FEBS Letters 443 (1999) 136–138 FEBS 21424

Proton gradient-induced changes of the interaction between CF_0 and CF_1 related to activation of the chloroplast ATP synthase

Sophia Ponomarenko[1], Irina Volfson, Heinrich Strotmann*

Institut für Biochemie der Pflanzen, Heinrich Heine Universität Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany

Received 4 December 1998

Abstract Thylakoid energization by light causes destabilization of CF_0CF_1 so that the peripheral CF_1 sector is more readily detached from the membrane by intermediate concentrations of the chaotropic salt NaSCN. Here we have investigated the correlation between the proton gradient-induced change of CF_0CF_1 interaction and CF_0CF_1 activation. The results indicate a close relationship between the two phenomena. The effect is most probably due to reduction of the electrostatic interaction between the two subcomplexes CF_0 and CF_1 as a consequence of protonations in the interface region.

© 1999 Federation of European Biochemical Societies.

Key words: ATP synthase; CF_0CF_1; Chloroplast; Activation; Protein-protein interaction; Proton gradient

1. Introduction

The proton translocating reversible ATPase (ATP synthase, CF_0CF_1) of the thylakoid membrane catalyzes the formation of ATP from ADP and phosphate at the expense of the transmembrane electrochemical proton potential difference generated by photosynthetic electron transport. The membrane-integral subcomplex CF_0 enables proton translocation, while the attached extrinsic CF_1 sector forms the catalytic part [1]. Coupling between proton translocation and phosphorylation is explained by an energy-linked binding change mechanism [2] involving cyclic changes of the protein conformation.

ATP hydrolysis catalyzed by isolated F_1 or an isolated $\alpha_3\beta_3\gamma$ aggregate has been shown to be related to rotation of the central γ subunit in the $\alpha_3\beta_3$ ring [3–5] which may cause sequential opening and closing of the three catalytic sites. This finding has raised a coupling hypothesis based on the assumption that the H^+ flow-driven rotation of a cylinder consisting of 12 membrane-spanning proteolipid molecules (= subunits c or III) causes the rotation of γ subunit in F_1 in ATP formation [6–8]. Such a mechanism would require a connection between the proteolipid ring and γ and presume that non-moving parts of the enzyme ('stator') including the F_1 headpiece consisting of $\alpha_3\beta_3$ are fixed to the membrane. In spite of some experimental hints [9–11] these prerequisites are not yet experimentally settled.

The 'photosynthetic ATP synthases' in contrast to the 'respiratory ATP synthases' are latent enzymes that have to be activated in order to display catalytic activity. The physiological activation requires a proton gradient [12]. Hence the proton gradient is not only the driving force for ATP synthesis, but is also a control factor for the ATP synthase. Related to activation are energy-linked conformational changes in γ [13,14] and in the ε subunit [15] as well as the pmf-dependent release of a nucleotide [16] tightly bound to one of the three catalytic sites [17].

In a preceding paper we have reported on pmf-dependent changes of the physical interaction between CF_0 and CF_1 [18] which can be monitored by the enhanced release of CF_1 from the energized thylakoid membrane compared with the deenergized membrane with the chaotropic salt NaSCN. The present work shows that this effect is related to the pmf-induced activation of CF_0CF_1.

2. Methods

Thylakoids were isolated from spinach leaves as reported [19]. The experiments were conducted in cylindrical glass cells placed in a ΔpH clamp instrument as described [20] at constant temperature (20°C). The samples, which were continuously stirred, were illuminated with red light >630 nm to yield the indicated ΔpH values. The prechosen ΔpH was maintained by the instrumental device and continuously monitored by the fluorescence of 9-aminoacridine which was present at a concentration of 5 μM. The medium contained in addition 25 mM Tricine buffer, pH 8.0, 50 mM KCl, 5 mM $MgCl_2$ and 10 μM pyocyanin. The chlorophyll content during the reaction was 25 μg/ml. The 9-aminoacridine fluorescence signal was calibrated as reported [21].

For detachment of CF_1 concentrated NaSCN solution was added to yield a final concentration of 0.5 M [18]. After 15 s the reaction mix was diluted 1.6-fold with a solution of 50 mM NaCl, 1 mM $MgCl_2$, 2 mM Tricine buffer, pH 7.8 and 20 mM DTT and immediately centrifuged. The proteins of the clear supernatants were precipitated with 10% TCA. The pellets were redissolved in 0.1 ml 0.1 N NaOH. After addition of 0.025 ml of a solution containing 5% SDS, 25% glycerol, 25% mercaptoethanol, 0.25% bromophenol blue and 312.5 mM Tricine buffer pH 8.0, the samples were incubated for 1 h at 60°C. Aliquots were subjected to SDS-PAGE and after staining the protein bands were quantitatively analyzed colorimetrically as described [18]. For quantification standards of pure CF_1 exactly determined protein concentrations were used in each gel. The calculations are based on a CF_1 molecular weight of 396.5 kDa and a subunit stoichiometry of $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ [1]. The assay of activity of CF_0CF_1, measured by the ATP hydrolyzing capacity, and the technique of measurement of [^{14}C]ADP binding have been described previously [22].

*Corresponding author. Fax: (49) (211) 81 13706.
E-mail: strotman@uni-duesseldorf.de

[1]Permanent address: Institute of General Genetics and Cytology, KNAS, Almaty, Kazakhstan.

Abbreviations: CF_0CF_1, chloroplast ATP synthase; DCCD, dicyclohexyl carbodiimide; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; pmf, proton motive force; SDS, sodium dodecylsulfate; TCA, trichloroacetic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine

0014-5793/99/$19.00 © 1999 Federation of European Biochemical Societies. All rights reserved.
PII: S0014-5793(98)01681-0

3. Results and discussion

3.1. Formation of a destabilized CF_0CF_1 conformation in the light and its relaxation in the dark

Chaotropic salts like NaBr [23] or NaSCN [18] remove CF_1 from thylakoid membranes. In a previous paper we showed that upon treatment with 0.5 M NaSCN 20–25% CF_1 was detached from thylakoids kept in the dark, but 60–80% CF_1 was detached when the thylakoids were illuminated [18].

Fig. 1a shows the time course of formation of the proton gradient in the light and its relaxation in the dark as measured by the quenching of 9-aminoacridine fluorescence. In a parallel experiment (Fig. 1b) we investigated the time course of additional release of CF_1 in the light induced by 0.5 M NaSCN. The increment of CF_1 release in the light (Fig. 1b) follows the formation of the proton gradient with a small delay. In the subsequent dark CF_0CF_1 is restabilized, but the restabilization process is much slower than the decay of ΔpH. However, when a micromolar concentration of ADP is added at the light-dark transition, restabilization of the complex occurs much faster within a few seconds.

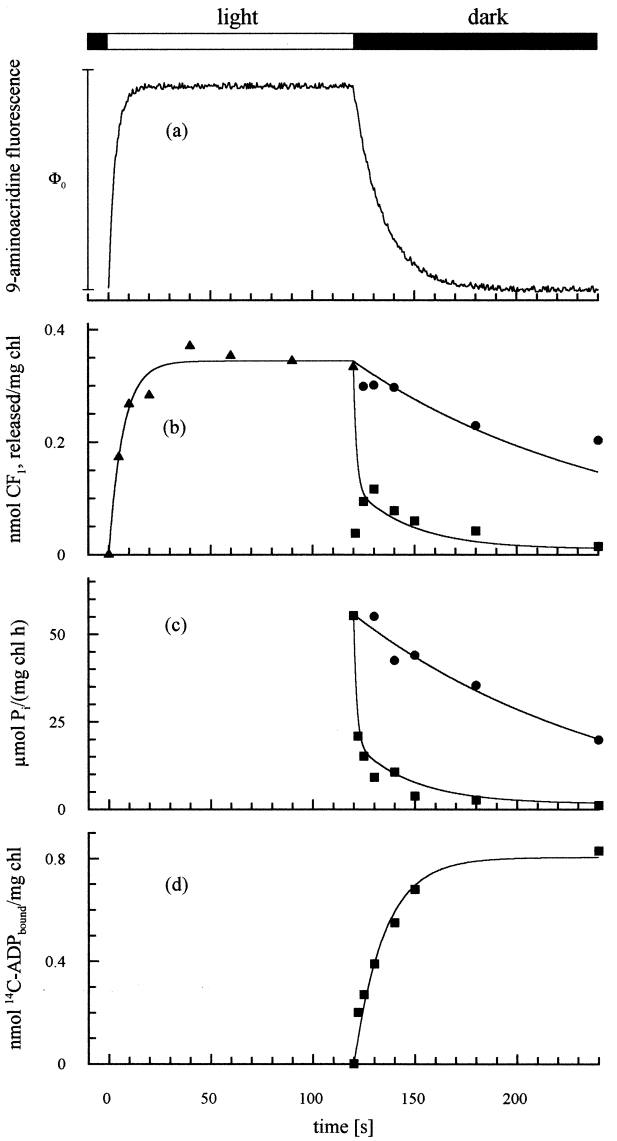

Fig. 1. a: Proton gradient formation as monitored by 9-aminoacridine fluorescence (ϕ_0: maximal fluorescence; calculated steady-state ΔpH in the light: 3.4). b: Formation of a destabilized CF_0CF_1 conformation (measured by the increment of CF_1 release on treatment of thylakoids with 0.5 M NaSCN) in the light (triangles) and relaxation in the following dark (dots). In a parallel series 10 μM ADP was added at the light-dark transition (squares). c: The decays of ATP hydrolyzing activity under the same conditions. d: The curve shows the time course of tight binding of ADP to CF_0CF_1. The medium contained 10 mM DTT.

While activation of CF_0CF_1 is usually related to release of a tightly bound nucleotide, fast deactivation is related to the tight binding of an ADP molecule to one of the catalytic sites [1,14,25–27]. As this nucleotide becomes completely undissociable it may be concluded that the site is closed against the medium. In the absence of medium nucleotide deactivation of the thiol-modulated CF_0CF_1 in the dark is slow (Fig. 1c). Enzyme deactivation and the relaxation of the destabilized CF_0CF_1 structure show comparable time courses each in the absence and presence of ADP. Fig. 1d shows the time course of 'tight' incorporation of the added [^{14}C]ADP. The time course is similar, but not identical to the time course of CF_0CF_1 deactivation in the presence of ADP (Fig. 1c) and the time course of restoration of a stable enzyme conformation in the presence of ADP (Fig. 1b), respectively. The results suggest that deactivation and the related structural rearrangement is triggered solely by the interaction of the nucleotide molecule with the binding site (which may be fast). The 'tight' incorporation of the ADP molecule, however, may be rate-limited by the subsequent occlusion of the site.

3.2. Effect of thiol modulation on the $\Delta\mu H^+$-induced formation of a destabilized CF_0CF_1

Reduction of a disulfide bridge in the γ subunit of CF_0CF_1 formed by the two cysteines γ-C199 and γ-C205 [1] causes a change of the energy threshold necessary for activation [12]. The process is called 'thiol modulation'. The natural reductant is thioredoxin-f [28] reduced by photosynthetic electron transport. As an in vitro thiol compound, dithiothreitol (DTT) may be employed. After reduction, the ΔpH profile of CF_0CF_1 activation is shifted by about one unit to lower ΔpH values [12]. In the following we have studied the ΔpH dependence of CF_0CF_1 destabilization with thylakoids in the absence and presence of DTT, respectively.

Fig. 2 shows the portion of CF_1 released by 0.5 M NaSCN due to ΔpH as a function of the size of ΔpH (points). The ΔpH profiles of activation for the oxidized and the reduced CF_0CF_1 obtained in acid-base energization experiments were taken from Junesch and Gräber [12] and are shown by the drawn curves. The activation profiles are similar to the profiles of extra CF_1 release obtained in light energization experiments. In particular the significant differences between the oxidized and the reduced enzyme are evident. For comparison we have also employed acid-base energization in the experiments with oxidized CF_0CF_1. The results fit to those obtained by light energization. As we included valinomycin+K^+ in the medium, we think that the electrochemical potential difference is a chemical proton potential only and that CF_0CF_1 activation – unlike oxidative phosphorylation in *Escherichia coli* [29] – is not obligatorily dependent on an electric potential difference.

As the F_0 blocker DCCD prevents the enhancement of CF_1 release [18], the generation of the destabilized CF_0CF_1 structure presumes the translocation of protons through CF_0.

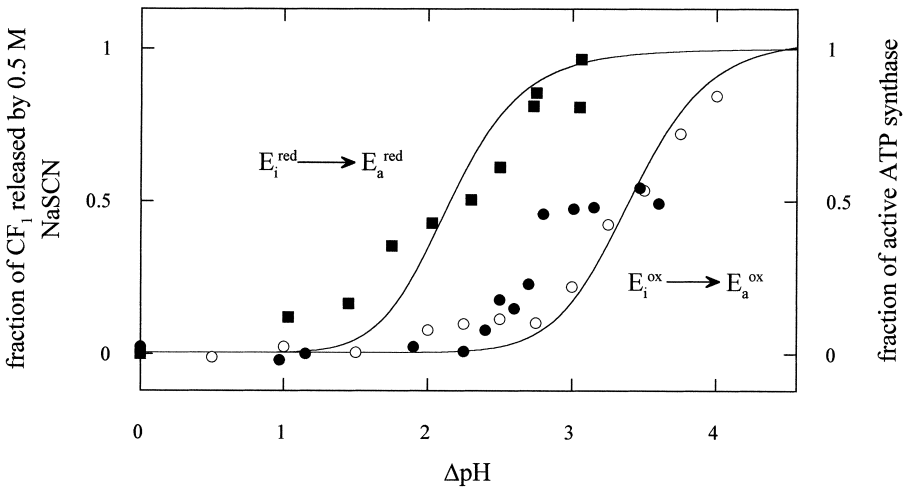

Fig. 2. Formation of a destabilized CF_0CF_1 conformation as measured by the increase of CF_1 detachment by 0.5 M NaSCN as a function of ΔpH in the absence (circles) and presence of 10 mM DTT (squares) and CF_0CF_1 activation. The proton gradient was generated either by light (filled circles) or by acid-base transition (open circles). The values (left scale) mean the differences of CF_1 release from energized minus deenergized membranes over the maximal difference (total CF_1 minus CF_1 release from deenergized membranes). Total CF_1 was ascertained by treatment with 2 M NaSCN which detaches all CF_1 [18]. The activation profiles (drawn curves, right scale) were taken from Junesch and Gräber [12].

Hence the protons seem to act at the CF_0-CF_1 interface and this may cause electrostatic repulsion or attenuation of the electrostatic interaction, respectively. This could be visualized as follows: the ground state of the enzyme may be stabilized by ionic bonds between basic amino acids on CF_0 and acidic amino acids on CF_1 or vice versa. Upon membrane energization these ionic bonds may be cleaved due to protonation of the acidic residues (p*K* values in the weakly acidic range) by protons coming from the thylakoid lumen and thus cause the observed attenuation of the CF_0-CF_1 interaction. As shown above, this structural destabilization is related to CF_0CF_1 activation. Accordingly activation seems to comprise unlocking of a catch between CF_0 and CF_1 as a prerequisite for catalytic turnover.

Acknowledgements: The authors thank Dr. Oliver Schwarz for valuable discussions. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 189), the Volkswagenstiftung and the Fonds der Chemischen Industrie.

References

[1] Strotmann, H., Shavit, N. and Leu, S. (1998) in: Advances in Photosynthesis (Rochaix, J.-D., Goldschmidt-Vlermont, M. and Merchant, S., Eds.), Vol. 7, pp. 477–500, Kluwer Academic, Dordrecht.
[2] Boyer, P.D. (1993) Biochim. Biophys. Acta 1140, 215–250.
[3] Duncan, T.M., Bulygin, V.V., Zhou, Y., Hutcheon, M.L. and Cross, R.L. (1995) Proc. Natl. Acad. Sci. USA 92, 10964–10968.
[4] Sabbert, D., Engelbrecht, S. and Junge, W. (1996) Nature 381, 623–625.
[5] Noji, H., Yasuda, R., Yoshida, M. and Kinosita Jr., K. (1997) Nature 386, 299–302.
[6] Vik, S.B. and Antonio, B.J. (1994) J. Biol. Chem. 269, 30364–30369.
[7] Junge, W., Lill, H. and Engelbrecht, S. (1997) Trends Biochem. Sci. 22, 420–423.
[8] Zhou, Y., Duncan, T.M. and Cross, R.L. (1997) Proc. Natl. Acad. Sci. USA 94, 10583–10587.
[9] Beckers, G., Berzborn, R.J. and Strotmann, H. (1992) Biochim. Biophys. Acta 1101, 97–104.
[10] Watts, S.D., Zhang, Y., Fillingame, R.H. and Capaldi, R.A. (1995) FEBS Lett. 368, 235–238.
[11] Lill, H., Hensel, F., Junge, W. and Engelbrecht, S. (1997) J. Biol. Chem. 271, 32737–32742.
[12] Junesch, U. and Gräber, P. (1987) Biochim. Biophys. Acta 893, 275–288.
[13] Schumann, J., Richter, M.L. and McCarty, R.E. (1985) J. Biol. Chem. 260, 11817–11823.
[14] Moroney, J.V., Fullmer, C.S. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7281–7285.
[15] Komatsu-Takaki, M. (1996) Eur. J. Biochem. 236, 470–475.
[16] Gräber, P., Schlodder, E. and Witt, H.T. (1977) Biochim. Biophys. Acta 461, 426–440.
[17] Zhou, J.-M., Xue, Z., Du, Z., Melese, T. and Boyer, P.D. (1988) Biochemistry 27, 5129–5135.
[18] Fiedler, H.R., Ponomarenko, S., von Gehlen, N. and Strotmann, H. (1994) Biochim. Biophys. Acta 1188, 29–34.
[19] Strotmann, H. and Bickel-Sandkötter, S. (1977) Biochim. Biophys. Acta 460, 126–135.
[20] Strotmann, H., Thelen, R., Müller, W. and Baum, W. (1990) Eur. J. Biochem. 193, 879–886.
[21] Kothen, G., Schwarz, O. and Strotmann, H. (1995) Biochim. Biophys. Acta 1229, 208–214.
[22] Kleefeld, S., Lohse, D., Mansy, A.R. and Strotmann, H. (1990) Biochim. Biophys. Acta 1019, 11–18.
[23] Kamienietzky, A. and Nelson, N. (1975) Plant Physiol. 55, 282–287.
[25] Lohse, D. and Strotmann, H. (1989) Biochim. Biophys. Acta 976, 94–101.
[26] Schumann, J. and Strotmann, H. (1981) in: Photosynthesis II. Electron Transport and Photophosphorylation (Akoyunoglou, G., Ed.), Vol. II, pp. 881–892, Balaban International Science Service, Philadelphia, PA.
[27] Carmeli, C. and Lifshitz, Y. (1972) Biochim. Biophys. Acta 267, 86–95.
[28] Schwarz, O., Schürmann, P. and Strotmann, H. (1997) J. Biol. Chem. 272, 16924–16927.
[29] Kaim, G. and Dimroth, P. (1998) FEBS Lett. 434, 57–60.