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Chloroplast ATP synthase: the clutch between proton flow and ATP synthesis is at the interface of subunit γ and CF_1

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Abstract

ATP synthase, CF_oCF_1, is both activated and driven by protonmotive force. Composed of more than 20 (8 different) subunits it probably functions as an electro-mechano-chemical transducer. We characterized conformational changes involving subunit γ of CF_1 in response to proton flow through CF_o. At the C-terminal end γ contains one cysteine (Cys^{322}, 'dark site'), which is readily accessible to modification by maleimides. Whereas the modification of this group with a monofunctional maleimide is without effect on the activity, the modification with the bifunctional ortho-phenylenebismaleimide (OPBM), in the presence of protonmotive force, causes a cross-link, induces proton leakage and inhibits photophosphorylation. We used these effects of OPBM to monitor the activity-linked conformational changes involving subunit γ. These conformational changes were induced by both modes of *proton flow* through the enzyme, *slipping*, without added nucleotides, and *coupled*, at catalytic concentration of ADP causing ATP synthesis. They were not induced when proton flow was blocked at intermediate ADP concentration. The implications are two-fold: (1) Proton slip, i.e., the idling of the proton transport machinery, induces the same type of conformational changes of γ as the coupled catalytic cycle. (2) The clutch to ATP synthesis resides beyond γ at its interface with the rest of CF_1, i.e., in the F_1-portion of this enzyme.

Keywords: ATP synthase; Proton flow; Conformational coupling; Bioenergetics

1. Introduction

ATP synthase, F_oF_1, catalyzes the formation of ATP from ADP and phosphate at the expense of protonmotive force. Its structure is bipartite with a membrane embedded, proton conducting portion, F_o, and a peripheral portion, F_1, which carries six nucleotide binding sites, three of which are potentially catalytic (for recent reviews see [1–5]). CF_1, the F_1-portion of the chloroplast enzyme, is composed of five subunits in stoichiometric proportion $\alpha_3\beta_3\gamma\delta\epsilon$. Recently a partial structure of F_1 from beef heart mitochondria has been disclosed at 2.8 Å resolution [6]. It provides a 'snapshot' of the ADP-inhibited state of the enzyme where the symmetry of the $\alpha_3\beta_3$-moeity is broken by different nucleotides but also by γ. The spatial arrangement of γ relative to the three pairs of $\alpha\beta$ differs depending on whether the respective β contains ADP, AMP-PNP (a non-hydrolysable ATP analogue) or nothing. The three positions of γ relative to β are highly suggestive of rotatory mechanical coupling steps between proton flow and ATP synthesis [7]. This is in line with electron microscopic evidence showing different relative arrangements of the smaller subunits depending on the nucleotide load of the enzyme [8]. The detailed structure of the channel portion and the arrangement of the functionally essential smaller subunits, of $\delta\epsilon$ and one portion of γ, that are supposedly at the interface between F_o and F_1, remains to be elucidated.

Protonmotive force not only drives ATP synthesis by CF_oCF_1 but also activates the enzyme [9–13]. The conformational changes which accompany activation and/or turnover have been indirectly assayed by the following

Abbreviations: 9-AA, 9-aminoacridine; AdN, adenine nucleotides; AMP-PNP, 5′-adenylyl-imidodiphosphate; CF_o, proton channel of ATP synthase; CF_1, coupling factor 1; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Me_2SO, dimethylsulfoxide; NEM, *N*-ethylmaleimide; OPBM, *N,N′-ortho*-phenylenebismaleimide; PEP, phosphoenolpyruvate; PK, pyruvate kinase; pmf, protonmotive force; TCA, trichloroacetic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycin.

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indicators: the release of tightly bound nucleotides [14–18], the accessibility of the regulatory disulfide bridge in γ (Cys^{199} and Cys^{205} in spinach) to added reductants [10,19] and the accessibility of the covalently bound probe eosin-isothiocyanate to the triplet quencher dioxygen [20]. Another approach to monitor conformational changes was based on the accessibility to SH-reagents of Cys^{89} on subunit γ [19,21–27]. Cys^{89} has been coined 'light accessible cysteine' to indicate that it is buried in the dark and only exposed to added maleimides like *N*-ethylmaleimide (NEM) [21] and *N,N'-ortho*-phenylenebismaleimide (OPBM) [24] in the presence of protonmotive force as induced by *light* (see Section 4).

Cys^{322} at the C-terminal end of γ is readily and rather specifically modified by maleimides even in the absence of protonmotive force. This covalent modification is without effect on the activity of ATP synthase. If Cys^{322}, however, is modified by the bifunctional maleimide, OPBM, and exposed to light, CF_oCF_1 becomes leaky to protons and ATP synthesis is inhibited [24,28]. Based on a series of experiments, this has been ascribed to the cross-linking of two cysteines on subunit γ, namely Cys^{322} and Cys^{89}, by OPBM [24–27]. That these two residues are closely apposed to each other was backed up by resonant energy transfer. A distance of 2.7 ± 0.6 nm between fluorescence probes covalently bound to Cys^{89} and Cys^{322} has been inferred from such studies [29]. This close proximity is difficult to reconcile with the partial atomic structure of the F_1-portion from beef heart mitochondria [6], which represents the C-terminal part of γ (with Cys^{322} in spinach) in the central cleft at the top of MF_1, whereas the small helical portion which contains Cys^{78} in MF_1 (equivalent to Cys^{89} in spinach) is located at the bottom, nearer to the stalk region and to the DELSEED-segment on β. The distance between these two sulfhydryl groups amounts to more than 7 nm, whereas the distance between the maleimide functions of OPBM amounts to less than 1 nm. This discrepancy between results from R. McCarty's and J. Walker's laboratories remains to be solved. It is beyond doubt, however, that the 'dark accessible' site for the binding of maleimides is situated on the C-terminal fragment of γ which contains Cys^{322} [27,39]. In the context of the question addressed in this article, it is without relevance, whether OPBM which is bound to γ-Cys^{322} is cross-linked intra-γ to γ-Cys^{89} or intra-γ to another residue on the same subunit or even intra-CF_1 to another subunit.

The OPBM-induced effects, proton leakage and inhibition of ATP synthesis, are related to subunit γ. Thus they are useful as markers of conformational changes involving this subunit. This property is used in this article.

There are two modes of proton flow through the membrane-bound holoenzyme in thylakoids: proton slip in the absence of nucleotides and coupled proton flow under ATP synthesis (see Ref. [36] and references therein). Proton slip represents the idling of the proton translocating device, and it is interesting to know to what extent the idling motion involves subunits and domains of the catalytic portion. We scrutinized our previous proposal that proton slip drives certain types of relative motion of subunits in CF_oCF_1 that are also carried out if ATP synthesis is coupled in.

The experimental procedure was three-stepped: (1) dark incubation with OPBM to saturate the 'dark accessible' Cys^{322}, (2) preillumination of thylakoids to generate sufficient protonmotive force for cross-linking by the second maleimide function of OPBM and (3) assay of the rate of ATP synthesis in a second illumination period. Following this protocol we found that both types of proton flow, slipping and coupled, likewise exposed buried portions of the enzyme to OPBM. No such exposure was observed, when proton slip was blocked by ADP ($+P_i$) or GDP ($+P_i$).

2. Materials and methods

2.1. Preparation of stacked thylakoids

Experiments were carried out either with spinach (*Spinacia oleracea*) from the local market or with 10 to 12 days old laboratory grown pea seedlings (*Pisum sativum* var. Kleine Rheinländerin). Stacked thylakoids were prepared as previously described [31]. Concentrated stock was resuspended in a medium containing 10 mM tricine/NaOH (pH 7.8), 100 mM sorbitol, 10 mM NaCl, and 5 mM $MgCl_2$. The concentrated suspension contained between 3 and 5 mM chlorophyll as determined according to Ref. [32]. It was stored on ice and lasted for at least 4 h without significant aging.

2.2. Incubation, preillumination and cross-linking with OPBM

Thylakoids were diluted to a chlorophyll concentration of 10 μM in a reaction medium containing 100 mM sorbitol, 50 mM tricine/NaOH (pH 8.0), 10 mM NaCl, 5 mM $MgCl_2$, 50 μM phenazinemethosulfate, a mediator of cyclic electron transport, and 10 μM *N,N'-ortho*-phenylenebismaleimide (OPBM) in dimethylsulfoxide (Me_2SO) or the same volume of Me_2SO. The control with Me_2SO was treated in the same way as the sample with OPBM. The mixture was incubated for 10 min on ice in the dark. Further components (e.g., ADP, P_i, tentoxin, dequalinium chloride) were then added as indicated. The mixture was preilluminated for 90 s with strong white light (150 mW/cm^2, filtered through 4 cm of water and a KG2 filter from Schott), if not otherwise specified. After preillumination 3 mM dithiothreitol was added to stop any further reaction of remaining maleimide functions.

2.3. Measurement of photophosphorylation

5 mM ADP and 5 mM phosphate were added to the mixture. The sample was then illuminated for 1 min by white light (150 mW/cm^2), reactions were stopped by addition of TCA, and ATP synthesis was measured by standard luciferin/luciferase assay (LKB) as previously described [33]. The rates of photophosphorylation were typically about 1300 μmol ATP/(mg chlorophyll · h) in fresh samples with Me_2SO and without preillumination, typically 1200 with preillumination (chosen as controls) and down to typically 500 in samples that were preilluminated in the presence of OPBM. Because preillumination itself lessened the rate of photophosphorylation, we used as control a sample with Me_2SO which was also preilluminated rather than a sample with OPBM which was kept in the dark.

The rate of ATP synthesis in the presence of OPBM was related to the control rate. The relative loss of ATP synthesis by preillumination in the presence of OPBM in percent of the control was named 'inhibition' in the following. To merge several series of experiments with various batches of thylakoids that differed in the absolute activity of the control and the inhibited samples, we compared the 'relative inhibition', i.e., the inhibition divided by the maximum inhibition in a given series (e.g., under variation of the ADP concentration). Usually 3–5 data sets from different batches of material sets were averaged.

The protective action of nucleotides against inhibition was phenomenologically interpreted in terms of a binding isotherm:

$$Ir = 1 - \left(P_{\max} \cdot \frac{c}{K_p + c} \right) \quad (1)$$

with Ir: relative inhibition, P_{max}: maximum protection, c: concentration of nucleotides, and K_p: protection constant.

The mechanism of inhibition of ATP synthesis by cross-linking is complex; it involves both the deactivation of the catalytic function of certain molecules and induction of proton leaks. The latter affects the protonmotive force and therewith the activity of even those enzyme molecules that were untouched by OPBM. By using the above phenomenological equation, we did not attempt to explicitly model these features.

2.4. The acidification of the lumen of thylakoids

Stacked thylakoids were incubated with OPBM or Me_2SO in the dark as described above. Then 2 μM dequalinium chloride or 10 μM tentoxin were added to block the catalytic turnover of CF_OCF_1. The sample was kept for 10 min in the dark. It was then preilluminated with strong white light for 3 min. 6 μM 9-AA was added and the sample was transferred into a spectrofluorophotometer (RF-5001PC, Shimadzu). Photosynthetic electron transport and proton pumping were initiated by red light (longpath filter RG610, Schott). The acidification of the thylakoid lumen was followed by the quenching of fluorescence of 9-aminoacridine [34,35]. The excitation and emission wavelengths were 400 nm and 455 nm.

2.5. Biochemicals

Nucleotides, pyruvate kinase (PK), phosphoenolpyruvate (PEP) and OPBM were purchased from Sigma. The ATP monitoring kit for measurement of photophosphorylation was from BioOrbit. Additional chemicals of analytical grade were supplied by Biomol and Roth. The AdN contamination of GDP was checked by the ATP bioluminescence assay from Boehringer. The ADP content was determined by conversion of ADP into ATP using a PEP/PK-coupled assay. The assay medium contained 50 mM HEPES (pH 7.5), 100 mM KCl, 20 mM $MgSO_4$, 2 mM PEP, 1 mM GDP and 10 units/ml PK. The conversion was completed after 20 min at room temperature. We determined an AdN contamination of GDP as below 0.01%.

3. Results

3.1. The protective effect of nucleotides

We used the inhibition of photophosphorylation during the second illumination period to estimate the degree of cross-linking by OPBM as induced during preillumination. When ADP was absent but OPBM present during preillumination, the rate of ATP synthesis after addition of ADP

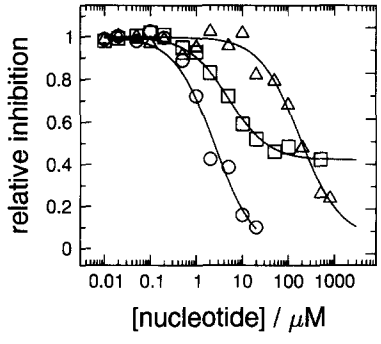

Fig. 1. Nucleotides protect against inhibition of photophosphorylation by cross-linking with OPBM. Stacked thylakoids were incubated in the dark with OPBM or Me_2SO as described in Section 2. Before preillumination the given concentration of ADP (plus 1 mM phosphate) (circle), of ADP alone (square), or of GDP (plus 1 mM phosphate) (triangle) was added. The mixture was preilluminated for 90 s with strong white light (150 mW/cm^2). To stop further reactions of OPBM 3 mM DTT was added. 5 mM ADP and 5 mM phosphate were added and the sample was again illuminated with white light for 60 s. The average rate of photophosphorylation was determined and the relative inhibition calculated. In the case of ADP and P_i we plotted only data with nucleotide concentration below 20 μM; data at higher concentrations are shown in Fig. 2B.

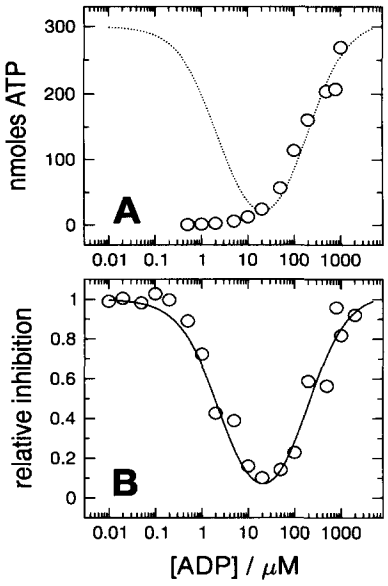

Fig. 2. Effect of ADP and phosphate on the inhibition of photophosphorylation by OPBM. The experimental procedure was as described in Fig. 1. The concentration of ADP was as indicated, the one of phosphate was held constant, 1 mM. After preillumination trichloroacetic acid was added to one batch of the control sample and the amount of ATP synthesized during preillumination was determined. The final amount of ATP after preillumination and illumination were corrected by this value before calculating the rates of photophosphorylation. Data were fitted with a binding isotherme for two binding sites:

$$Ir = 1 - \left(P_{max} \cdot \frac{c}{K_p + c} \right) + \frac{c}{K_m + c}$$

with Ir: relative inhibition, P_{max}: maximum protection, c: concentration of ADP, K_p: protection constant, K_m: apparent Michaelis constant. Graph B shows the relative inhibition (circle: data, line: fit curve). Graph A shows the amount of synthesized ATP during preillumination. The dotted curve is the same as in B. This demonstrates the correspondence between ATP synthesis and inhibition by OPBM.

and P_i decreased typically from 1200 μmol ATP/(mg chlorophyll · h) in controls (no OPBM) down to 500. We took this value as a relative inhibition of 1. Added nucleotides protected against inhibition. Fig. 1 shows the relative inhibition of ATP synthesis as function of the nucleotide concentration during preillumination. Phosphate or arsenate shifted the protective effect to lower concentrations of ADP (see Fig. 2). Whereas ADP, alone, protected against inhibition with $K_p = 5.7$ μM, its K_p decreased to 2.5 μM in the presence of 1 mM P_i. The greater effect of ADP in the presence of P_i paralleled the enhanced binding affinity for ADP in the presence of P_i which has been reported elsewhere [36,37]. It was taken as evidence for a stochastic binding mechanism for this pair of substrate molecules.

Each set of data was fitted with a binding isotherme that contained only one protection constant (Table 1). Values of these constants were higher than expected for the binding of nucleotides to the particular site of CF_1 which inhibits proton slip (see Section 4. We asked for the dependence of K_p on the light intensity and the preillumination time. As Table 2 shows, the absolute extent of the inhibition differed depending on the light intensity, but the ADP concentration at half maximal protection remained almost constant over a range from 37 to 280 mW/cm^2. The same constancy resulted from a variation of the time of preillumination from 30 s to 180 s at constant intensity (Table 2). Longer preillumination did not result in greater extent of inhibition.

Table 1
Protection constants for nucleotides

Nucleotide during preillumination	Maximum protection	K_p (μM)
ATP	63%	3.0
ADP	58%	5.7
ADP + 1 mM arsenate	84%	2.5
ADP + 1 mM phosphate	100%	2.5
GDP	66%	346
GDP + 1 mM phosphate	95%	180

Relative inhibition of photophosphorylation by OPBM was measured as function of added nucleotides as documented in Fig. 1. Data were fitted by equation 1 (see Section 2). The fit parameters, maximum protection and protection constant, are given. For ADP and P_i only data with nucleotide concentration up to 20 μM were fitted, not taking into account the rise of the inhibition at higher concentrations, i.e., during synthesis as shown in Fig. 2B.

Not only ADP and ATP but also GDP was competent to protect against inhibition of photophosphorylation (Fig. 1). Again the presence of phosphate during preillumination enhanced the effect by a factor of two (Table 1). In both cases the half maximal concentration was about 60 to 70 times higher than with ADP.

3.2. Cross-linking during ATP synthesis

When both ADP and P_i were present during preillumination, ATP was synthesized already during preillumination. Its amount was determined separately after the end of

Table 2
Effect of various light intensities and preillumination times on the protection of photophosphorylation against inhibition by OPBM

Light intensity mW/cm^2)	Time of preillumination (s)	Maximum inhibition (%)	K_p (μM)
37	180	22	1.4
126	180	39	1.6
194	180	52	1.6
280	180	58	1.9
150	30	26	1.7
150	60	36	1.7
150	90	45	1.9
150	180	47	1.3

ADP (concentrations as in Fig. 1) and 1 mM arsenate were added before preillumination. The protection constants and the maximum of inhibition of photophosphorylation are given. Lower light intensity and shorter time of preillumination diminished the extent of inhibition without affecting the protection constants.

the reaction with OPBM during preillumination. The total amount attributable to ATP formed during the first and the second illumination was determined and the amount from the first period was subtracted to yield the amount of the second illumination interval. The effect of ADP and phosphate on the relative inhibition of photophosphorylation during the second illumination period is shown in Fig. 2B. Low concentrations of ADP protected against inhibition with maximum protection at 20 μM ADP (1 mM P_i), but the protection was relieved at higher ADP concentrations, i.e., when ATP was produced during preillumination. The amount of ATP produced during preillumination as function of the concentration of ADP is given in Fig. 2A.

3.3. Effects of inhibitors of phosphorylation on cross-linking

We asked which steps of proton flow were responsible for the conformational change as probed by OPBM cross-linking. Two inhibitors of phosphorylation were added before preillumination, tentoxin and dequalinium chloride, both of which prevent catalysis but allow certain conformational changes that have been associated with the activation of ATP synthase (see Refs. [30,42] and literature cited therein). As these agents block photophosphorylation, its rate can no longer serve as an indicator of cross-linking. We used increased proton leakage through CF_OCF_1 instead, the other consequence of cross-linking by OPBM. It was evident from a diminished pH-difference under continuous illumination. The steady pH-difference was monitored by the fluorescence quenching of 9-AA. Fig. 3 shows the relative fluorescence quenching of 9-AA in light–dark

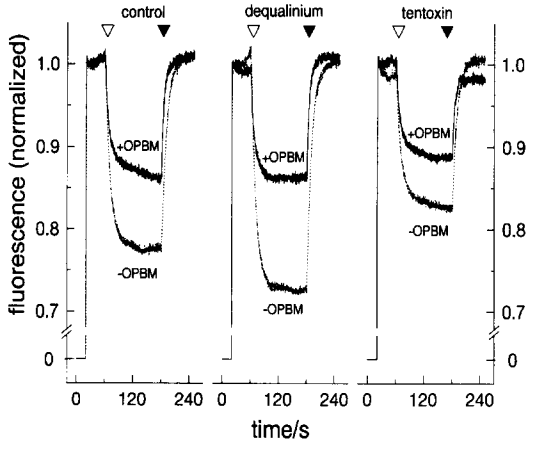

Fig. 3. Effect of inhibitors of photophosphorylation on cross-linking with OPBM. Tentoxin or dequalinium chloride were added before preillumination and the quenching of fluorescence of 9-AA was determined in a second illumination period as described in Section 2. In both cases and in the control without inhibitor the acidification of the thylakoidal lumen was less when OPBM was present during preillumination, indicating that cross-linking did occur. Quenching with tentoxin was smaller because of the longer incubation time, but the difference between a mixture with OPBM and the control with Me_2SO was still obvious.

transition with 120-s light intervals. In all three samples (the control without tentoxin and dequalinium, one sample with tentoxin, the other one with dequalinium) the pH-difference was greater without than with OPBM. Accordingly, the proton leak, which is attributable to cross-linking by OPBM, occurs even in the presence of tentoxin and dequalinium both of which block ATP synthesis but not the limited proton flow which has been ascribed to the activation of the enzyme [30,42].

3.4. The number of enzyme turnovers required for cross-linking with OPBM

Cross-linking with OPBM necessitated rather long exposure to high protonmotive force during preillumination. Typically 30 s of preillumination caused half maximum inhibition at a given light intensity (not shown). Under synthesis conditions this corresponds to at least 10000 catalytic turnovers. This estimate was based on the following assumptions: the pertinent stoichiometric ratio is 1000 mol chlorophyll per mol CF_OCF_1 and the turnover number is 333 nmol ATP/(μmol chlorophyll · s).

4. Discussion

OPBM, as a maleimide, reacts mainly with sulfhydryl groups [38]. The interaction of maleimides with thylakoid membranes has been extensively studied by Richard McCarty's group [21–27]. Incubation of thylakoid membranes with OPBM *in the dark* causes OPBM-binding to CF_1, predominantly to its γ subunit. No other chloroplast protein (except ϵ of CF_1) is labeled by OPBM to any significant extent. The most readily accessible site in the dark is located on a particular trypsin fragment of γ that contains Cys^{322} [27,39]. Dark incubation with OPBM and the subsequent protection of its second maleimide function by added DTT in the dark does not affect ATP synthesis. Illumination of OPBM-labeled thylakoids produces proton leakage through CF_OCF_1 and inhibits ATP synthesis [24,28]. This has been ascribed to the exposure of γ-Cys^{89} to the second maleimide function of bound OPBM and the formation of a cross-link within γ under the action of protonmotive force [24,22]. This interpretation has been backed up by the observation that incubation of illuminated thylakoids with NEM covalently modifies another trypsin fragment of γ that contains Cys^{89} [39]. It has been mentioned in the introduction that this assignment is difficult to reconcile with the atomic structure of MF_1 [6]. With this discrepancy still pending, we use the following assignments: the 'dark site' accessible to maleimides is γ-Cys^{322} and the '*light site*', whether on γ or on another subunit remains unspecified.

The reviewed and further evidence suggests that the *light site* is protected from the attack by maleimides in the

absence of protonmotive force, and only exposed in its presence. Our results imply that the *light site* approaches Cys^{322} at least transiently to about 0.6 nm distance (the length of OPBM) to yield the OPBM-induced cross-link under both types of proton flow through CF_OCF_1.

There were two such conditions, proton slip in the absence of nucleotides and coupled proton flow in the presence of catalytic amounts of ADP and P_i. The mere exposure of CF_OCF_1 to protonmotive force, but without proton flow, as at intermediate concentrations of ADP, was not sufficient. We also found that tentoxin, an inhibitor of the catalytic turnover but not of the conformational changes accompanying activation, did not prevent the transitory close proximity of the *light site* and Cys^{89}. We have recently demonstrated limited proton flow across the enzyme in the presence of tentoxin [30,42]. Under flashing light a prompt onset of ('activating') proton flow was observed as contrasted with the delayed ('catalytic') flow. About 10000 turnovers of the proton conducting machinery of CF_OCF_1 were required for cross-linking. It is conceivable that the time window of the close apposition of residue 322 and the *light site* covers only a short segment of the proton conducting cycle.

The nucleotide dependence of cross-linking by OPBM, as monitored by the relative inhibition of ATP synthesis, qualitatively followed the behavior of proton flow from a slipping over a blocked into a coupled state of the enzyme as known from previous work [36]. Even the synergetic effect of ADP and P_i and the sensitivity to GDP was reproduced, which qualified the protective nucleotide binding site(s) into the potentially catalytic subset of three out of a total of six [6,40]. But the half-maximal concentrations differed drastically from those determined by studying proton flow under flashing light [36]. In the case of ADP + 1 mM P_i it was about 2.5 μM, with arsenate substituting for phosphate it was the same. This value was higher than expected, because 0.2 μM ADP is sufficient to halve the rate of proton slip in the presence of phosphate [36]. We concluded that the protection attributed to ADP in the presence of P_i/arsenate was rather caused by ATP and ADP · AsO_4 that were synthesized during preillumination. Indeed the K_ps for ADP + P_i/AsO_4 (2.5 μM) and ATP (3.0 μM) were very close to the inhibition constant of ATP (namely 2.0 μM) for proton slip [36]. The lifetime of ADP · AsO_4 in solution is about 500 ms [41] and therefore was long enough so that a considerable steady concentration of ADP · AsO_4 was accumulated which inhibited proton slip. According to Fig. 2A ADP was quantitatively converted into ATP during preillumination at low ADP concentrations.

In conclusion, the conformational change in CF_OCF_1 that can be trapped by the bifunctional maleimide OPBM is intimately linked to partial reactions of proton transport. The conformational change is executed both under coupled as well as under slipping proton flow. The internal motion in CF_1 which brings an otherwise buried group, the *light site*, into close proximity to γ-Cys^{322} with OPBM was inhibited when proton flow was blocked at intermediate concentrations of nucleotides. It was not evoked by transmembrane protonmotive force alone, that might cause proton binding/liberation without proton conduction. The conformational change was, however, still induced in the presence of limited ('activating') proton flow [30,42] as in the presence of tentoxin and dequalinium, both inhibitors of ATP synthesis.

Our previous studies have shown that proton slip represents the idling of the proton carrier function in CF_OCF_1 which is controlled by the nucleotide concentration [36]. This gave evidence for long-range interaction between CF_O and the nucleotide binding sites mainly on the β-subunits of CF_1. Our studies on the selective effects of ADP binding to the central pocket in subunits β, and of dequalinium, binding to its DELSEED domain, on different steps of proton transfer under flashing light corroborated this long range interaction [42]. In the present work we showed that subunit γ underwent the same type of a conformational change as function of proton flow in the slipping and in the coupled mode. It is obvious that the clutch between proton transfer and ATP synthesis is located beyond γ, perhaps between γ and β in the headpiece of ATP synthase.

Acknowledgements

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