

The prokaryotic thermophilic TF_1 -ATPase is functionally compatible with the eukaryotic CF_o -part of the chloroplast ATP-synthase

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Abstract

The ATP synthase from chloroplasts, $CF_o \cdot F_1$, was reconstituted into liposomes, from which most of CF_1 was removed by a short treatment with guanidinium chloride. ATP-dependent proton uptake was restored with these CF_o -liposomes even better by the addition of the bacterial TF_1 - than of the related CF_1 -part. This proton uptake was prevented by tentoxin, a specific inhibitor of the CF_1 -ATPase, in these $CF_o \cdot F_1$ -liposomes, but not in the hybrid $CF_o \cdot TF_1$ -liposomes. Venturicidin, a specific inhibitor of proton flow through CF_o , was able to block it in both the hybrid $CF_o \cdot TF_1$ -liposomes and reconstituted $CF_o \cdot F_1$ -liposomes. These results indicate that the bacterial TF_1 -part binds to the eukaryotic CF_o -part of four subunits forming a functional $CF_o \cdot TF_1$ -ATPase.

Key words: Thermophilic bacterium PS3; Spinach chloroplast; $CF_o \cdot TF_1$ -ATP synthase; Reconstruction (in vitro); Functional compatibility

1. Introduction

The ATP synthase of chloroplasts, bacteria and mitochondria consists of two sectors, the F_1 -part, which bears the catalytic sites for ATP synthesis and the F_o -part, that functions to transfer protons [1] or in some bacteria sodium ions [2] across the correlated membranes. Only Na^+ ions allowed to show that the F_o -part of a bacterial $F_1 \cdot F_o$ ATPase from *Propionigenium modestum* binds ions [3] involved in energy transduction. This ion specificity of the F_o -part could be supported by a hybrid constructed in vitro with F_1 from *E. coli* and F_o from *P. modestum* [4]. Thus first energy conservation occurs in the F_o -part, probably by binding Na^+ or H_3O^+ ions [5]. The crucial question is now, in which way this energy is

transferred from F_o to F_1 to release tightly bound ATP, i.e. which subunits are involved in the interaction between F_o and F_1 .

We report here the formation of a hybrid constructed in vitro with prokaryotic TF_1 from the thermophilic bacteria PS3 and eukaryotic CF_o from spinach chloroplast on $CF_o \cdot CF_1$ liposomes, which were largely depleted of CF_1 . This $CF_o \cdot TF_1$ hybrid is more active in ATP-driven ACMA quenching than the $CF_o \cdot F_1$ ATPase, reconstituted with isolated CF_1 .

2. Materials and methods

$CF_o \cdot F_1$ was prepared according to the method of Fromme et al. [6], CF_1 according to Berger et al. [7], and TF_1 according to Kagawa and Yoshida [8]. Protein concentration in CF_1 and TF_1 was measured by the method of Lowry and by optical absorbance at 280 nm using an extinction coefficient of 0.48 cm²/mg.

500 mg of phosphatidyl choline type II S from Sigma were solubilised in 10 ml buffer of 10 mM Tricine, pH 8, 0.2 mM EDTA, 5 mM DTT containing 100 mg of desoxycholate and 200 mg of cholate. The suspension was stirred at room temperature until homogeneity, then cooled to 0°C and sonicated for 1 min with the standard tip at full energy (Labsonic U, Braun Biotech Int.; 350 W/min). The lipid solution was centrifuged in a Beckman rotor SW 60 for 10 min at 27,000 rpm. 2 mg $CF_o \cdot F_1$ in 0.32 ml were mixed with 3.68 ml of the phospholipid solution and submitted to 10 times 0.5 s bursts (with 0.5 s cooling between each) with the needle tip of the sonicator at half energy at 0°C. Liposomes were kept in ice for 1 h. This sample of 4 ml was dialysed against 2 liters of buffer containing 10 mM Tricine pH 8, 0.2 mM EDTA, 2.5 mM $MgSO_4$, 130 mM K_2SO_4 and 0.25 mM DTT preequil-

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Abbreviations: ACMA, 9-amino-6-chloro-2-methoxy-acridine; CHAPS, 3-[(cholamidopropyl)-dimethyl-ammonio]-1-propane sulfate; DCCD, dicyclohexylcarbodiimide; DTT, D,L-dithio-threitol; EDTA, ethylenediaminetetraacetic acid; FCCP, carbonyl cyanide-*p*-trifluorohydroxyphenyl hydrazine; GCL, guanidinium chloride; [¹²⁵I]ASA- β -ala-OH, 3-[¹²⁵Iodo]-4-azido-2-hydroxybenzoyl- β -alanine; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl] glycine.

ibrated at 30°C. Dialysis was run for 20 h at 30°C with one change of the dialysis buffer. The $CF_0 \cdot F_1$ liposomes suspension contained 0.5 mg of $CF_0 \cdot F_1$ per 46 mg phospholipid in 1 ml.

CF_1 was depleted from the $CF_0 \cdot F_1$ liposomes by guanidinium chloride treatment according to McEnery et al. [9]. 0.5 ml of the $CF_0 \cdot F_1$ liposomes were mixed with 3 ml buffer containing 10 mM Tricine pH 8, 0.2 mM EDTA and centrifuged in a Beckman rotor SW 60 for 1 h at 50,000 rpm. Pellets were resuspended in 1 ml buffer containing 10 mM Tricine, pH 8, 5 mM EDTA and 315 mg guanidinium chloride (GCl). The suspension was kept in ice for 5 min, then mixed with 6 ml of the same buffer but without GCl and cooled at 0°C. After centrifugation as described above, the pellets were resuspended in 3.5 ml of the same buffer without GCl at 0°C and centrifuged again as above. The pellet of CF_1 depleted $CF_0 \cdot F_1$ liposomes was resuspended in 0.5 ml buffer containing 10 mM Tricine pH 8, 0.2 mM EDTA, 2.5 mM $MgSO_4$ and 130 mM K_2SO_4 , and stored in ice. In photolabeling experiments the same procedure was used after incubation with 0.5 μM [^{125}I]ASA- β ala-OH (10 Ci/mmol) and irradiation by wavelengths longer than 300 nm.

The CF_1 depleted liposomes were reconstituted with CF_1 or TF_1 , as follows: 20 μl of CF_1 depleted liposomes were mixed with 0 to 20 μg of CF_1 or TF_1 in a buffer containing 10 mM Tricine pH 8, 0.2 mM EDTA, 2.5 mM $MgSO_4$ and 130 mM K_2SO_4 , and kept in ice for at least 2 h. Not incorporated protein was separated from the $CF_0 \cdot F_1$ liposomes by flotation in a discontinuous sucrose gradient according to Rigaud [10].

By trypsin treatment of the $CF_0 \cdot F_1$ liposomes ATPase molecules facing the outside medium were degraded. The remaining ATPase activity in the liposomes after their solubilization in 1.5% Triton X-100 allowed to determine the amount of ATPase molecules facing the inside of the liposomes.

ATPase activity of soluble F_1 -ATPase as well as $F_0 \cdot F_1$ liposomes (each containing 35–70 μg protein/ml) were measured. They were incubated for 10 min at 37°C in a buffer containing 50 mM Tricine pH 8.0, 37.5 mM $NaHCO_3$, 6.8 mM $MgSO_4$, 15 mM ATP and 1.5% Triton X-100 with various amounts of trypsin. Reactions were quenched by perchloric acid and inorganic phosphate was determined colorimetrically as molybdeneblue.

3. Results

3.1. Reconstitution of $CF_0 \cdot F_1$ into liposomes

The chloroplasts ATP synthase $CF_0 \cdot F_1$ was reconstituted into liposomes, which were separated by a discontinuous sucrose gradient according to Rigaud [10]. The proteoliposomes were found between 10–20% sucrose. The protein, which was not incorporated into the liposomes, was detected on the bottom of the tube. After the proteoliposome fraction was dissolved by Triton X-100, its ATPase activity represented 85%, and that of the protein about 15% of the total activity measured.

When the proteoliposomes were incubated with trypsin for two hours and their ATPase activity was determined, after they were dissolved by Triton X-100, only 8% of the original 85% were found. Thus apparently 90% CF_1 of $CF_0 \cdot F_1$ reconstituted into the proteoliposomes, is located on the outer surface.

3.2. Removal of CF_1 from $CF_0 \cdot F_1$ -liposomes

CF_1 depleted liposomes were obtained, when $CF_0 \cdot F_1$ liposomes were incubated with 1.4 M guanidinium chloride. ATP-dependent proton uptake was restored best by the addition of TF_1 -ATPase, when only a short incubation time of 5 min with guanidinium chloride in diluted liposomes samples was used before (Table 1). These CF_1

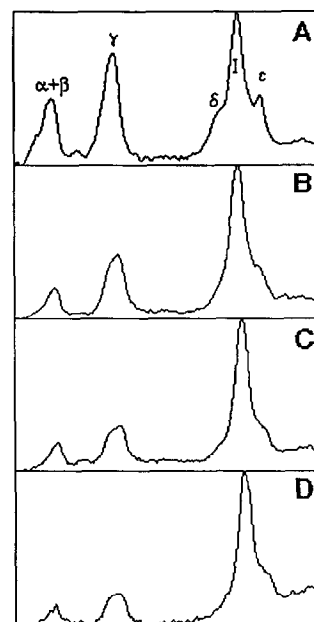


Fig. 1. Solubilisation of the CF_1 part from CF_0F_1 liposomes after treatment with (A) 10 mM Tricine, 0.2 mM EDTA, pH 8.0 (reconstitution buffer), (B) 0.7 mM EDTA, 1 mM Tricine, pH 8.0, (C) 1.4 M guanidinium chloride, 5 mM EDTA, 10 mM Tricine, pH 8.0, (D) 2 M NaBr, 10 mM Tricine, pH 8.0^[9]. A concentration of 0.5 μM [^{125}I]ASA- β ala-OH (10 Ci/mmol) was used for photolabeling before solubilisation procedures. The peak of subunit I was used as internal standard.

depleted liposomes remain active at least for one week, when stored at 0°C. After photolabeling experiments with [^{125}I]ASA- β ala-OH incubation with guanidinium chloride yielded depletion of CF_1 subunits, the efficiency of which was placed between the incubation with EDTA and NaBr. (Fig. 1).

Table 1

ATP-dependent proton uptake after addition of various amounts of CF_1 and TF_1

TF1 added μg per 5 μg of CFOF1 initially present	CFOF1 liposomes treated with 1.4 M guanidinium hydrochlorid for different periods		
	5 min	10 min	20 min
0	0	0	0
1.56	1.12	0.65	0.28
3.9	0.87	0.67	–
7.8	0.81	0.63	0.35
15.6	0.79	0.57	0.33

CF_1 was depleted from $CF_0 \cdot F_1$ liposomes by treatment with 1.4 M guanidinium chloride in 5, 10 and 15 min. Reconstitution with TF_1 to the hybrid $CF_0 \cdot TF_1$ ATPase was determined by measuring the rate of the initial ATP-dependent ACMA fluorescence quenching. CF_1 depleted liposomes were incubated in the dialysis medium with increasing amounts of TF_1 (μg of TF_1 per 5 μg of $CF_0 \cdot F_1$ initially present in the liposomes). Rate was ACMA fluorescence quenching per second expressed as percent of the fluorescence observed before ATP addition.

3.3. Reconstitution of ATP-dependent proton uptake by addition of CF_1 or TF_1 to CF_0 -liposomes

CF_1 was treated with 50 mM DTT before addition to CF_0 liposomes, what is not necessary for TF_1 . ATP-dependent proton uptake had to be measured by quenching of the fluorescent dye ACMA in the presence of valinomycin, when CF_1 was added to CF_0 -liposomes. Without valinomycin no ACMA quenching could be observed. With TF_1 added, valinomycin was not necessary, but in its presence this proton uptake was faster and greater. After addition of increasing amounts of CF_1 and TF_1 resp. the initial rate of fluorescence quenching, $R\%/s$, was determined as a measure for proton uptake (Fig. 2). Maximal initial rates of ACMA quenching were obtained by addition of $0.8\ \mu\text{g}$ CF_1 and $0.44\ \mu\text{g}$ TF_1 resp. to CF_0 liposomes, prepared from $5\ \mu\text{g}$ $CF_0 \cdot F_1$. This corresponds to a maximal reconstitution of 16% active $CF_0 \cdot F_1$ -ATPase and 8.8% hybrid $CF_0 \cdot TF_1$ -ATPase resp. The maximal initial rate of reconstituted $CF_0 \cdot F_1$ -ATPase with $0.8\ \mu\text{g}$ CF_1 was found to be 36% of that which was obtained by the hybrid $CF_0 \cdot TF_1$ -ATPase, using $0.44\ \mu\text{g}$ TF_1 . The maximal final fluorescence quenching, $Q\%$, (measured in % of the fluorescence observed just after ATP addition) was 44% with $0.16\ \mu\text{g}$ CF_1 and 57% with $0.44\ \mu\text{g}$ TF_1 .

3.4. Action of the CF_1 -specific inhibitor, tentoxin and of the CF_0 -specific inhibitor venturicidin on $CF_0 \cdot F_1$ - and the hybrid $CF_0 \cdot TF_1$ -liposomes

A final concentration of $12\ \mu\text{M}$ tentoxin inhibited completely quenching of ACMA, which is obtained by

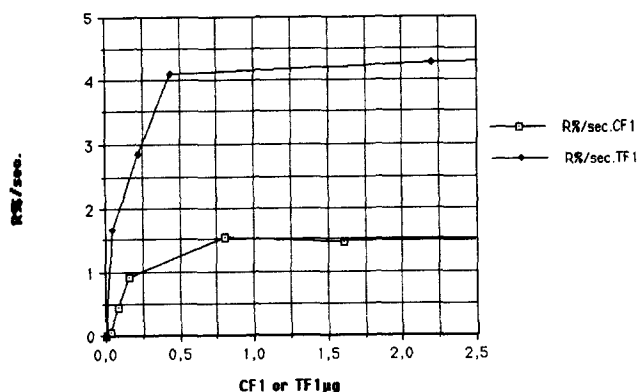


Fig. 2. ATP-dependent proton uptake of CF_0 -liposomes after addition of various amounts of CF_1 and TF_1 . ATP induced fluorescence quenching was measured in 10 mM tricine pH 8, 0.2 mM EDTA, 2.5 mM $MgSO_4$, and $0.25\ \mu\text{M}$ ACMA. 1.25 mM ATP was added, then $0.9\ \text{nM}$ valinomycin. $R\%$ was the initial rate of fluorescence quenching per second. R was measured in % of the fluorescence observed just after ATP addition to take into account the direct fluorescence quenching induced by ATP on the ACMA dye. For reconstitution CF_1 depleted proteoliposomes (prepared from $CF_1 \cdot F_0$ liposomes, containing $5\ \mu\text{g}$ $CF_1 \cdot F_0$) were mixed with various amounts of CF_1 (50 mM DTE treated) or TF_1 in dialysis medium.

CF_1 , —□—; TF_1 , —◆—;

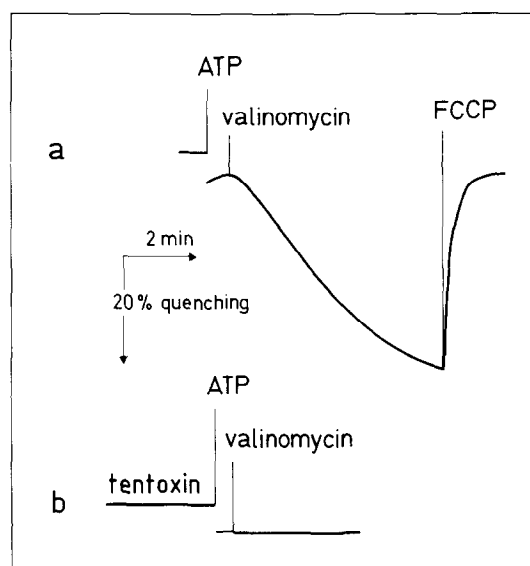


Fig. 3. Quenching of ACMA fluorescence by CF_0 -liposomes, reconstituted with CF_1 , in the presence or absence of tentoxin. CF_1 depleted $CF_0 \cdot F_1$ liposomes were incubated with $6\ \mu\text{g}$ CF_1 for $10\ \mu\text{g}$ $CF_0 \cdot F_1$ initially present before guanidinium chloride treatment. $10\ \mu\text{l}$ of these liposomes, (corresponding to $500\ \mu\text{g}$ of lipids) were added to $0.4\ \mu\text{l}$ of the medium: 10 mM tricine pH 8, 0.2 mM EDTA, 2.5 mM $MgSO_4$ and $0.25\ \mu\text{M}$ ACMA. Valinomycin was added to a final concentration of $1\ \text{nM}$, ATP to $1.25\ \text{mM}$ and FCCP to $250\ \text{nM}$. When added tentoxin ATP dependent fluorescence quenching of reconstituted $CF_1 \cdot F_0$ liposomes in the absence (curve a) or in the presence (curve b) of tentoxin.

ATP-dependent proton uptake in liposomes, into which $CF_0 \cdot F_1$ -ATPase was reconstituted as a whole (Fig. 3) and CF_0 -liposomes, with which $CF_0 \cdot F_1$ was reconstituted by the addition of CF_1 (Fig. 4). This inhibition of ACMA quenching could not be found, after the hybrid $CF_0 \cdot TF_1$ -ATPase was formed by adding TF_1 to CF_0 -liposomes (Fig. 5). In all liposomes, studied above, $13\ \mu\text{M}$ venturicidin, a specific inhibitor of the CF_0 -part, as well as $63\ \mu\text{M}$ DCCD, a specific inhibitor of all F_0 -parts known, prevented quenching of ACMA, driven by ATP-dependent proton uptake (not shown here). The addition of TF_1 to CF_0 -liposomes created obviously an hybrid $CF_0 \cdot TF_1$ -ATPase, which transported protons into the liposomes by hydrolysis of ATP.

4. Discussion

Hybrid $F_0 \cdot F_1$ -ATPases which are reconstituted with F_1 - and F_0 -parts from different species, are useful in studying function and interaction of these two parts. Recently it was demonstrated by F_1 -stripped everted membrane vesicles that F_1 and F_0 -parts from *E. coli* and the thermophilic bacterium PS3 [11] as well as those from the closely related bacteria *E. coli* and *Salmonella typhimurium* [12] are functionally compatible. Furthermore a hybrid ATPase, composed of F_1 from *E. coli* and

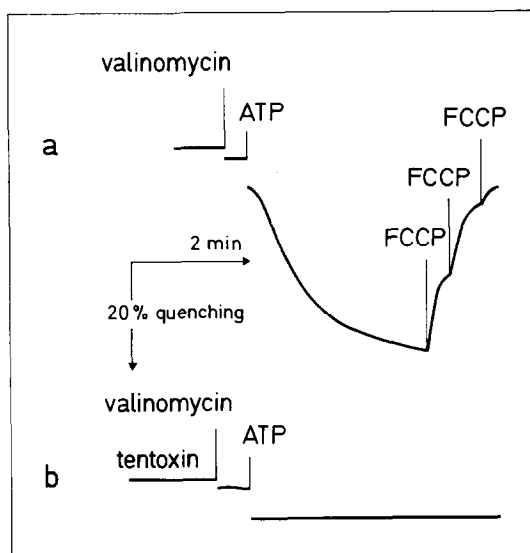


Fig. 4. ATP-dependent quenching of ACMA fluorescence by $\text{CF}_0 \cdot \text{F}_1$ liposomes in the presence or absence of tentoxin. $10 \mu\text{l}$ of the $\text{CF}_0 \cdot \text{F}_1$ liposomes (corresponding to $250 \mu\text{g}$ of lipids) were added to $0.4 \mu\text{l}$ of the medium containing 10 mM EDTA, 2.5 mM MgSO_4 and $0.25 \mu\text{M}$ ACMA. Valinomycin was added to a final concentration of 0.36 nM , ATP to 2 mM , FCCP to 125 nM . When added tentoxin was $12 \mu\text{M}$ and liposomes were preincubated for 5 min with tentoxin ATP-dependent fluorescence quenching by $\text{CF}_1 \cdot \text{F}_0$ liposomes in the absence (curve a) or in the presence (curve b) of tentoxin was recorded.

F_0 from *Propionigenium modestum* in a reconstituted system, was shown to be a pump of sodium ion as the original $\text{F}_0 \cdot \text{F}_1$ -ATPase from *P. modestum*. It was concluded that the F_0 -part determines ion specificity [4]. This essential finding was supported by extended experiments with F_1 and F_0 parts of *P. modestum* and the thermophilic bacterium PS3, demonstrating that the hybrid $\text{PF}_0 \cdot \text{TF}_1$ -ATPase is a sodium pump and the hybrid $\text{TF}_0 \cdot \text{PF}_1$ -ATPase a proton pump. Both transports could be inhibited by DCCD (E.B., unpublished). A first hybrid ATPase from prokaryotic and eukaryotic F_1 and F_0 parts was formed by binding of *E. coli* F_1 to membranes of rat liver mitochondria and the related mitochondrial F_1 to *E. coli* everted membranes [14]. In a second approach the thermophilic TF_1 was incubated with lettuce chloroplasts, which were partially depleted of CF_1 by EDTA. Because the photophosphorylation activities after reconstitution with CF_1 as well as with TF_1 were inhibited by tentoxin in both cases, it was concluded that TF_1 sealed only the membrane, and endogenous CF_1 promoted tentoxin-sensitive ATP synthesis [15].

The hybrid $\text{CF}_0 \cdot \text{TF}_1$ -ATPase, however, reconstituted here in liposomes, was completely insensitive to tentoxin (Fig. 5c). Furthermore rebinding of TF_1 to CF_0 -liposomes was better reproducible than that of CF_1 , reflecting the extraordinary stability of TF_1 . This parallels our experience, mentioned above, in the reconstitution of a sodium ion pump by adding TF_1 to liposomes with F_0 of *P. modestum*, where the hybrid $\text{PF}_0 \cdot \text{TF}_1$ -ATPase

was ten times better coupled (ratio: Na^+/ATP) (E.B., unpublished) than the related hybrid with F_1 from *E. coli* [4]. It was, therefore, surprising that only about 1 of 10 CF_0 had bound TF_1 forming an active ATPase. This was the case, though about 90% CF_1 was on the outside of $\text{CF}_0 \cdot \text{F}_1$ liposomes and most of CF_1 was removed by guanidinium chloride (Table 1). This removal could be evaluated semiquantitatively by a new water soluble, radioactive photolabel [^{125}I]ASA- β Ala-OH, which was introduced for bulk phase modification of membrane proteins (Fig. 1). Apparently most of the subunits α , β and δ were removed, whereas some ϵ and predominantly remained on the liposomes. The treatment with NaBr appeared in our hands the most effective one for the removal of CF_1 subunits, but not in reconstitution experiments with CF_1 or TF_1 resp. After treatment with guanidinium chloride for 5 min none of the CF_0 -parts appeared to be leaky under the conditions used for ACMA quenching (Fig. 5a).

One possible explanation may be that these CF_0 moieties do not function as open channels, but by a carrier-type mechanism, as found in studies with the F_0 -part of

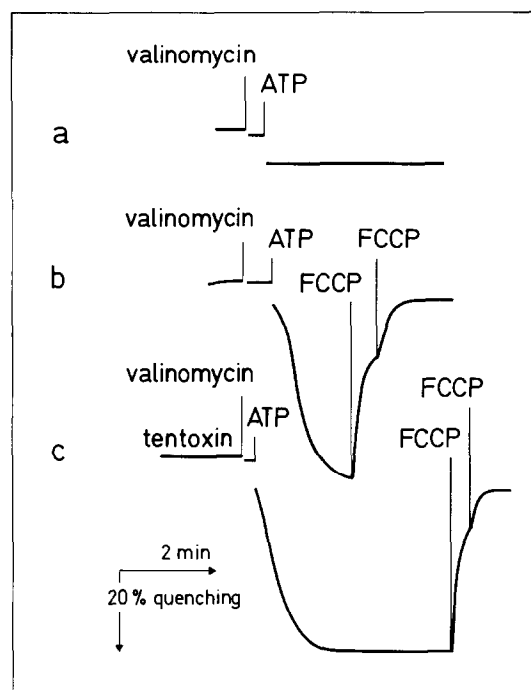


Fig. 5. ATP-dependent quenching of ACMA fluorescence by $\text{CF}_0 \cdot \text{F}_1$ liposomes and $\text{CF}_0 \cdot \text{TF}_1$ liposomes in presence and absence of tentoxin. CF_1 depleted $\text{CF}_0 \cdot \text{CF}_1$ liposomes were incubated or not with $25.2 \mu\text{g}$ TF_1 for $10 \mu\text{g}$ $\text{CF}_0 \cdot \text{F}_1$, initially present before guanidinium chloride treatment. $10 \mu\text{l}$ of the liposomes (corresponding to $250 \mu\text{g}$ of lipids) were added to $0.4 \mu\text{l}$ of the medium containing 10 mM tricine pH 8, 0.2 mM EDTA, 2.5 mM MgSO_4 and $0.25 \mu\text{M}$ ACMA. Valinomycin was added to a final concentration of 0.36 nM , ATP to 2 mM and FCCP to 125 nM . When added tentoxin was $12 \mu\text{M}$ and liposomes were preincubated for 5 min with tentoxin, ATP-dependent fluorescence quenching was prevented. Fluorescence quenching of CF_1 depleted liposomes (curve a), of $\text{TF}_1 \cdot \text{CF}_0$ liposomes in the absence (curve b) or in the presence (curve c) of tentoxin was recorded.

P. modestum [3]. On the other hand even short treatment of $CF_o \cdot F_1$ liposomes with guanidinium chloride may have changed composition and structure of many CF_o parts, because longer treatment decreased more and more proton induced ACMA quenching after addition of TF_1 (not shown here).

Obviously is the membrane part CF_o of spinach chloroplast ATP synthase functionally compatible with the TF_1 moiety of the bacterial ATP synthase $TF_o \cdot F_1$, though CF_o has two different b subunits, $b(CF_oI)$ and $b'(CF_oII)$, whereas all bacterial F_o possess two identical subunits b. In an *E. coli* mutant carrying a chain termination in the unc F gene, subunit b may be replaced by spinach subunit $b(CF_oI)$ yielding a strain of *E. coli*, which was now able to grow on succinate-minimal medium [16]. This result reflects the possibility that spinach subunit b may interact functionally with EF_1 without any subunit $b'(CF_oII)$ present. On the other hand evidence was presented that subunit $b'(CF_oII)$ is an essential subunit of the photosynthetic ATP synthase $CF_o \cdot F_1$ [17,18] and is necessary for rebinding of CF_1 to EDTA-treated thylakoids [17]. In addition remarkable similarity in secondary structure predictions allows to suggest that subunit $b(CF_oI)$ and subunit $b'(CF_oII)$ possess a similar membrane topography. Because subunit $b'(CF_oII)$ has been found in all photosynthetic bacterial and chloroplast enzymes (for review see [18]) and appears to be necessary for their structure and function, it is an open question, if the bacterial TF_1 interacts with subunit $b'(CF_oII)$ or not in the above described $CF_o \cdot TF_1$ hybrid.

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References

- [1] Fillingame, R.H. (1990) in: *The Bacteria*, Vol. XII, Bacterial Energetics (T.A. Krulwich, Ed.) Academic Press, New York, pp. 345–391.
- [2] Laubinger, W. and Dimroth, P. (1989) *Biochemistry* 28, 7194–7198.
- [3] Kluge, C. and Dimroth, P. (1992) *Biochemistry* 31, 12665–12672.
- [4] Laubinger, W., Deckers-Hebestreit, G., Altendorf, K. and Dimroth, P. (1990) *Biochemistry* 29, 5458–5463.
- [5] Boyer, P.D. (1988) *Trends Biochem. Sci.* 13, 65–7.
- [6] Fromme, P., Boekema, E.J. and Gräber, P. (1987) *Z. Naturforsch.* 42c, 1239–1245.
- [7] Berger, G., Girault, G., Andre, F. and Galmiche, J.M. (1987) *J. Liquid. Chrom.* 10, 1507–1517.
- [8] Kagawa, Y. and Yoshida, M. (1979) *Methods Enzymol.* 55, 781–787.
- [9] McEnery, M.W., Hülliken, J. and Pedersen, P.L. (1989) *J. Biol. Chem.* 264, 12029–12036.
- [10] Rigaud, J.L., Paternostre, M.-T. and Bluzat, A. (1988) *Biochemistry* 27, 2677–2668.
- [11] Steffens, K., Di Gioia, A., Deckers-Hebestreit, G. and Altendorf, K. (1987) *J. Biol. Chem.* 262, 6334–6338.
- [12] Hsu, S.Y., Scuda, M., Kanazawa, H., Tschuchiya, T. and Futai, M. (1984) *Biochemistry* 23, 988–993.
- [13] Laubinger, W., Deckers-Hebestreit, G., Altendorf, K. and Dimroth, P. (1990) *Biochemistry* 29, 5458–5463.
- [14] Liu, S.-S., Gao, F.H., Tsai, H.L. and Ding, Y.Z. (1982) *Biophys. J.* 37, 88–91.
- [15] Bar-Zvi, D., Yoshida, M. and Shavit, N. (1985) *Biochim. Biophys. Acta*, 806, 341–347.
- [16] Schmidt, G., A.J. W. Rodgers, S.M. Howitt, A.L. Munn, G.S. Hudson, T.A. Holten, P.R. Whitfeld, W. Bottomley, F. Gibson and G.B. Cox (1990) *Biochim. Biophys. Acta*, 1015, 195–199.
- [17] Berzborn, R.J., L. Klein-Hitpaß, J. Otto, S. Schünemann, R. Oworah-Nkruma and H.E. Meyer (1990) *Z. Naturforsch.* 45c, 772–784.
- [18] Hermann, R.G., J. Stepphun, G.S. Herrmann and N. Nelson (1993) *FEBS Lett.* 326, 192–198.
- [19] Grotjohann, I. and P. Graeber (1990) *Biochim. Biophys. Acta* 1017, 177–180.