ELSEVIER
Biochimica et Biophysica Acta 1555 (2002) 140–146
BIOCHIMICA ET BIOPHYSICA ACTA BBA
www.bba-direct.com

Molecular devices of chloroplast F_1-ATP synthase for the regulation

Toru Hisabori [a,b,*], Hiroki Konno [a,b], Hiroki Ichimura [a], Heinrich Strotmann [c], Dirk Bald [a,d]

[a] *Chemical Resources Laboratory, Tokyo Institute of Technology, Nagatsuta 4259, Midori-ku, Yokohama, Kanagawa 226-8503, Japan*
[b] *ATP System Project, Exploratory Research for Advanced Technology (ERATO), Japan Science and Technology Corporation (JST), 5800-3 Nagatsuta-cho, Midori-ku, Yokohama 226-0026, Japan*
[c] *Institut für Biochemie Pflanzen, Heinrich-Heine Universität Düsseldorf, D-40225 Düsseldorf, Germany*
[d] *Department of Structural Biology, Free University of Amsterdam, De Boelelaan 1087, 1081 Amsterdam, Netherlands*

Received 4 March 2002; received in revised form 22 April 2002; accepted 22 April 2002

This paper is dedicated to the memory of Dr. Mizuho Komatu-Takaki, whose 48 years of life was dedicated to the research on the chloroplast ATP synthase

Abstract

In chloroplasts, synthesis of ATP is energetically coupled with the utilization of a proton gradient formed by photosynthetic electron transport. The involved enzyme, the chloroplast ATP synthase, can potentially hydrolyze ATP when the magnitude of the transmembrane electrochemical potential difference of protons ($\Delta\mu H^+$) is small, e.g. at low light intensity or in the dark. To prevent this wasteful consumption of ATP, the activity of chloroplast ATP synthase is regulated as the occasion may demand. As regulation systems $\Delta\mu H^+$ activation, thiol modulation, tight binding of ADP and the role of the intrinsic inhibitory subunit ε is well documented. In this article, we discuss recent progress in understanding of the regulation system of the chloroplast ATP synthase at the molecular level.

Keywords: Chloroplast; ATP synthase; Redox regulation; γ subunit; ε subunit

1. Introduction

F_oF_1 ATP synthase, which is a constituent of the chloroplast thylakoid membranes, mitochondrial inner membranes, and bacterial plasma membranes, synthesizes ATP from ADP and phosphate at the expense of a proton gradient across the membranes [1]. The water-soluble part of ATP synthase, the F_1 region, which is capable of catalyzing ATP hydrolysis, consists of five subunits with a stoichiometry of $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ [2]. The hydrophobic F_o part is composed of $a_1b_2c_{10-14}$. Different numbers of the *c* subunits were reported for the complexes from the different organelles and also depending on the employed method for the determination [3–5].

In 1994, Abrahams et al. [6] reported a high resolution X-ray crystal structure of a major part of the bovine heart mitochondrial F_1. The α and β subunits in the $\alpha_3\beta_3$ hexagon are alternately arranged and surround the γ subunit as a central axis. Three catalytic sites are located on the β subunits at the interfaces to the α subunits and three non-catalytic nucleotide binding sites on the α subunits at the interfaces to the β subunits. A subcomplex of F_1, $\alpha_3\beta_3\gamma$ is the minimum complex that is capable of ATP hydrolysis [7]. By rotation within the $\alpha_3\beta_3$ ring, the γ subunit induces a structural and functional asymmetry among three αβ pairs [6,8]. Rotation of the γ subunit in the $\alpha_3\beta_3$ hexagon was first postulated by P.D. Boyer based on the analysis of the catalytic site cooperativity [9]. Subsequently, the rotation of γ related with catalytic turnover was suggested by biochemical experiments [10] and biophysical measurements [11]. Direct visualization of the rotation of an actin filament attached to the γ subunit of an $\alpha_3\beta_3\gamma$ complex of F_1 of the thermophilic bacterium PS3 unequivocally showed that γ can rotate unidirectionally during ATP hydrolysis [12]. Moreover, rotation of the γ subunit was proven in *Escherichia coli* F_1 [13,14] and chloroplast F_1 [15] by using the same method. Rotation of the central γ subunit includes three 120° steps, suggesting that every step may be associated to hydrolysis of a single ATP molecule at each of the three catalytic sites [16].

* Corresponding author. Chemical Resources Laboratory, Tokyo Institute of Technology, Nagatsuta 4259, Midori-ku, Yokohama, Kanagawa 226-8503, Japan. Fax: +81-45-924-5277.
E-mail address: thisabor@res.titech.ac.jp (T. Hisabori).

0005-2728/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0005-2728(02)00269-4

In contrast to other F_oF_1 types, the chloroplast ATP synthase/ATPase is a latent enzyme. Without preceding activation, the activity is zero. Physiological activation of the membrane-bound enzyme is caused by the transmembrane proton gradient. Artificial activation may be achieved by certain alcohols and other organic solvents [17], limited tryptic cleavage [18], and heat [19], etc. Moreover, there are other regulatory devices that shall be discussed in the following (for review, see also Ref. [20]). The molecular structure of F_oF_1 ATP synthase revealed that some of these devices are located on the central axis of the enzyme. However, on the molecular level, our understanding of the regulation is not very deep.

2. Observed phenomena

Solubilized CF_1 is a latent ATPase that shows no ATP hydrolyzing activity unless activated. Likewise, membrane-bound CF_oCF_1 is an inactive enzyme. This is demonstrated by the observation that chloroplasts or thylakoids kept in the dark do not catalyze ATP hydrolysis. However, on illumination of thylakoids, the enzymatic activity of CF_oCF_1 rises with the rise of the proton gradient across the thylakoid membranes. This must be concluded from the fact that ATP is formed in the light. Upon turning off the light, the enzymatic activity disappears in parallel with the decay of the gradient. Hence, in addition to its role as the free energy input for ATP synthesis, the transmembrane electrochemical potential difference of protons ($\Delta\mu H^+$) is a kinetic factor acting on the involved enzyme. The two components of $\Delta\mu H^+$, ΔpH and the membrane potential ($\Delta\Psi$), were reported to be equivalent with regard to the driving force as well as for the activation process [21]. ATP formation is thermodynamically as well as kinetically promoted by the proton gradient, whereas wasteful ATP hydrolysis is thermodynamically inhibited when the gradient is high, or kinetically inhibited when the gradient is low. This simple but subtle control device avoids energy losses in the chloroplast although the energy source light is a highly variable parameter (day/night, transient shading by clouds, light flecks, etc.).

The strict light–dark control, however, is partially abolished by thiol modulation. Thiol modulation is attained by illumination of chloroplasts in the presence of thiols (see below). After this pretreatment, chloroplasts are capable of hydrolyzing ATP in the subsequent dark. That means the deactivation of the enzyme is retarded in comparison with the decay of $\Delta\mu H^+$ due to a lower deactivation rate of the thiol-modulated enzyme compared to that of the demodulated enzyme. Accordingly, in a time window of about 1 min after light–dark transition, when $\Delta\mu H^+$ is declining below a critical value, ATP hydrolysis coupled with proton pumping is both thermodynamically and kinetically possible.

As the active state of CF_oCF_1 in membrana in the light is the result of simultaneous activation and deactivation, we may conclude a higher equilibrium activity when the enzyme is thiol modulated. Actually, at thiol modulation, in comparison to demodulation, the $\Delta\mu H^+$ dependency of ATP formation is shifted towards lower values [22,23]. At a given limiting $\Delta\mu H^+$, a higher rate of ATP formation is observed under thiol-modulated conditions. Accordingly, the less strict control of ATP hydrolysis at light–dark transition allows of a better utilization of light energy at light limitation.

What is the physiological significance of thiol modulation of CF_oCF_1 on the thylakoid membrane? The answer for this question is still ambiguous. The CF_1-γ subunit of cyanobacterium *Synechocystis* lacks nine amino acids including two regulatory cysteines in the switch region (Fig. 1). When the regulatory sequence from spinach CF_1-γ was conferred by genetic engineering, the obtained cyanobacterium has no obvious benefit from it [24]. Similarly *Chlamydomonas*, which lost the regulatory cysteines by site-directed mutagenesis, had no obvious disadvantage over the wild type [25]. In contrast, when Gabrys et al. [26] selected mutants using the strategy that the growth rate of the mutant plants is normal under the high-light conditions but slow under the weak-light conditions after the random mutagenesis of *Arabidopsis thaliana*, they obtained the possible mutants at the regulatory region of the γ subunit of CF_1. This suggests that the thiol-modulation system in the higher plants may indeed provide a benefit to the plants under the insufficient light conditions.

In part, the insufficient control of ATP hydrolysis by the thiol-modulated enzyme is cured by another control mechanism, the nucleotide-dependent deactivation. Addition of ADP, even at micromolar concentrations, greatly enhances

```
                 190       200       210       220       230       240
                -+----'----+----'----+----'----+----'----+----'----+----
S.oleracea      LLPLSPKGEICDINGKCVDAAEDELFRLTTKEGKLTVERDMIKTETPAFSPILEFE
P.sativum       LLPLSPKGEICDINGNCVDAAEDELFRLTTKEGKLTVERDVIRSKTVDFSPILQFE
A.thariana      LLPLSPKGEICDINGTCVDAAEDEFFRLTTKEGKLTVERETFRTPTADFSPILQFE
C.reinhardtii   LLPMTPMGELCDVDGKCVDAADDEIFKLTTKGGEFAVEREKTTIETEALDPSLIFE
Syn.sp.PCC 6803 LFPLSPQG---------LEAPDDEIFRLITRGGKFQVEREKVEAPVESFPQDMIFE
Syn.sp.PCC 6716 LLPLDPQG---------LEAADDEIFRLTTRASHLEVNREKVTSNLPALPPDMIFE
Ana.sp.PCC 7120 LLPLDTQG---------LEAADDEIFRLTTRGGQFQVERQTVTSQARPLPRDSIFE
E.coli          LLPLP-----------------------------------ASDDDDLKHKSWDYLYE
Bacillus PS3    LLPLT-----------------------------------DLAENKQRTVYEFE
```

Fig. 1. The comparison of the amino acid sequences of the γ subunit at around the regulatory region. Residue numbers are those for spinach CF_1-γ subunit. Two regulatory cysteines are reversed.

the deactivation of CF_oCF_1 at light–dark transition. If ATP is present at light–dark transition, ATP is hydrolyzed until a critical ADP/ATP ratio is attained. Then, ADP-dependent deactivation also provides decay of ATP hydrolyzing activity. This process is related with tight binding of ADP to the CF_1 sector of the ATP synthase. Reactivation, on the other hand, effects re-release of this tightly bound nucleotide. The tightly bound nucleotide resides on one of the three catalytic sites [27]; occupation of one site with ADP is sufficient to obtain the ADP-inhibited state of the enzyme.

In terms of the rotational coupling mechanism, the inhibited state may be visualized as an arrested state caused by the occasional trapping of the product ADP at the catalytic site(s) during ATP hydrolysis, and the active state as being capable of rotating. The binding of ATP to the noncatalytic site(s) can facilitate the release of this trapped ADP and recover the enzyme from the inhibited state [28]. From the analysis of the rotation mode of the single molecule enzyme at the inhibited state and the active state, Hirono-Hara et al. [29] recently reported that the pausing position of the γ subunit deviates from the ATP-waiting position when the enzyme dropped into the inhibited states. This position seemed to be most likely the recently found intermediate 90° position of the γ subunit [16] when the first ATP-waiting position was set as 0°.

3. Regulation assisted by the γ subunit

3.1. Molecular mechanism of redox regulation

The enzyme activity of CF_oCF_1 from green algae and higher plants is modulated by the reversible reduction of a disulfide bridge in the γ subunit by thiols and therefore called "thiol modulation" [22,30]. In the chloroplast, the reductant is reduced thioredoxin, which is formed by transfer of electrons from the photosynthetic electron transport chain via ferredoxin and ferredoxin–thioredoxin reductase [31,32]. Thioredoxin-*f* is by a factor 5 more efficient than thioredoxin-*m* [32]. In vitro thioredoxin may be substituted by DTT [33]. The disulfide bond is formed between the two cysteines ^{199}Cys and ^{205}Cys of γ (numbering of spinach γ). In the F_oF_1 complex, the region including these two cysteines must be located in the region of γ, which is located between the $\alpha_3\beta_3$ ring and F_o, but more close to F_o and ε. [34] This switch region in γ is characteristic for CF_1 from green algae and higher plants, and consists of 37 amino acid residues (^{194}Pro–^{230}Ile in case of the spinach chloroplast enzyme, Fig. 1) [35]. This segment is lacking in other γ subunits including those from cyanobacterial F_1 [36] and CF_1 from the diatom *Odontella sinensis* [37].

As mentioned in the previous chapter, for the fast reduction of the disulfide bond, a certain magnitude of $\Delta\mu H^+$ in addition to thioredoxin is necessary, suggesting that an energy-linked conformational change in the F_oF_1 complex is involved. It was concluded that by this conformational change exposure of the hidden disulfide bridge is affected [38]. Conformational changes in the γ and ε subunits induced by $\Delta\mu H^+$ have been demonstrated to occur by differential chemical modification of certain amino acid residues [39,40], and differential reactivity against anti-ε subunit antibody [41].

The ε subunit (in the case of the mitochondrial F_1, the counterpart is designated as δ) is known as an endogenous inhibitor of ATP synthase. Recently, a drastic conformational change, with a noninhibitory 'down-form' and an inhibitory 'up-form', of this subunit in the F_1 complex was revealed from the crystal structure analyses [34,42] and biochemical studies [43–45]. Hence, we may discuss the regulatory function of this ε subunit in context with γ subunit on the basis of this structural information (see Section 4).

Moreover, reduction of the disulfide bond on the γ subunit unmasks the latent ATP hydrolyzing activity (ATPase activity) of solubilized CF_1 [46]. However, higher concentrations of reductant and longer incubation times are required than in case of thiol modulation of membrane-bound CF_oCF_1. Redox regulation is a common property of the so-called thiol enzymes in chloroplasts-like glyceraldehyde 3-phosphate dehydrogenase, fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase, phosphoribulokinase (thioredoxin-*f*) and NADP-malate dehydrogenase (thioredoxin-*m*) [47]. However, we still cannot figure out the molecular events at the 'lower' region of the γ subunit promoted by $\Delta\mu H^+$, and by the reduction or oxidation, as structural information of this region is lacking so far.

Redox regulation is independent of the origin of the $\alpha_3\beta_3$ moiety and can be transferred, e.g. on $\alpha_3\beta_3$ of bacterial F_1 from the γ subunit of CF_1 from spinach chloroplasts [48,49]. The same is true for a chimeric γ subunit (γ_{TCT}) comprising the N-terminal and the C-terminal regions from thermophilic F_1, but the regulatory region from CF_1 [50]. As this chimeric γ subunit or the whole $\alpha_3\beta_3\gamma$ complex ($\alpha_3\beta_3\gamma_{TCT}$) has only two cysteines, which are located at the introduced regulatory region, the observed change of the activity must be the direct reflection of the change of the redox state of the introduced regulatory region (Fig. 2). However, we had no answer why the enzyme activity decreases to 30–40% of the full activity under the oxidizing conditions.

To address this question, observation of the rotation of F_1-ATPase seemed to be the most powerful means because it allowed us to observe the real-time behavior of the enzyme at the single molecule level.

3.2. Regulation of rotation

We investigated the differences of the rotation behavior of the γ subunit in the engineered F_1 complex, $\alpha_3\beta_3\gamma_{TCT}$ under reduced and oxidized conditions [51]. Instead of the fluorescein-labeled actin filament, of which fluorescence is easily quenched under the oxidizing conditions, we adopted the polystyrene microsphere technique for the detection of

rotation [16] (Fig. 3). We first observed the rotation of the complex under the reducing conditions. Averaged rotation rate of 13 microspheres, attached to F_1, for a 5-min observation period were 48 revolutions/min. After switching to the oxidizing conditions, the rotating probes (microspheres) often showed characteristic, long pauses, and the averaged rotation rate of these 13 microspheres came down to 13 revolutions/min. In spite of the long pauses, the rotation in most cases did not stop completely over the whole phase. Interestingly, for the periods between the long pauses, i.e. when the enzyme in the oxidizing conditions did rotate, the rotation rate was almost same as that observed in the reducing conditions. When the complex was switched back to the reducing conditions, the average rotational rate increased again (22 revolutions/min) although it was lower than in the first reducing conditions due to lags.

As the observed regulation of the rotation reflects the redox-regulated behavior of enzyme activity (see Fig. 4 in Ref. [51]), this finding reliably provides new insight into the molecular mechanism of F_1-ATPase, especially in the suppressed state under the oxidizing conditions (Fig. 3). Detailed analysis of the rotation indicated that the rotating particle shows two different patterns under the oxidizing conditions; the counter-clockwise rotation whose rate is indistinguishable from that of the enzyme under the reducing conditions, and the very long pause. These two different patterns can be explained when the oxidized complex was assumed to take two independent states: in one state, F_1 works in the usual manner as under the reducing conditions, in the other, it assumes a hardly rotating form. From these observations, we assumed that the regulatory region of the γ subunit controls how easy the γ subunit rotates in the central cavity of $\alpha_3\beta_3$ dependent on the redox states of the regulatory cysteines. Under the oxidizing conditions, the oscillation between the two states induced by the oxidized regulatory region lowers the number of revolutions of the γ subunit, resulting in lower enzyme activity.

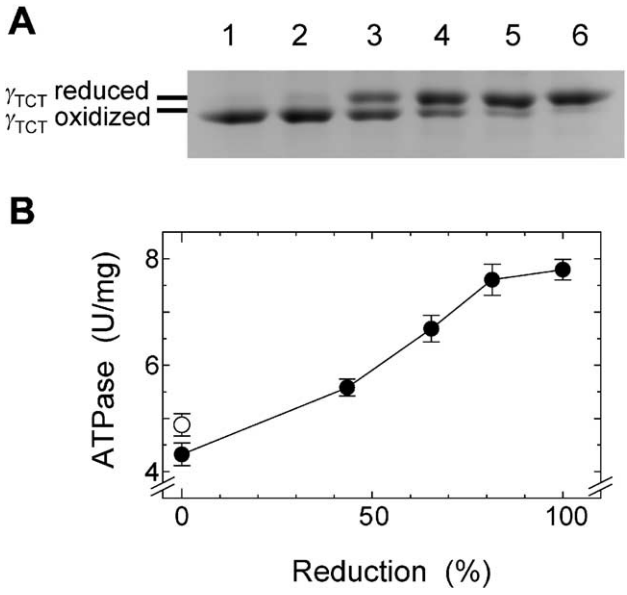

Fig. 2. Reduction of the γ_{TCT} subunit in the partial complex $\alpha_3\beta_3\gamma_{TCT}$ and the ATPase activity. (A) $\alpha_3\beta_3\gamma_{TCT}$ complex was prepared as described [50]. γ_{TCT} in the partial complex $\alpha_3\beta_3\gamma_{TCT}$ was reduced with 5 μM Trx-*f* and the indicated concentration of DTT or oxidized with 50 μM $CuCl_2$ for 30 min at 30 °C. Then the samples were separated on 15% (w/v) SDS-PAGE and the gel was stained with CBB. Only the region around γ subunit is shown. The reduction levels were visualized by the incorporation of 4-acetamido-4′-maleimidyl-stilbene-2, 2′-disulfonate (for details, see Ref. [61]); fully oxidized complex (lane 1), the complex incubated with Trx-*f* without DTT (lane 2), with 1 μM DTT (lane 3), with 2 μM DTT (lane 4), with 4 μM DTT (lane 5) and with 10 μM DTT (lane 6) were used. (B) The ATPase activities of these complexes were then measured and plotted against the extents of the reduction of γ_{TCT} estimated from the intensities of the CBB-stained bands on the gel shown in (A). The band intensities were calculated using the image processing and analysis software, NIH-image. The activity of the fully oxidized complex was shown with the open circle.

4. The role of ε subunit in regulation

There are two reports that suggest conformational changes of ε subunit dependent on $\Delta\mu H^+$ [39,41]. Komatsu-Takaki [39] studied the differential reactivity of Lys-109 of the ε subunit to pyridoxal 5′ -phosphate, when the membranes were energized by illumination or deenergized in the dark. The experiments revealed that this amino acid must change its position in the process of activation of CF_oCF_1. Based on the reported three-dimensional structure of the ε subunit of *Escherichia coli* F_1 [52,53], Lys-109 of CF_1-ε must be located on the short loop between two α-helices at the C-terminal end. The recently resolved two different structures (of ε subunit of *E. coli* F_1, and of the homologous δ subunit of MF_1) strongly suggest that these two helices may easily change their relative position in the complex. During the preparation of this review, $\Delta\mu H^+$-dependent conformational change of the C-terminal α-helices of the ε subunit was confirmed by using the antibodies, which specifically react with this region [62].

Furthermore, these conformational changes were recently confirmed from studies of bacterial F_1-ATPase, and a drastic conformational change of the ε subunit in the complex from a noninhibitory ‘down-form’ to an inhibitory ‘up-form’ were indicated [34,42–45]. These two forms were first postulated from kinetic analysis of the time-dependent alteration of the ATPase activity of thermophilic F_1 [54]. Using cross-linking after introducing cysteines in appropriate positions of the ε subunit of *E. coli* F_1, Tsunoda et al. [45] investigated whether the ε subunit actually can assume two different conformations in the complex or not. With the ‘down-form’, the ε subunit did not inhibit both ATP hydrolysis and ATP synthesis. Surprisingly, the ε subunit only inhibited ATP hydrolysis when the subunit is with the ‘up-form.’ This study strongly demonstrates the physiological importance of the conformational change of the ε subunit in the F_oF_1 complex. The opposite conformational change of the ε subunit leading to activation must be induced by $\Delta\mu H^+$.

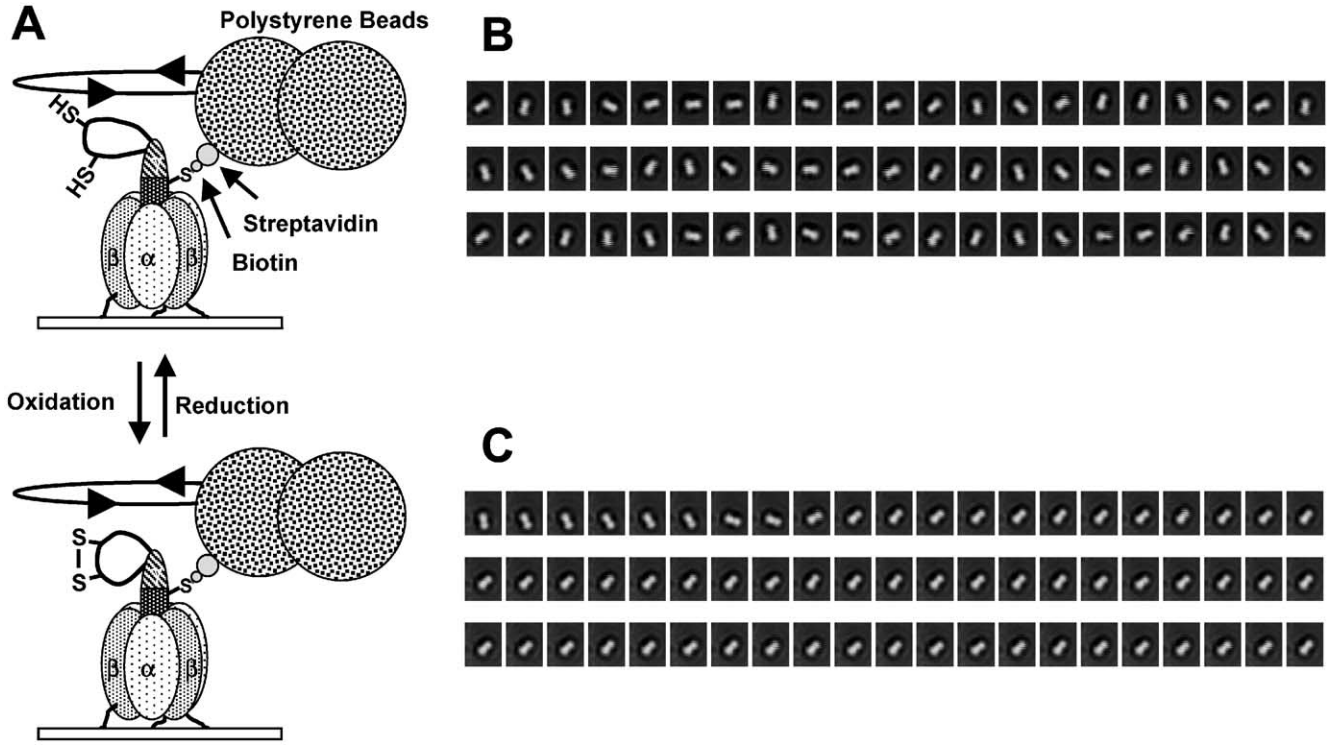

Fig. 3. Measurement of the redox regulation of the rotation of F_1-ATPase. (A) The experimental set-ups (for details, see Ref. [51]). (B) Sequential images of a rotating microsphere attached to the γ subunit on the $\alpha_3\beta_3\gamma_{TCT}$ complex. The complex was in the reducing condition. Time interval between images was 33 ms. (C) Rotation images in the oxidizing conditions.

From a similar cross-linking study between cysteines introduced into the two different domains of the ε subunit, we proposed that a conformational change may occur upon nucleotide binding to the complex. When the inhibition was released by the incubation of the complex with ATP or ADP, a cross-linking between the two domains was easily formed, suggesting a noninhibitory 'down-form' must be induced by the nucleotide binding to the catalytic sites [43]. The crystal structures of F_1-complex or the partial subunit complex, respectively, strengthened this view [34,42]. Electrostatic interactions between basic residues in the C-terminal α-helix of the ε subunit and acidic residues of the conserved 'DELSEED' region of the β subunit appear to stabilize the association between the ε and β subunits when the ε subunit is in the 'up-form.' This strong interaction might be essential cause for the inhibition [44].

The interaction between the CF_1-γ subunit and the ε subunit is strongly affected by the redox states of the regulatory cysteines of the γ subunit [55–57]. In the case of the authentic CF_1 complex, the affinity of the ε subunit for the complex decreased more than 10-fold upon reduction of the γ subunit. In contrast, the chimeric complex formed from the thermophilic α and β subunits and the recombinant γ subunit of CF_1 showed the opposite tendency [48]. The cause of this discrepancy is not known yet. Nevertheless, the redox state of the regulatory disulfide certainly affects the conformation of the interacting regions of the γ and ε subunits.

Vice versa, we recently found that in the F_oF_1 complex, the ε subunit is likewise important for redox regulation. We have constructed a chimeric F_oF_1 complex, in which all subunits were from the thermophilic *Bacillus* PS3 except the γ subunit, which contained the regulatory region from CF_1. The whole complex was expressed in *E. coli*, and was assembled on the plasma membrane of the bacterium. Then, we studied the redox regulation of this chimeric complex on the membrane. Interestingly, the redox regulation properties were observed only when the ε subunit was also from CF_1 (Konno and Hisabori, unpublished results). These results strongly suggest that the disulfide group on γ subunit and the ε subunit complement each other in regulation.

5. Molecular devices for the regulation

In Fig. 4, the conformational change of the ε subunit and the states of the redox disulfide on the γ subunit are summarized. As suggested by the investigation of the chemical modification conditions, the disulfide bond on the γ subunit is expected to become accessible from the medium phase upon membrane energization (from state I to state II). The conformational change of the ε subunit may be responsible for the extent of accessibility of the disulfide group to the reductant. This expectation is consistent with the results that the disulfide reduction by the reduced form of thioredoxin is easier when ε is not involved [58–60]. The

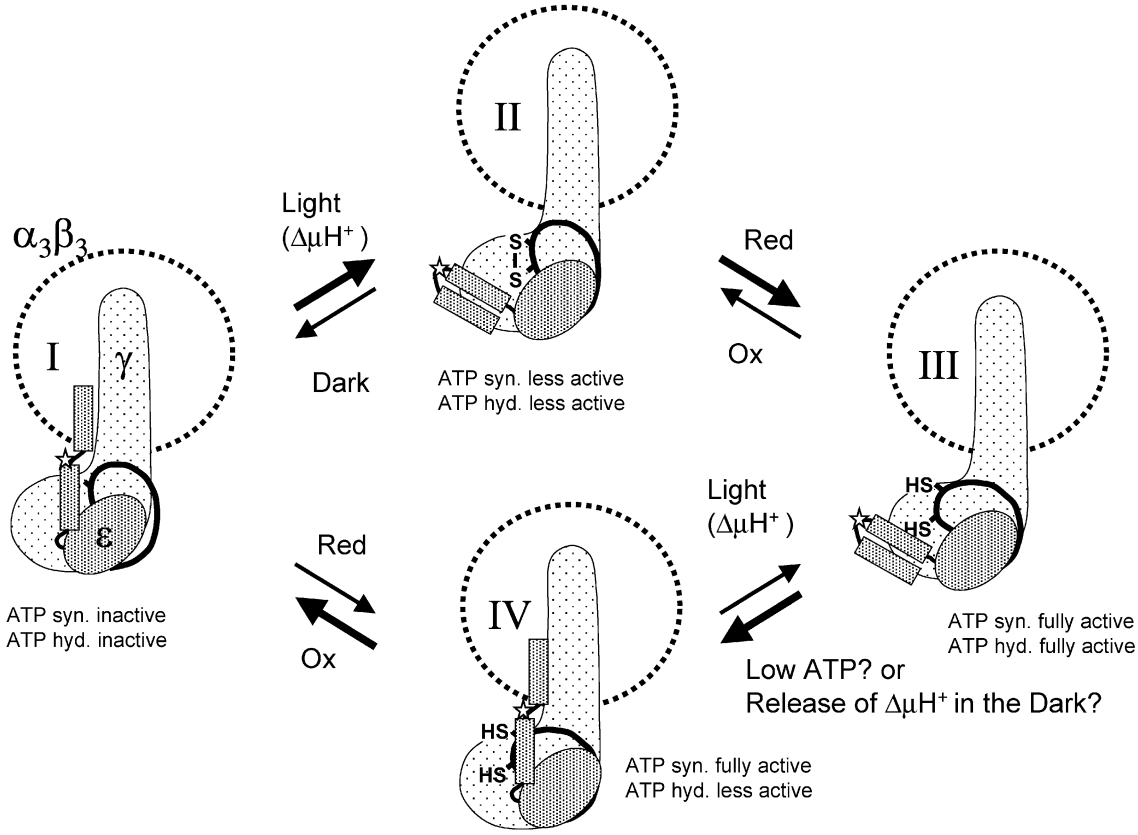

Fig. 4. The involvement of the γ and ε subunits in the regulation of the CF_oCF_1 activity. Schematic conformation of the ε subunit is postulated based on the recently provided crystal structure of the bacterial F_1-ε [42] and the mitochondrial counterpart [34]. The position of Lys-109 of the ε subunit is shown as an open star.

deactivation pathway from state III to state I is not known very well. The question is if the state IV actually occurs or not. This question directly links to the order of the decay of $\Delta\mu H^+$ and the accumulation of the oxidants in the chloroplasts in the dark. In addition, the concentration of ATP or ADP in the chloroplasts may directly affect to the inhibitory effect of ε if the similar conformational change as observed in F_1-ATPase is the critical one for the function of this subunit in the membrane bound F_oF_1 complex.

Another important device for the regulation is the tightly bound ADP, which is trapped at a catalytic site and inhibits the transition of the γ subunit to the next catalytic site. However, it is still difficult to sum up all of the regulation devices to describe the fine tuning system of the enzymatic activity of F_oF_1 ATP synthase under the various physiological conditions. One of the contradiction is the role of the nucleotide binding to the catalytic site(s) as stated above; the inhibitory role of the tightly bound ADP and the effect for the release of the enzyme from the ε-inhibition. Reevaluation of the many results obtained for the regulation of the membrane bound CF_oCF_1 from the view of the rotational coupling mechanism and the possible drastic conformational change of the ε subunit in the complex must reveal the genuine regulation mechanism of this extraordinary enzyme complex.

Acknowledgements

This work was supported in part by Grants-in-Aid for science research to T.H. (No. 13440238) from the Ministry of Education, Science, Sports, Culture, and Technology of Japan and in part by JST. We thank M. Yoshida, R. Yasuda, T. Suzuki, Y. Kato-Yamada, and K. Y. Hara for fruitful discussion.

References

[1] P.D. Boyer, Annu. Rev. Biochem. 66 (1997) 717–749.
[2] M. Yoshida, N. Sone, H. Hirata, Y. Kagawa, J. Biol. Chem. 252 (1977) 3480–3485.
[3] W. Jiang, J. Hermolin, R.H. Fillingame, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4966–4971.
[4] H. Seelert, A. Poetsch, N.A. Dencher, A. Engel, H. Stahlberg, D.J. Müller, Nature 405 (2000) 418–419.
[5] H. Stahlberg, D.J. Müller, K. Suda, D. Fotiadis, A. Engel, T. Meier, U. Matthey, P. Dimroth, EMBO Rep. 2 (2001) 229–233.
[6] J.P. Abrahams, A.G. Leslie, R. Lutter, J.E. Walker, Nature 370 (1994) 621–628.
[7] T. Matsui, M. Yoshida, Biochim. Biophys. Acta 1231 (1995) 139–146.
[8] C. Kaibara, T. Matsui, T. Hisabori, M. Yoshida, J. Biol. Chem. 271 (1996) 2433–2438.

[9] M.J. Gresser, J.A. Myers, P.D. Boyer, J. Biol. Chem. 257 (1982) 12030–12038.
[10] T.M. Duncan, V.V. Bulygin, Y. Zhou, M.L. Hutcheon, R.L. Cross, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 10964–10968.
[11] D. Sabbert, S. Engelbrecht, W. Junge, Nature 381 (1996) 623–625.
[12] H. Noji, R. Yasuda, M. Yoshida, K. Kinosita Jr., Nature 388 (1997) 299–302.
[13] H. Noji, K. Häsler, W. Junge, K. Kinosita Jr., M. Yoshida, S. Engelbrecht, Biochem. Biophys. Res. Commun. 260 (1999) 597–599.
[14] H. Omote, N. Sambonmatsu, K. Saito, Y. Sambongi, A. Iwamoto-Kihara, T. Yanagida, Y. Wada, M. Futai, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 7780–7784.
[15] T. Hisabori, A. Kondoh, M. Yoshida, FEBS Lett. 463 (1999) 35–38.
[16] R. Yasuda, H. Noji, M. Yoshida, K. Kinosita Jr., H. Itoh, Nature 410 (2001) 898–904.
[17] H. Sakurai, K. Shinohara, T. Hisabori, K. Shinohara, J. Biochem. (Tokyo) 90 (1981) 95–102.
[18] J.V. Moroney, R.E. McCarty, J. Biol. Chem. 257 (1982) 5910–5914.
[19] F. Farron, Biochemistry 9 (1970) 3823–3828.
[20] Y. Evron, E.A. Johnson, R.E. McCarty, J. Bioenerg. Biomembranes 32 (2000) 201–506.
[21] P. Gräber, U. Junesch, G.H. Schatz, Ber. Bunsenges. Phys. Chem. 88 (1984) 599–608.
[22] J.D. Mills, P. Mitchell, FEBS Lett. 144 (1982) 63–67.
[23] U. Junesch, P. Gräber, Biochim. Biophys. Acta 893 (1987) 275–288.
[24] S. Werner-Grüne, D. Gunkel, J. Schumann, H. Strotmann, Mol. Gen. Genet. 244 (1994) 144–150.
[25] S.A. Ross, M.X. Zhang, B.R. Selman, J. Biol. Chem. 270 (1995) 9813–9818.
[26] H. Gabrys, D.M. Kramer, A.R. Crofts, D.R. Ort, Plant Physiol. 104 (1994) 769–776.
[27] J.M. Zhou, Z.X. Xue, Z.Y. Du, T. Melese, P.D. Boyer, Biochemistry 27 (1988) 5129–5135.
[28] T. Matsui, E. Muneyuki, M. Honda, W.S. Allison, C. Dou, M. Yoshida, J. Biol. Chem. 272 (1997) 8215–8221.
[29] Y. Hirono-Hara, H. Noji, M. Nishiura, E. Muneyuki, K.Y. Hara, R. Yasuda, K. Kinosita Jr., M. Yoshida, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 13649–13654.
[30] J.D. Mills, P. Mitchell, Biochim. Biophys. Acta 679 (1982) 75–83.
[31] J.D. Mills, P. Mitchell, P. Schürmann, FEBS Lett. 112 (1980) 173–177.
[32] O. Schwarz, P. Schürmann, H. Strotmann, J. Biol. Chem. 272 (1997) 16924–16927.
[33] S.R. Ketcham, J.W. Davenort, K. Warncke, R.E. McCarty, J. Biol. Chem. 259 (1984) 7286–7293.
[34] C. Gibbons, M.G. Montgomery, A.G. Leslie, J.E. Walker, Nat. Struct. Biol. 7 (2000) 1055–1061.
[35] J. Miki, M. Maeda, Y. Mukohata, M. Futai, FEBS Lett. 232 (1988) 221–226.
[36] S. Werner, J. Schümann, H. Strotmann, FEBS Lett. 261 (1990) 204–208.
[37] P.G. Pancic, H. Strotmann, FEBS Lett. 320 (1993) 61–66.
[38] J. Schümann, M.L. Richter, R.E. McCarty, J. Biol. Chem. 260 (1985) 11817–11823.
[39] M. Komatsu-Takaki, J. Biol. Chem. 264 (1989) 17750–17753.
[40] M. Komatsu-Takaki, Eur. J. Biochem. 236 (1996) 470–475.
[41] M.L. Richter, R.E. McCarty, J. Biol. Chem. 262 (1987) 15037–15040.
[42] A.J. Rodgers, M.C. Wilce, Nat. Struct. Biol. 7 (2000) 1051–1054.
[43] Y. Kato-Yamada, M. Yoshida, T. Hisabori, J. Biol. Chem. 275 (2000) 35746–35750.
[44] K.Y. Hara, Y. Kato-Yamada, Y. Kikuchi, T. Hisabori, M. Yoshida, J. Biol. Chem. 276 (2001) 23969–23973.
[45] S.P. Tsunoda, A.J. Rodgers, R. Aggeler, M.C. Wilce, M. Yoshida, R.A. Capaldi, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 6560–6564.
[46] C.M. Nalin, R.E. McCarty, J. Biol. Chem. 259 (1984) 7275–7280.
[47] J.-P. Jacquot, J.-M. Lancelin, Y. Meyer, New Phytol. 136 (1997) 543–570.
[48] T. Hisabori, Y. Kato, K. Motohashi, H. Strotmann, P. Kroth-Pancic, T. Amano, Eur. J. Biochem. 247 (1997) 1158–1165.
[49] W.C. Tucker, Z. Du, R. Hein, M.L. Richter, Z. Gromet-Elhanan, J. Biol. Chem. 275 (2000) 906–912.
[50] D. Bald, H. Noji, M.T. Stumpp, M. Yoshida, T. Hisabori, J. Biol. Chem. 275 (2000) 12562–12757.
[51] D. Bald, H. Noji, M. Yoshida, Y. Hirono-Hara, T. Hisabori, J. Biol. Chem. 276 (2001) 39505–39507.
[52] U. Uhlin, G.B. Cox, J.M. Guss, Structure 5 (1997) 1219–1230.
[53] S. Wilkens, R.A. Capaldi, J. Biol. Chem. 273 (1998) 26645–26651.
[54] Y. Kato, T. Matsui, N. Tanaka, E. Muneyuki, T. Hisabori, M. Yoshida, J. Biol. Chem. 272 (1997) 24906–24912.
[55] P. Soteropoulos, K.-H. Süss, R.E. McCarty, J. Biol. Chem. 267 (1992) 10348–10354.
[56] P.J. Andralojc, D.A. Harris, Biochim. Biophys. Acta 1016 (1990) 55–62.
[57] R.J. Duhe, B.R. Selman, Biochim. Biophys. Acta 1017 (1990) 70–78.
[58] M.S. Dann, R.E. McCarty, Plant Physiol. 99 (1992) 153–160.
[59] M.T. Stumpp, K. Motohasi, T. Hisabori, Biochem. J 341 (1999) 157–163.
[60] X. He, M. Miniginiac-Maslow, C. Sigalat, E. Keryer, F. Haraux, J. Biol. Chem. 275 (2000) 13250–13258.
[61] T. Kobayashi, S. Kishigami, M. Sone, H. Inokuchi, T. Mogi, K. Ito, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11857–11862.
[62] E.A. Johnson, R.E. McCarty, Biochemistry 41 (2002) 2446–2451.