Proton flux through the chloroplast ATP synthase is altered by cleavage of its gamma subunit

ARTICLE *in* BIOCHIMICA ET BIOPHYSICA ACTA · AUGUST 2007

Impact Factor: 4.66 · DOI: 10.1016/j.bbabio.2007.04.008 · Source: PubMed

2 AUTHORS, INCLUDING:

Richard E Mccarty
Johns Hopkins University
132 PUBLICATIONS **3,827** CITATIONS

SEE PROFILE

Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1767 (2007) 974–979
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbabio

Proton flux through the chloroplast ATP synthase is altered by cleavage of its gamma subunit

Jeremy R. McCallum, Richard E. McCarty *

Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218, USA

Received 20 February 2007; received in revised form 2 April 2007; accepted 23 April 2007
Available online 10 May 2007

Abstract

Electron transport, the proton gradient and ATP synthesis were determined in thylakoids that had been briefly exposed to a low concentration of trypsin during illumination. This treatment cleaves the γ subunit of the ATP synthase into two large fragments that remain associated with the enzyme. Higher rates of electron transport are required to generate a given value of the proton gradient in the trypsin-treated membranes than in control membranes, indicating that the treated membranes are proton leaky. Since venturicidin restores electron transport and the proton gradient to control levels, the proton leak is through the ATP synthase. Remarkably, the synthesis of ATP by the trypsin-treated membranes at saturating light intensities is only slightly inhibited even though the proton gradient is significantly lower in the treated thylakoids. ATP synthesis and the proton gradient were determined as a function of light intensity in control and trypsin-treated thylakoids. The trypsin-treated membranes synthesized ATP at lower values of the proton gradient than the control membranes. Cleavage of the γ subunit abrogates inhibition of the activity of the chloroplast ATP synthase by the ε subunit. Our results suggest that overcoming inhibition by the ε subunit costs energy.

Keywords: Chloroplast ATP synthase; Thylakoid membranes; Electron transport; ATP synthesis; Proton flux; γ subunit; ε subunit

1. Introduction

Light-dependent ATP formation in chloroplasts is catalyzed by an enzyme, the chloroplast ATP synthase, that has many similarities to its counterparts in other coupling membranes [1]. ATP synthases are composed of two separable parts: F1 and Fo. F1 is a hydrophilic protein complex that bears the nucleotide binding sites of the enzyme. Chloroplast F1 (CF1) has five different polypeptides in the stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$. Chloroplast Fo (CFo), a hydrophobic integral membrane protein, binds CF1 and conducts protons across the thylakoid membrane.

Although the structures and mechanisms of action of the proton-linked ATP synthases are similar, the chloroplast ATP synthase differs from ATP synthases from the coupling membranes of mitochondria and eubacteria in that its activity is stringently regulated. The enzyme is virtually inactive in the dark, but is very active in the light. (See references [2–4] for reviews.) *In vitro* studies have identified four mechanisms that may contribute to this unusual regulation: the electrochemical proton gradient (hereafter referred to as the proton gradient or ΔpH) [2], the binding of Mg^{2+} ADP [5,6], the oxidation state of the disulfide/dithiol of the γ subunit [7–9] and the ε subunit [10,11]. The ε subunit is a potent inhibitor of the activity of the (CF1). The γ subunit of CF1 contains an extra domain of approximately 44 amino acids that is missing in mitochondrial and bacterial F1s [12]. This domain contains the only two redox active Cys residues. Reduction of the γ subunit disulfide stimulates the activity of CF1, in part by weakening the interaction between the γ and ε subunits [13,14].

Illumination of thylakoids in the presence of the protease, trypsin, results in specific cleavage of the γ subunit into two large fragments that remain associated with the enzyme [15,16]. Trypsin cuts the γ subunit of CF1 in illuminated thylakoids at

Abbreviations: F1, catalytic portion of ATP synthases; CF1, chloroplast F1; Fo, portion of the ATP synthase intrinsic to the membrane; CFo, chloroplast Fo; CF1-ε, CF1 deficient in its ε subunit

* Corresponding author. Fax: +1 410 516 5213.
E-mail address: rem1@jhu.edu (R.E. McCarty).

0005-2728/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2007.04.008

Arg 215 and Lys 231. These residues are present in the domain of the γ subunit that is absent in MF1 and ECF1.

Uncoupling and an increase in the proton permeability of the trypsin-treated thylakoids were seen [17,18]. The illumination of thylakoids in the presence of trypsin enhances the ATPase activity of CF1, assayed either in solution or when bound to the membrane. Although the physiologically significant substrates of the chloroplast ATP synthase are Mg^{2+} ATP and Mg^{2+} ADP, Ca^{2+} ATPase activity is a useful property of the enzyme. The Ca^{2+} ATPase activity of CF1 in thylakoid membranes or in solution is quite low [19,20]. CF1 stripped of its ε subunit (CF1-ε), however, has high Ca^{2+} ATPase activity whether it is membrane bound [21] or in solution [11] and this activity is readily inhibited by the rebinding of the ε subunit. The Ca^{2+} ATPase activity of CF1-ε from trypsin-treated thylakoids is not inhibited by the ε subunit [14]. The fact that the Ca^{2+} ATPase activity of thylakoid membranes illuminated in the presence of trypsin is high [20] is, therefore, a clear indication that cleavage of the γ subunit abolishes inhibition of activity by the ε subunit.

Conditions were found under which substantial activation of the ATPase activity of CF1 in thylakoids by trypsin was obtained with minimal loss of photophosphorylation [20]. In this paper we report the results of a study of electron transport, proton gradient formation and ATP synthesis by control and trypsin-treated membranes. Although by some criteria the trypsin-treated membranes are uncoupled, they catalyze higher rates of light-dependent ATP synthesis at low values of the proton gradient than the control thylakoids. It is concluded that overcoming inhibition of activity by the ε subunit requires energy.

2. Materials and methods

Thylakoids were isolated from market spinach as described [20] and were incubated on ice with 0.1 mM $K_3Fe(CN)_6$ for 30 min to assure that the ATP synthase was oxidized. The thylakoids (0.2 mg chlorophyll ml^{-1}) were then incubated at room temperature with 5 μg ml^{-1} trypsin. The incubation medium (5 ml total volume) contained 50 mM Tricine-NaOH (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$ and 0.025 mM pyocyanine. After 1 min in the light (2 kw/m^2) the samples were transferred to centrifuge tubes that contained 0.15 mg of soybean trypsin inhibitor. The control thylakoids were treated in the same manner, except that no trypsin was added. The suspensions were then centrifuged at 6000 ×*g* for 5 min and the pellets were resuspended in 0.6 ml of STN (400 mM sucrose, 20 mM Tricine-NaOH (pH 8.0), and 10 mM NaCl).

Photophosphorylation was determined in a reaction mixture that contained 50 mM Tricine-NaOH (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM ADP (treated with hexokinase and glucose to reduce the ATP content), 1 mM Pi, either 0.025 mM pyocyanine or 0.3 mM $K_3Fe(CN_6)$ and thylakoids equal to 20 or 50 μg of chlorophyll per ml. When photophosphorylation was assayed by ATP formation using the luciferase method, diadenosine pentaphosphate (10 μM) was also added. In some experiments, photophosphorylation rates with pyocyanine as the mediator of cyclic electron transport were determined by disappearance of Pi. In experiments in which photophosphorylation and ΔpH were estimated in the same sample, 9-aminoacridine was present at 2 μM.

ΔpH (pHout–pHin) was calculated from the light-dependent quenching of the fluorescence (excitation 399 nm; emission 430 nm) of 9-aminoacridine as described [22], assuming a ratio of external volume to internal thylakoid volume of 2500 to 1. Actinic light was provided by a cluster of light-emitting diodes that emitted at 660 nm with an intensity of 200 μmol m^{-2} s^{-1}. Light intensity was varied either by the use of calibrated wire screens or by neutral density filters. The stirred reaction mixture (2 ml) contained 50 mM Tricine-NaOH (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$, thylakoids equal to 40 μg of chlorophyll, either 0.025 mM pyocyanine or 0.3 mM $K_3Fe(CN)_6$ and other additions as noted in the legends to figures and the table. Rates of ferricyanide reduction were determined by the simultaneous assay of ΔpH and electron transport [23].

3. Results

Although many of the properties of CF1 from thylakoids that had been incubated with trypsin in the light have been thoroughly characterized, electron transport and associated ATP synthesis by the trypsin-treated membranes have not received much attention. The dependence of the rate of ATP synthesis (Fig. 1A) and the quenching of the fluorescence of 9-aminoacridine (Fig. 1B) on light intensity were determined in separate experiments using pyocyanine as the mediator of electron flow. The trypsin treatment inhibited ATP synthesis at all light intensities, but the inhibition was not marked. The quenching of the fluorescence of 9-aminoacridine was more severely impaired by the trypsin treatment, especially at lower light intensities. The rate of ATP synthesis by thylakoids is sharply dependent on the magnitude of ΔpH; a decrease in the ΔpH by 0.3 unit is associated with a ten-fold decline in the rate of ATP synthesis [24,25]. The quenching of 9-aminoacridine

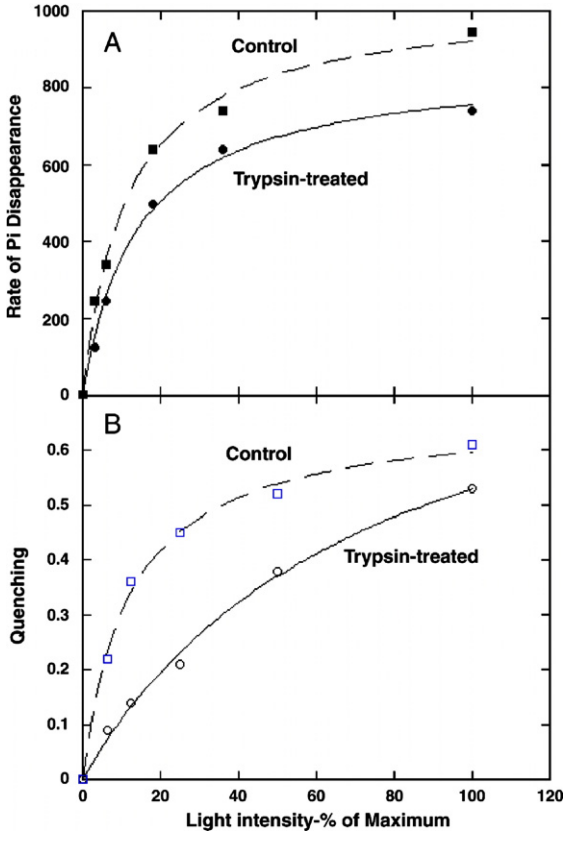

Fig. 1. A. Light intensity dependence of ATP synthesis in control and trypsin-treated thylakoids. ATP synthesis with pyocyanine as the mediator of cyclic electron flow was determined by measuring Pi disappearance. 100% light intensity was 2 kw/m^2 0f white light and the intensity was varied by the use of calibrated wire screens. Rates are expressed as μmol Pi disappeared/hr/mg chlorophyll. B. Light intensity dependence of the quenching of the fluorescence of 9-aminoacridine in control and trypsin-treated thylakoids. The actinic light intensity was varied by the use of neutral density filters.

fluorescence in the control and trypsin-treated thylakoids at 25% of full light intensity (Fig. 1B) is 0.44 and 0.20, respectively. These quenching values translate to ΔpH values of 3.30 for the control thylakoids and 2.80 for the treated thylakoids. Thus, based on the quenching values, a much greater inhibition on ATP synthesis by the trypsin-treated membranes would have been expected.

Thylakoids were either incubated in the dark with trypsin for 1 min or incubated in the light with trypsin that had been incubated with the trypsin inhibitor prior to the addition of the thylakoids. The properties of these control thylakoids did not differ from the control thylakoids to which no trypsin had been added.

As a test of our methods, the effect of reduction of the γ disulfide bond on the relationship between the rates of ATP synthesis and ΔpH was determined. Reduction of the disulfide bond shifts the curve that relates phosphorylation rates to ΔpH to lower ΔpH values than in oxidized thylakoids [8,9]. We confirmed these results with our methods. Reduction shifted the curve by about 0.3 pH unit to lower values of the ΔpH (data not shown). Thus, the apparent discrepancy between ΔpH and the rate of ATP synthesis in the trypsin-treated thylakoids is not likely to be an artifact of the methods used.

ATP synthesis rates were determined in control and trypsin-treated thylakoids as a function of ΔpH which was varied by light intensity. Pyocyanine was the mediator of electron flow (Fig. 2). The rates of ATP synthesis by the trypsin-treated membranes were found to be higher than those in the control thylakoids at the lower values of the ΔpH.

These results seem to be at odds with the conclusion that trypsin treatment uncouples phosphorylation from electron transport. A closer look at the coupling in the control and treated thylakoids seemed warranted. We used the simultaneous method for the determination of rates of electron transport (water to ferricyanide) and ΔpH at several different light intensities [23]. At the higher light intensities, the rate of electron transport in the trypsin-treated thylakoids was higher than that

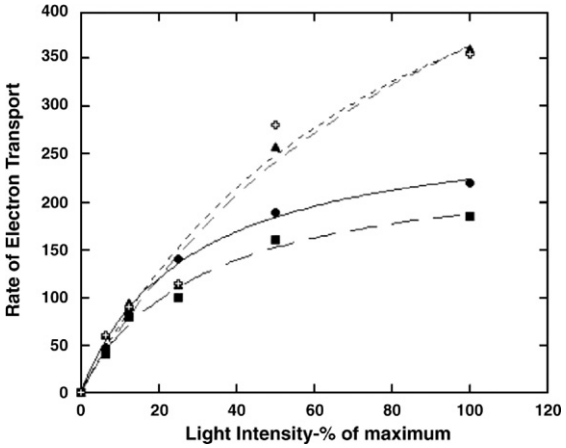

Fig. 3. The effects of ADP on electron transport. Electron transport (μmol ferricyanide reduced/hr/ mg chlorophyll) was determined by the simultaneous assay in the presence and absence of 50 μM ADP. Circles: control thylakoids, no ADP. Squares: control thylakoids plus ADP. Triangles: Trypsin-treated thylakoids no ADP. Crosses: trypsin-treated thylakoids plus ADP.

in the control thylakoids (Fig. 3). This result is consistent with uncoupling by the trypsin treatment. Note as well that ADP inhibits electron transport in the control thylakoids, but not in the treated thylakoids. ADP partially prevents a proton leak or slip through the ATP synthase that is elicited at high ΔpH values [26,27]. Even though electron transport at high light intensities is accelerated by the incubation of thylakoids with trypsin, the ΔpH values achieved by the treated membranes are 0.4 to 0.5 pH unit lower than those reached by the control membranes (Fig. 4). This result is another indication of uncoupling. ADP partially blocks proton leakage in control thylakoids, enhancing ΔpH, but there is less effect of ADP on the ΔpH generated by the trypsin-treated thylakoids.

In coupled thylakoids, the rate of electron transport is stimulated by phosphorylation. At high light intensity, the already fast rate of electron flow in trypsin-treated thylakoids was further enhanced by ADP and Pi (Table 1). ADP and Pi

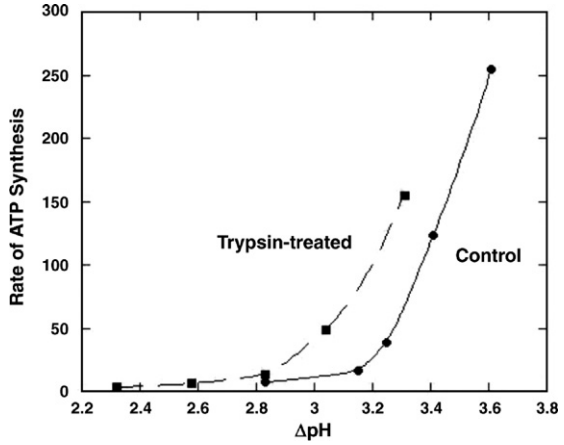

Fig. 2. Trypsin-treated thylakoids catalyze higher rates of ATP synthesis at low values of the ΔpH than control thylakoids. Pyocyanine was the mediator of cyclic electron flow. ΔpH was calculated from the quenching of the fluorescence of 9-aminoacridine and ATP was determined in aliquots of the same samples by the luciferin/luciferase method.

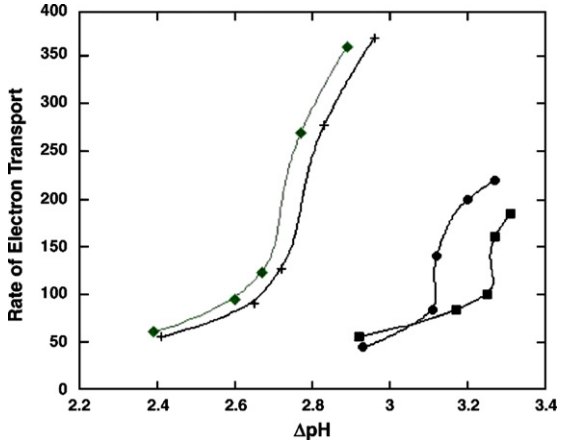

Fig. 4. The relationship between the rate of electron transport and ΔpH. Electron transport and ΔpH were determined at five light intensities by the simultaneous assay in the presence and absence of 50 μM ADP. Circles: control thylakoids no ADP. Squares; control thylakoids plus ADP. Diamonds: trypsin-treated thylakoids no ADP. Crosses: trypsin-treated thylakoids plus ADP.

Table 1
Effect of ATP Synthesis on the Rate of Electron Transport and the Quenching of the Fluorescence of 9-Aminoacridine

Thylakoids	Electron Transport		Quenching	
	+ADP	+ADP and Pi	+ADP	+ADP and Pi
Control	172	300	0.39	0.26
+Trypsin	323	450	0.31	0.16

Electron transport and the quenching of the fluorescence of 9-aminoacridine were determined at the highest light intensity using the simultaneous assay in the presence of either 50 μM ADP or of 1 mM ADP and 1 mM Pi. Electron transport is given as μmol ferricyanide reduced/hr/mg chlorophyll.

decreased the extent of the light-dependent quenching of 9-aminoacridine fluorescence (Table 1) in both the control and treated thylakoids, indicating that ATP synthesis was occurring in both preparations. As shown in Fig. 5, the ΔpH values generated by electron transport in the presence of ADP and Pi in trypsin-treated thylakoids were lower than those in control thylakoids at the same light intensity. Yet, the rate of ATP synthesis by the treated membranes at a given value of the ΔpH was higher than that of the control membranes. At high light intensity the phosphorylation efficiencies (the ratio of the rate ATP synthesis to that of ferricyanide reduction divided by two or P/e_2 ratio) of the control and treated membranes were similar (0.65 for the control thylakoids and 0.52 for the treated thylakoids). At lower light intensities, however, the P/e_2 ratio of the trypsin-treated thylakoids at a given ΔpH was higher than that of the control. For example, at a ΔpH of 2.60, the P/e_2 ratio for the trypsin-treated thylakoids was 0.36 and that of the control thylakoids, 0.28. Thus, thylakoid membranes that contain a cleaved γ subunit have the enigmatic property of being loosely coupled in one sense and better coupled than control thylakoids, in another. Reduction of the disulfide bond has similar effects on ATP synthesis [8,9] and electron transport [23] and it is perhaps not a coincidence that trypsin cuts the γ subunit in the same region of the γ subunit.

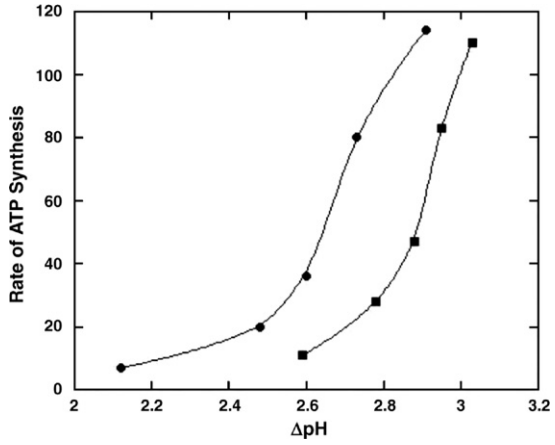

Fig. 5. ATP synthesis as a function of ΔpH. ATP synthesis and the quenching of the fluorescence of 9-aminoacridine were determined at five light intensities. Ferricyanide was the electron acceptor. The rates of ATP synthesis are expressed as μmol ATP formed/hr/mg chlorophyll. Squares: control thylakoids. Circles: trypsin-treated thylakoids.

Table 2
Effect of Venturicidin and Tentoxin on Electron Transport and the Quenching of 9-Aminoacridine Fluorescence

Additions	Control Thylakoids			Trypsin-treated Thylakoids		
	Electron Transport	Q	ΔpH	Electron Transport	Q	ΔpH
None	145	0.33	3.09	262	0.27	2.97
Venturicidin	107	0.39	3.20	109	0.38	3.19
ADP and Pi	237	0.23	2.88	333	0.14	2.61
Venturicidin plus ADP and Pi	109	0.34	3.13	150	0.33	3.09
Tentoxin plus ADP and Pi	n.d.			262	0.26	2.95

Electron transport (expressed as μmol $Fe(CN)_6^{3-}$ reduced/hr/ mg chlorophyll) and the light-dependent quenching (Q) of the fluorescence of 9-aminoacridine were determined at full light intensity using the simultaneous assay. n. d. stands for not determined.

The shift of the curve that relates the rates of ATP synthesis to lower values of the ΔpH by either reduction or trypsin treatment is not simply a result of the increased permeability of the treated membranes to protons. The light intensity dependence of ATP synthesis in control thylakoids as well as in the presence of a fixed concentration of the protonophores, carbonyl cyanide m-chlorophenylhydrazone or gramicidin was determined [28]. No shift was detected.

The effects of the incubation with trypsin on electron transport are indirect. Control and trypsin-treated thylakoids had the same rate of electron transport (1100 μmol $Fe(CN)_6^{3-}$ reduced/hr/mg chlorophyll) at full light intensity in the presence of 2.5 μM gramicidin and 4 mM NH_4Cl. The uncoupling induced by the trypsin treatment was fully reversed by venturicidin which blocks proton transport through CFo (Table 2) [29]. Venturicidin also blocks the stimulation of electron transport and decrease in ΔpH seen in the presence of ADP and Pi in both control and treated thylakoids. Tentoxin, which inhibits ATP synthesis but does not block proton transport [30], blocked only that part of the stimulation of electron transport in trypsin-treated thylakoids that is associated with ATP synthesis.

4. Discussion

There are pitfalls in the use of proteases to probe the actions of a complicated enzyme in a membrane. Many targets for protease action are possible. Several facts support the notion that the γ subunit is the major target of trypsin action in our studies. The exposure of the thylakoids to the enzyme is short and the concentration of the trypsin is low. Moreover, when the γ subunit of CF1 is oxidized, light is required for trypsin to act [15]. The effect of light is indirect. Light-driven electron transport generates the proton gradient that in turn alters the structure of CF1. Evidence for energy-dependent changes in the γ and ε subunits includes the light-dependent alkylation of Cys89 of the γ subunit [31], the large acceleration of the rate of reduction of the γ disulfide bond by illumination [9,19] the energy-dependent modification of Lys109 of the ε subunit [32] and the light-dependent exposure of the C-terminus of the ε subunit to antibodies [33].

The C-terminus of the ε subunit likely interacts with the γ subunit in CF1 in thylakoids in the dark in such a way as to inhibit activity and to block the access of external reagents to the domain of the γ subunit that bears the Cys residues that are redox active and that is absent in the γ subunits of the ATP synthases of bacteria and mitochondria [12]. The ΔpH-induced movement of the C-terminus of the ε subunit could nullify inhibition by the ε subunit and expose the γ subunit to reduction as well as to trypsin. The rate of reduction of the γ subunit disulfide of CF1 is markedly enhanced by the removal of the ε subunit [34] and the addition of the ε subunit retards the rate of reduction [35]. ε subunit from which 45 amino acids were deleted from its C-terminus did not slow the rate of reduction. The truncated ε was, however, as active as full-length ε in restoring ATP synthesis to thylakoid membranes that contain CF1-ε [36].

The rate of specific cleavage of the γ subunit of CF1 in solution by trypsin is also enhanced by removal of the ε subunit. The fragments of the γ subunit that are generated by the incubation of oxidized CF1-ε with trypsin in solution and by the incubation of thylakoids in the light with trypsin are identical (fragment 1, amino acids 1-215 and fragment 2, 232-323) [16]. These large fragments remain tightly associated with the $\alpha_3\beta_3$ hexamer. Trypsin cuts the γ subunit close to the disulfide bond which is formed by residues 199 and 205. The activity of trypsin treated CF1-ε is not inhibited by the ε subunit [14].

The evidence that the γ subunit is the site of action of the protease is further strengthened by comparison of the effects of other specific modifications of the γ subunit on the activity and proton flux through the synthase to those of the trypsin treatment. Alkylation of Cys89 of the γ subunit by N-ethylmaleimide increases the proton permeability of the membranes and this effect is reversed by the presence of nucleotides in the medium [27]. Cross-linking of γCys322 to another group within the γ subunit increased the proton leakiness of the membranes and inhibited ATP synthesis [37]. The modification of the γ subunit whose effects most closely resemble those of the trypsin cleavage of γ is the reduction of the γ subunit disulfide. Both reduction [19,38] and trypsin cleavage [17,18,39] stimulate the ATPase activity of thylakoid membranes. Both reduction and trypsin cleavage permit ATP synthesis to occur at lower values of the ΔpH than in oxidized thylakoids. Reduction [23] and cleavage [17,18] of the γ subunit increase the leakiness of thylakoid membranes to protons and venturicidin blocks this leak. Both reduction and trypsin cleavage of the γ subunit decrease the affinity of CF1 for the ε subunit. Moreover, Samra et al. [40] found that the Ca^{2+} ATPase activity of $\alpha_3\beta_3\gamma$ complexes that contained γ subunits with deletions in the disulfide region was less sensitive to inhibition by the ε subunit than the complex that contained the full-length ε subunit.

There are, however, significant differences in the properties of reduced and trypsin-treated membranes. The Ca^{2+} ATPase activity of reduced thylakoids is very low, but that of thylakoids incubated with trypsin in the light is at least an order of magnitude higher [20]. CF1-ε bound to thylakoid membranes has high Ca^{2+} ATPase activity [11,34]. Sulfite, which reverses inhibition by Mg^{2+} and Mg^{2+} ADP [38,41], does not stimulate the Mg^{2+} ATPase activity of reduced membranes as much as that of trypsin-treated membranes [20]. These differences may be explained by the facts that the activity of the reduced enzyme in solution is inhibited by added ε subunit, whereas the activity of CF1 from trypsin-treated membranes is not [14]. Thus, the activity of the reduced enzyme may be limited by the residual inhibition by the ε subunit.

Another difference between the two preparations is that the Mg^{2+} ATPase activity of the enzyme in reduced thylakoids is sensitive to ADP, whereas the activity of the enzyme in thylakoids incubated with trypsin is not [20]. Electron transport in trypsin-treated thylakoids is also not affected by ADP.

How can the ATP synthase in the trypsin-treated thylakoids catalyze relatively rapid ATP synthesis at low values of the ΔpH? One plausible explanation involves the effects of the cleavage of the γ subunit on interactions with the ε subunit. The ε subunit is an inhibitor of the ATPase activity of the synthase. It must also inhibit ATP synthesis as well and energy in the form of the proton gradient must be expended to overcome this inhibition. Since inhibition by the ε subunit is obviated in the trypsin-treated thylakoids, less energy may be needed to convert the ATP synthase an active state. The curve that relates the rates of ATP synthesis to ΔpH is shifted by about 0.25 pH unit to lower values by the trypsin treatment. This shift may seem small, but in view of the sharp dependence of the rate of photophosphorylation on the magnitude of ΔpH [24,25], it has easily discernable effects on the coupling of ATP synthesis to proton transport.

Acknowledgements

This research was supported in part by a grant from the National Science Foundation (MCB-0110232). This article is based in part on the Master's thesis of JRM (Johns Hopkins University, 2006). We thank Dr. Eric A. Johnson for many helpful discussions and his help in the preparation of the manuscript.

References

[1] P.L. Pedersen, Y.H. Ko, S. Hong, ATP synthases in the year 2000. Evolving views about the structures of these remarkable enzyme complexes, J. Bioenrg. Biomembr. 32 (2000) 325–332.
[2] D.R. Ort, K. Oxborough, In situ regulation of chloroplast coupling factor activity, Annu. Rev. of Plant Physiol. and Plant Mol. Biol. 43 (1992) 269–291.
[3] T. Hisabori, H. Konno, H. Ichimura, H. Strotmann, D. Bald, Molecular devices of chloroplast F-1-ATP synthase for the regulation, Biochim. Biophys Acta 1555 (2002) 140–146.
[4] M.L. Richter, Gamma-epsilon interactions regulate the chloroplast ATP synthase, Photosyn. Res. 79 (2004) 319–329.
[5] Y. Hochman, A. Lanir, C. Carmeli, Binding of cations, ATP and cation-ATP complexes as measured by EPR and ATPase activity in coupling factor from chloroplasts, Israel J. Med. Sci. 12 (1976) 1366–1366.
[6] H. Strotmann, S. Bickel, B. Huchzermeyer, Energy-dependent Release of adenine-nucleotides tightly bound to chloroplast coupling factor CF1, FEBS Lett. 61 (1976) 194–198.

[7] C.M. Nalin, R.E. McCarty, Role of a disulfide bond in the γ subunit in activation of the ATPase of chloroplast coupling factor1, J. Biol. Chem. 259 (1984) 7275–7280.
[8] J.D. Mills, P. Mitchell, Thiol modulation of the chloroplast protonmotive ATPase and its effect on photophosphorylation, Biochim. Biophys. Acta 764 (1984) 93–104.
[9] S.R. Ketcham, J.W. Davenport, K. Warncke, R.E. McCarty, Role of the gamma-subunit of chloroplast coupling factor-I in the light-dependent activation of photophosphorylation and ATPase activity by dithiothreitol, J. Biol. Chem. 259 (1984) 7286–7293.
[10] M.L. Richter, W.D. Patrie, R.E. McCarty, Preparation of the ε subunit and ε subunit deficient chloroplast coupling factor 1 in reconstitutively active forms, J. Biol. Chem. 259 (1984) 7371–7373.
[11] J.A. Cruz, B. Harfe, C.A. Radkowski, M.S. Dann, R.E. McCarty, Molecular dissection of the epsilon-subunit of the chloroplast ATP synthase of spinach, Plant Physiol. 109 (1995) 1379–1388.
[12] J. Miki, M. Maeda, Y. Mukohata, M. Futai, The gamma-subunit of ATP synthase from spinach-chloroplasts- Primary structure deduced from the cloned cDNA sequence, FEBS Lett. 232 (1988) 221–226.
[13] R.J. Duhe, B.R. Selman, The dithiothreitol-stimulated dissociation of the chloroplast coupling factor-1 epsilon-subunit is reversible, Biochim. Biophys. Acta 1017 (1990) 70–78.
[14] P. Soteropoulos, K.H. Suess, R.E. McCarty, Modifications of the gamma-subunit of chloroplast coupling factor-I alter interactions with the inhibitory epsilon-subunit, J. Biol. Chem. 267 (1992) 10348–10354.
[15] J. Schumann, M.L. Richter, R.E. McCarty, Partial proteolysis as a probe of the conformation of the gamma-subunit in activated soluble and membrane-bound chloroplast coupling factor-1, J. Biol. Chem. 260 (1985) 1817–1823.
[16] K.E. Hightower, R.E. McCarty, Proteolytic cleavage within a regulatory region of the gamma subunit of chloroplast coupling factor 1, Biochemistry 35 (1996) 4846–4851.
[17] W.S. Lynn, K.D. Straub, ADP kinase and ATPase in chloroplasts, Proc. Natl. Acad. Sci. U. S. A. 63 (1969) 540–547.
[18] J.V. Moroney, R.E. McCarty, Light-dependent cleavage of the γ subunit of coupling factor 1 causes activation of Mg^{2+}-ATPase activity and uncoupling of photophosphorylation, J. Biol. Chem. 257 (1982) 5915–5920.
[19] R.E. McCarty, E. Racker, Partial resolution of the enzymes catalyzing photophosphorylation III. Activation of adenosine triphosphatase and ^{32}P-labeled orthophosphate-adenosine triphosphate exchange of chloroplasts, J. Biol. Chem. 243 (1968) 129–137.
[20] R.E. McCarty, ATP synthase of chloroplast thylakoid membranes: A more in depth characterization of its ATPase activity, J. Bioenerg. Biomembr. 37 (2005) 289–297.
[21] J.A. Cruz, C.A. Radkowski, R.E. McCarty, Functional consequences of deletions of the N-terminus of the ε subunit of the chloroplast ATP synthase, Plant Physiol. 113 (1997) 1185–1192.
[22] S. Schuldiner, G. Rottenberg, M. Avron, Determination of ΔpH in chloroplasts.2. Fluorescent amines as a probe for the determination of ΔpH in chloroplasts, Eur. J. Biochem. 25 (1972) 64–70.
[23] Y. Evron, R.E. McCarty, Simultaneous measurement of ΔpH and electron transport in spinach chloroplast thylakoids by 9-aminoacridine, Plant Physiol. 124 (2000) 407–414.
[24] A.R. Portis, R.E. McCarty, Effects of adenine nucleotides and of illuminated photophosphorylation on H^+uptake and the magnitude of the H^+ gradient in chloroplasts, J. Biol. Chem. 249 (1974) 6250–6254.
[25] J.W. Davenport, R.E. McCarty, An analysis of proton fluxes coupled to electron transport and ATP synthesis in chloroplast thylakoids, Biochim. Biophys. Acta 766 (1984) 363–374.
[26] R.E. McCarty, J.S. Fuhrman, Y. Tsuchiya, Effects of adenine nucleotides on hydrogen ion transport in chloroplasts, Proc. Natl. Acad. Sci. U. S. A. 68 (1971) 2522–2526.
[27] Y. Evron, M. Avron, Characterization of an alkaline pH-dependent proton slip in the ATP synthase of lettuce chloroplasts, Biochim. Biophys. Acta 1019 (1990) 115–120.
[28] J.W Davenport, R.E. McCarty, Relationships between rates of steady state ATP synthesis and the magnitude of the proton gradient across thylakoid membranes, Biochim. Biophys. Acta 851 (1986) 136–145.
[29] P.E. Linnett, R.B. Beechy, Inhibitors of the ATP synthase system, Meth. Enzymology 545 (1979) 472–518.
[30] J.A. Steele, T.E. Uchytil, R.D. Durbin, P. Bhatnagar, D.H. Rich, Chloroplast coupling factor 1: A species-specific receptor for tentoxin, Proc. Natl. Acad. Sci. U. S. A. 73 (1976) 2245–2248.
[31] R.E. McCarty, J. Fagan, Light-stimulated incorporation of N-ethylmaleimide into coupling factor 1 in spinach chloroplasts, Biochemistry 12 (1973) 1503–1507.
[32] M. Komatsu-Takaki, Energy-dependent conformational-changes in the epsilon-subunit of the chloroplast ATP synthase (CFoCF1), J. Biol. Chem. 264 (1989) 17750–17753.
[33] E.A. Johnson, R.E. McCarty, The carboxyl terminus of the ε subunit of the chloroplast ATP synthase is exposed during illumination, Biochemistry 41 (2002) 2446–2451.
[34] M.L. Richter, B. Snyder, R.E. McCarty, G.G. Hammes, Binding stoichiometry and structural mapping of the ε polypeptide of chloroplast coupling factor 1, Biochemistry 24 (1985) 5755–5763.
[35] K.F. Nowak, R.E. McCarty, Regulatory role of the C-terminus of the ε subunit from the chloroplast ATP synthase, Biochemistry 43 (2004) 3273–3279.
[36] K.F. Nowak, V. Tabidze, R.E. McCarty, The C-terminal domain of the chloroplast ATP synthase is not required for ATP synthesis, Biochemistry 41 (2002) 15130–15134.
[37] M.A. Weiss, R.E. McCarty, Cross-linking within a subunit of coupling factor 1 increases the proton permeability of spinach chloroplast thylakoids, J. Biol. Chem. 252 (1977) 8007–8012.
[38] B. Petrack, A. Craston, F. Sheppy, F. Farron, Studies on the hydrolysis of adenosine triphosphate by spinach chloroplasts, J. Biol. Chem. 240 (1965) 906–914.
[39] E.M. Larson, A.T. Jagendorf, Sulfite stimulation of chloroplast coupling factor ATPase, Biochim. Biophys. Acta 973 (1989) 67–77.
[40] H.S. Samra, F. Gao, F. He, E. Hoang, Z. Chen, P.A. Gegenheimer, C.L. Berrie, M.L. Richter, Structural analysis of the regulatory dithiol-containing domain of the chloroplast ATP synthase gamma subunit, J. Biol. Chem. 281 (2006) 31041–31049.
[41] J.D. Digel, N.D. Moore, R.E. McCarty, Influence of divalent cations on nucleotide exchange and ATPase activity of chloroplast coupling factor 1, Biochemistry 49 (1998) 17209–17215.