were then reconstituted with various samples. These vesicles were nearly completely inhibited, as was evident from the very low photophosphorylation rate (rates uncorrected for intrinsic ATP amounted to 20–25 µmol ATP/mg chlorophyll). A shift of tentoxin from binding sites on remaining CF_1 to added samples could be excluded because both $CF_1(-\delta)$ and DCCD-$CF_1(-\delta)$ were practically ineffective on tentoxin vesicles.

Any restoration of photophosphorylation in tentoxin vesicles would indicate functional reconstitution. CF_1 improved

Table 1. *Electron-transport rates as measured with a Clark-type electrode*
nig, 500 μM nigericin; n.d., not determined. All vesicle samples contained 60 μg chlorophyll

Sample	Addition	Experiment 1		Experiment 2	
		−nig	+nig	−nig	+nig
		mmol $e^- \cdot s^{-1} \cdot$ mol chlorophyll^{-1}			
Thylakoids	–	25	104	38	132
Vesicles	–	138	145	143	144
	300 μg CF_1	95	117	83	112
	300 μg DCCD-CF_1	76	121	83	114
	300 μg $CF_1(-\delta)$	116	138		
	300 μg DCCD-$CF_1(-\delta)$	114	131		
	25 μM DCCD	46	n.d.		

Table 2. *The half-decay times (t) of the flash light-induced electrical potential across the thylakoid membrane, as determined via the electrochromic absorption change at 522 nm [21]*
Tx, tentoxin. All vesicle samples contained 40 μg chlorophyll

Sample	Addition	*t*	
		−Tx	+Tx
		ms	
Thylakoids	–	125	125
Vesicles	–	6	8
	200 μg CF_1	41	41
	200 μg DCCD-CF_1	42	39
	200 μg $CF_1(-\delta)$	22	21
	200 μg DCCD-$CF_1(-\delta)$	23	23

the photophosphorylation rate 5−7-fold, whereas DCCD-CF_1 was significantly less active. The remaining activity of DCCD-CF_1 was probably due to a (minor) DCCD-free subpopulation (note the different effectiveness of the two different preparations of DCCD-CF_1 in Figs 1 and 2).

The ineffectiveness of $CF_1(-\delta)$ and DCCD-$CF_1(-\delta)$ was explained as follows: the relative level of extraction of the vesicles was determined by rocket immunoelectrophoresis. It indicated a 52% loss in CF_1 and a 45% loss in subunit δ. Hence only 7% of the exposed CF_0 could be expected to retain subunit δ. A functional reconstitution by $CF_1(-\delta)$ could be expected only if all proton leaks through open CF_0 channels were overcome by rapid proton pumping, which was highly unlikely. Therefore, a slight impairment of proton efflux through the CF_0 channel was the most probable explanation for the reconstitutive activity of $CF_1(-\delta)$ in EDTA vesicles.

This view was supported by the data shown in Tables 1 and 2: $CF_1(-\delta)$ improved the electron-transport rates of EDTA-treated vesicles and it increased the decay time of the membrane potential in these vesicles generated by a short flash of light and indicating a decrease in membrane permeability.

In conclusion functional reconstitution was only obtained with CF_1, that is in the presence of subunit δ, whereas all other samples were effective only at the structural level.

It would have been desirable to determine the number of CF_1 rebound directly after reconstitution. However, with the vesicle preparations currently used this was not possible. Any determination of membrane-bound CF_1 must discriminate between CF_0CF_1 and CF_1 that just sticks to the membrane. In order to remove the latter, the membrane would have to be washed and this washing in our experience always resulted in further depletion. This is the reason why all reconstitutions were carried out without removal of excess sample.

The DCCD-derived samples might have had an impaired ability to rebind (correctly) to CF_1-depleted CF_0 [14]. This possibility was checked by measuring the ratio of the rates of uncoupled to coupled oxygen evolution and, additionally, by measuring the decay of the electrochromic absorption changes at 522 nm [21], both of which can be used as indicators of the proton leak conductance of the membrane. As shown in Tables 1 and 2, the leak-plugging action of added CF_1 and DCCD-CF_1 on one hand, and of $CF_1(-\delta)$ and DCCD-$CF_1(-\delta)$ on the other, were practically the same and therefore the rebinding ability of the DCCD-modified samples was not changed.

How do these results relate to earlier work? In contrast to data in [9], where the reconstitutive activity of $CF_1(-\delta)$ could be abolished by immunodecoration of residual subunit δ; in this study, $CF_1(-\delta)$ obviously blocked the proton leak through open CF_0, although to a lesser extent than CF_1. The reasons for this discrepancy have remained unclear. The high excess of $CF_1(-\delta)$ to chlorophyll, which was used here, might be relevant. The antibodies which masked remaining subunit δ [9] also decreased the rate of photophosphorylation. This could be explained simply by further detachment of CF_1 from the vesicles [9] but it might have affected the potential for reconstitution in these vesicles in a more complicated way. The easiest explanation for the reconstitutive activity of $CF_1(-\delta)$ is a small amount of subunit δ which was not removed but undetected. This was disproved by the finding that in rocket electrophoresis there was no subunit δ detectable, even with large amounts of sample. The homogeneity of the $CF_1(-\delta)$ preparations was further demonstrated by the nearly complete lack of effectiveness of $CF_1(-\delta)$ and DCCD-$CF_1(-\delta)$ on the tentoxin vesicles (an improvement in the photophosphorylation rate by 13−17 μmol $ATP_{synthesized} \cdot h^{-1} \cdot$ mg chlorophyll^{-1} was not considered to be relevant).

Similar data were also obtained with thylakoids from peas which were treated, at very low chlorophyll concentrations, with EDTA (data not shown). Proton conduction in these vesicles was previously investigated [21, 22] and it was deduced that, partly due to subunit δ remaining on CF_0 and partly due to a rearrangement of most of the CF_1-depleted CF_0 which were thus converted from a high to a low conductance state, only very few highly conducting CF_0 were left over. These were able to rebind CF_1 while the majority were badly conducting channels which had lost this ability [23]. According to these results, the minimal and maximal restoration of photophosphorylation is predetermined by the level of extraction of CF_1 (50% extraction means a yield after reconstitution of 50% of the initial rate of photophosphorylation). The degree of functional reconstitution is negligible because of the small number of reconstitutable CF_0, in the range of one or two/vesicle.

Clearly, this prediction was not verified in this study. Restoration of photophosphorylation was, at least partly, functional and it was significantly smaller than the predicted value. The discrepancy might be related to the different time scales and energization at which the various experiments were carried out. Energization of intact thylakoids in single-flash experiments produces a ΔpH of ≈ 0.05 and a membrane voltage

of ≈ 50 mV for about 10 ms, whereas photophosphorylation with saturating white light for 1 min in the presence of 50 μM phenazine methosulphate will produce Δ pH $\geqslant 3$ and a negligible transmembrane voltage.

The current data can be explained by the assumption that a substantial number of CF_1-depleted CF_0 no longer reacted with CF_1. The remaining (moderately conducting) fraction, however, reacted with CF_1 with moderate affinity and every member of this population started ATP synthesis and simultaneously switched on about one CF_0CF_1 that remained on the membrane. Proton leaks were still effective to an extent which prevented photophosphorylation rates comparable to the total number of intact CF_0CF_1. It therefore appears that CF_0 can assume various conformations with different proton conduction and rebinding affinity. A similar conclusion was drawn by Nelson [24].

This point is also illustrated by the effect of $CF_1(-\delta)$ on open CF_0 and is further demonstrated by the following observation: in the usual reconstitution assay involving a 10-min incubation of vesicles with freshly prepared CF_1, the rate of photophosphorylation was increased by 247 μmol $ATP_{synthesized} \cdot h^{-1} \cdot$ mg chlorophyll^{-1}. The same CF_1 sample, after two weeks of storage at 4 °C in the presence of 1 mM ADP (and without any sign of denaturation), increased the ATP synthesis rate by only 139 μmol $\cdot h^{-1} \cdot$ mg chlorophyll^{-1} after a 10-min incubation but by 274 μmol $\cdot h^{-1} \cdot$ mg chlorophyll^{-1} after a 60-min incubation. In the same experiment, the EDTA extract of the vesicle preparation (mainly CF_1 [7]) restored the photophosphorylation rate to the same extent, irrespective of the incubation time. A possible explanation (see also Nelson [24]) is that conformational rearrangements which are necessary for effective reconstitution, depending on the initial conformation of both CF_0 and CF_1, can be rather slow processes: an induced fit over many or large energy barriers.

We gratefully acknowledge the expert technical assistance provided by Karin Schürmann and preparation of the figures by Hella Kenneweg. We thank the *Deutsche Forschungsgemeinschaft* for funding (SFB 171/B3).

REFERENCES

1. Senior, A. E. (1988) *Physiol. Rev. 68*, 177–231.
2. Schneider, E. & Altendorf, K. (1987) *Microbiol. Rev. 51*, 477–497.
3. Junge, W., Hong, Y. Q., Qian, L. P. & Viale, A. (1984) *Proc. Natl Acad. Sci. USA 81*, 3078–3082.
4. Lill, H., Engelbrecht, S. & Junge, W. (1987) in *Progress in photosynthesis research* (Biggins, J., ed.) vol. III, pp. 141–144, Martinus Nijhoff Publishers, Dordrecht.
5. Lill, H., Engelbrecht, S. & Junge, W. (1988) *J. Biol. Chem. 263*, 14518–14522.
6. Engelbrecht, S. & Junge, W. (1987) *FEBS Lett. 219*, 321–325.
7. Engelbrecht, S. & Junge, W. (1988) *Eur. J. Biochem. 172*, 213–218.
8. Junge, W. (1987) *Proc. Natl Acad. Sci. USA 84*, 7084–7088.
9. Engelbrecht, S., Schürmann, K. & Junge, W. (1989) *Eur. J. Biochem. 179*, 117–122.
10. Andreo, C. S., Patrie, W. J. & McCarty, R. E. (1982) *J. Biol. Chem. 257*, 9968–9975.
11. Leckband, D. & Hammes, G. G. (1988) *Biochemistry 27*, 3629–3633.
12. Engelbrecht, S., Lill, H. & Junge, W. (1986) *Eur. J. Biochem. 160*, 635–643.
13. Selman, B. R. & Durbin, R. D. (1978) *Biochim. Biophys. Acta 502*, 29–37.
14. Shoshan, V. & Selman, B. R. (1980) *J. Biol. Chem. 255*, 384–389.
15. Bar-Zvi, D., Yoshida, M. & Shavit, N. (1985) *Biochim. Biophys. Acta 806*, 341–347.
16. Berzborn, R. J. & Schröer, P. (1976) *FEBS Lett. 70*, 271–275.
17. Hesse, H., Jank-Ladwig, R. & Strotmann, H. (1976) *Z. Naturforsch. 31c*, 445–451.
18. Apley, E., Wagner, R. & Engelbrecht, S. (1985) *Anal. Biochem. 150*, 145–154.
19. Wagner, R., Apley, E. C., Engelbrecht, S. & Junge, W. (1988) *FEBS Lett. 230*, 109–115.
20. Shoshan, V. & Shavit, N. (1973) *Eur. J. Biochem. 37*, 355–360.
21. Lill, H., Althoff, G. & Junge, W. (1987) *J. Membr. Biol. 98*, 69–78.
22. Lill, H., Engelbrecht, S., Schönknecht, G. & Junge, W. (1986) *Eur. J. Biochem. 160*, 627–634.
23. Lill, H. & Junge, W. (1989) *Eur. J. Biochem. 179*, 459–467.
24. Nelson, N. (1980) *Ann. N. Y. Acad. Sci. 258*, 25–35.