THE CHLOROPLAST H⁺-ATPase: PARTIAL REACTIONS OF THE PROTON

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Summary

This article reviews proton intake, charge transfer and proton release by F-ATPases, based in part on flash spectrophotometric studies on the chloroplast ATPase in thylakoid membranes, CF₁Fₒ. The synthesis-coupled translocation of charges by CF₁Fₒ (maximum rate <1500 s⁻¹) and the dissipative flow through its exposed channel portion, CFₒ (rate >10000 s⁻¹), are extremely proton-specific (selectivity H⁺:K⁺ >10⁷:1). The proton-specific filter is located in CFₒ. Proton flow through exposed CFₒ can be throttled by adding subunit δ or subunit β of CF₁. These subunits thus may provide energy-transducing contacts between CF₁ and CFₒ. Recently, we characterized two conditions where, in contrast to the above situation, proton intake by CF₁Fₒ was decoupled from proton transfer across the main dielectric barrier: (a) CF₁Fₒ structurally distorted by low ionic strength transiently trapped protons in a highly cooperative manner, but remained proton tight. This result has been interpreted in terms of Mitchell’s proton well. (b) In the absence of nucleotides there is a proton slip. Addition of nucleotides (100 nmol l⁻¹ ADP) abolished proton conduction but not proton intake by CF₁Fₒ. These experiments functionally tag proton binding groups on CF₁Fₒ that are located before the main dielectric barrier.

Introduction

The proton side of F-ATPases appears to be even more difficult to assess than the nucleotide processing side. The proton-motive force not only drives ATP synthesis but also regulates enzyme activity (Junge et al. 1970; Bakker-Grunwald and van Dam, 1974; Junesch and Gräber, 1985, 1991). Three protons are translocated per molecule of ATP formed, whether concertedely or each one serving a particular function is unknown. The equivalence of the electrical portion of the proton-motive force, ΔΨ, and the chemical portion, ΔpH, as driving forces for ATP synthesis is plausible for near-equilibrium situations but not at all trivial far from equilibrium as in kinetic experiments, where it is also experimentally established (Junesch and Gräber, 1991). Specific amino acid residues which are essential for proton transfer have been identified by mutational analysis (Fraga and Fillingame, 1991; Miller et al. 1990) but evidence for their direct involvement is lacking.

Among F-ATPases those in photosynthetic membranes offer themselves for detailed

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kinetic studies on reactions involving the proton. The proton pumps are driven by light flashes, and transients of the transmembrane voltage and of the pH on both sides of the coupling membrane are detectable spectroscopically by intrinsic and extrinsic indicator dyes (Witt, 1979; Junge, 1982). When chloroplasts of green plants are excited by light the lumen of inner vesicles, thylakoids, is positively charged and acidified. The lumen is equivalent to the exoplasmatic side of unicellular organisms. The basic proton conductance of the thylakoid membrane is rather low. Proton conduction is enhanced when CF$_1$F$_0$ synthesizes ATP or more dramatically when CF$_0$ is exposed by removal of the catalytic portion CF$_1$. Under pulse stimulation by short flashes of light, proton intake from the lumen, charge transfer across the dielectric barrier and proton release into the suspending medium can be monitored, both for proton flow related to the ATP-synthesizing CF$_1$F$_0$ (Junge, 1987) and for proton flow through the exposed channel portion CF$_0$ (Schönknecht et al. 1986; Lill et al. 1987; Althoff et al. 1989). In both cases the intake of protons from the lumen and the transport of their charge equivalent across the membrane appear to be synchronized. A typical spectrophotometric experiment deals with more than $10^{13}$ molecules of CF$_1$F$_0$ or CF$_0$ which may operate independently and in an uncorrelated manner even when subjected to a sudden step of the electrochemical driving force. Thus, partial reactions of the proton could be blurred over. They may become apparent, however, if some reactions are frozen and completion of the transport cycle is blocked.

This article first reviews physicochemical properties of proton conduction by CF$_1$F$_0$ and by CF$_0$ and then reports new results on proton binding by CF$_1$F$_0$ without concomitant proton transfer across the membrane.

The proton conductance of CF$_1$F$_0$ and of CF$_0$

The turnover rate of CF$_1$F$_0$ for protons has been inferred from measurements of the rate of ATP synthesis assuming a stoichiometry of 3H$^+$/ATP. Values of up to 400 mol ATP mol$^{-1}$ CF$_1$F$_0$ s$^{-1}$ have been found in CF$_1$F$_0$-reconstituted liposomes during the initial phase after a large pH jump (Schmidt and Gräber, 1987). Even higher figures (500 s$^{-1}$) can be observed with spinach thylakoids under continuous light and with phenazine methosulfate as cofactor for cyclic electron transport. This result implies maximum turnover numbers for protons of up to 1500 s$^{-1}$. It is noteworthy, that reported rates for F$_1$F$_0$ from all other sources are lower.

Fig. 1 documents proton flow through CF$_1$F$_0$ under flashing light, where the turnover number is smaller than in the work cited above because of a smaller proton-motive force. The figure is taken from earlier work (Junge, 1987). Fig. 1A shows transients of the transmembrane voltage and Fig. 1B of the luminal pH. They are elicited by excitation of thylakoids with two groups of three closely timed flashes of light. In the presence of a specific inhibitor of CF$_1$, tentoxin, the relaxation of both is slow. The transmembrane voltage (Fig. 1A) decays more rapidly than the acidification of the thylakoid lumen (Fig.1B). This result reflects an electrical leak conductance by ions other than the proton. In the absence of tentoxin and with substrates (ADP and inorganic phosphate) present, ATP synthesis causes an accelerated decay of both the transmembrane voltage and the
lumenal acidification. The difference between the two traces in Fig. 1A indicates the extra transmembrane charge flow that accompanies ATP synthesis. The difference between the two traces in Fig. 1B indicates extra proton intake from the lumen by CF₀. Both differences have been normalized and, for a short time segment, plotted on top of each other in Fig. 1C. Their coincidence within noise limits identifies the extra charges which pass the dielectric barrier in CF₁F₀ as protons taken up from the lumen (Junge, 1987). Proton intake and charge transfer appear to be synchronized. The accelerated charge flow stops at a certain driving force and this is due to the gating of enzyme activity by the transmembrane voltage (Junge et al. 1970; Junge, 1970) or, more generally, by the transmembrane proton-motive force (Junesch and Gräber, 1985, 1991).

For a while, published figures of the proton conductance of the channel portion, F₀, have fallen short of the above turnover numbers. Values ranging from 10 to 100 s⁻¹ have been obtained mainly with reconstituted archaeabacterial and eubacterial channels in liposomes and by pH electrode (Negrin et al. 1980; Sone et al. 1981; Schneider and Altendorf, 1987). These low values are incompatible with the proposed role of F₀ as a low-impedance access to the coupling site in the enzyme (Mitchell, 1966). One reason for these shortcomings might be an insufficient time resolution of the pH electrode (G. Althoff and G. Dekkers-Hebestreit, personal communication), another reason might be the survival of only a few active channels during reconstitution.
We investigated the protonic conduction of $\text{CF}_0$ by flash spectrophotometry. The catalytic portion, $\text{CF}_1$, was removed from thylakoid membranes by treatment with EDTA. The number of $\text{CF}_1$ molecules removed from the membrane was determined by electro-immunodiffusion. Thylakoids were excited by short light flashes and the relaxation of the transmembrane voltage and of the pH transients in the lumen and in the suspending medium was monitored as shown in Fig. 1 (Althoff et al. 1989; Lill et al. 1987; Schönnknecht et al. 1986). To label charge flow mediated by $\text{CF}_0$, signals were recorded pairwise, with and without blocking agent (DCCD, venturicidin or organotins; Linnett and Beechey, 1979). The area-specific electrical capacitance of thylakoid membranes and the specific number of $\text{CF}_1$ molecules removed were taken into account (Althoff et al. 1989). Assuming that any $\text{CF}_1$ which was removed leaves a conducting $\text{CF}_0$ behind, the average protonic conductance per exposed $\text{CF}_0$ was about 10$fS$. The average was taken over two domains, the ensemble of those $\text{CF}_0$ that had lost their $\text{CF}_1$ counterpart and the time (counting open and closed intervals of channels). At moderate driving force, 100 mV, this conductance is equivalent to a turnover number for protons of about 6000 s$^{-1}$. Under 200 mV driving force the turnover number is 12,000 s$^{-1}$, ohmic behaviour assumed. This number is fully compatible with the proposed role of $\text{CF}_0$ as the proton channel of the enzyme. As a time-averaged figure, it represents only a lower limit for the turnover number of active channels.

We attempted to determine the conductance of the subset of conducting $\text{CF}_0$ channels. The rationale was to use vesicles so small as to contain 0, 1, 2 or very few active channels only and to determine the time-averaged conductance of single channels by a statistical analysis of electrochromic absorption transients. Thylakoids were fragmented into very small vesicles and the analysis technique was calibrated with gramicidin as a channel with known properties. Its conductance in thylakoids (Lill et al. 1987; Althoff et al. 1991; Schönnknecht et al. 1992) was in the range of values published for its conductance in artificial bimolecular lipid membranes. The same type of analysis applied to $\text{CF}_0$ (without gramicidin present) led to the conclusion that only one out of 100 exposed $\text{CF}_0$ molecules per vesicle was conducting. Accordingly, the proton conductance of the few functional channels was much greater than the average conductance, namely 1 pS instead of 10 fS (Schönnknecht et al. 1986). This result was in line with another report of a very large proton conductance of $\text{CF}_0$, namely of 3.5 pS, which was obtained for $\text{CF}_1\text{F}_0$ by the (dip stick) patch-clamp technique (Wagner et al. 1989). We did not find any clear-cut mistake in our spectrophotometric analysis, particularly as it worked well with gramicidin. Still, the result is not compatible with theoretical and model studies. A proton conductance of 1 pS, particularly around neutral pH, exceeds by orders of magnitude (a) the calculated convergence conductance to a pore mouth of reasonable diameter (say 1 nm) (Peskoff and Bers, 1988), (b) calculated rates of net proton transfer through short hydrogen-bonded chains (Brünger et al. 1983), and (c) measured rates of proton transfer through other proton-specific channels (Veatch et al. 1975; Lear et al. 1988). Charge flow enhancing factors, a short selectivity filter and large channel mouth, as discussed in the context of K$^+$ maxi channels (Hille, 1984), the drag force in the coulomb cage (Peskoff and Bers, 1988) and mobile buffers (Decker and Levitt, 1988; Nunogaki and Kasai, 1988) do not really account for this discrepancy.
Other properties of this channel as determined by flash spectrophotometry (Althoff et al. 1989) are independent of the absolute value of the conductance. CF₀ is proton-specific even at pH 8 against a background of 300 mmol l⁻¹ NaCl or KCl, or 30 mmol l⁻¹ MgCl₂ in the medium. The selectivity filter is therefore a property of CF₀ and not of the rate-limiting (coupling) step in CF₁F₀. The proton specificity of over more than seven orders of magnitude is unparalleled by other known proton channels (e.g. gramicidin, selectivity for protons over other cations less than 100; Decker and Levitt, 1988). It is the more astounding, as a homologous enzyme to CF₁F₀ has been described for Propionigenium modestum that can act both as a sodium- and as a proton-translocating ATPase (Dimroth, 1991). The conductance of CF₀ is pH independent in the range between 5.6 and 8. In the same pH range there is a constant hydrogen/deuterium isotope effect of 1.7. Addition of glycerol decreases the conductance and abolishes the isotope effect. This finding suggests that the isotope effect may be caused by events in the channel and that the channel operates close to limitation by events in the water phase. The Arrhenius activation energy of proton conduction by CF₀ is 42 kJ mol⁻¹, intermediate between the respective figures of a channel-type (30 kJ mol⁻¹, gramicidin) and a carrier-type antibiotic (65 kJ mol⁻¹, valinomycin) (see Althoff et al. 1989, for details).

In conclusion, CF₀ is a very effective and selective proton channel. Its turnover number greatly exceeds that of the coupled enzyme. It can act as a low-impedance access channel to the coupling site. CF₀ is endowed with an extremely specific selectivity filter for protons, unparalleled by other types of proton channels.

Subunits of CF₁ that can throttle proton conduction by CF₀

Three subunits of F₁, namely, γ, δ and ε, of the thermophylic bacterium PS3 were reported to block proton flow through the respective F₀ channel (Kagawa, 1978). We asked whether single subunits of CF₁ can interact with exposed CF₀ in such a way as to block proton flow. Triggered by the observation that δ may remain on CF₀ after removal of CF₁, keeping CF₀ non-conducting (Junge et al. 1984), we found that isolated δ, when added back to CF₁-depleted thylakoids, can throttle proton flow through exposed CF₀ (Lill et al. 1988). Thereby, it restores photophosphorylation by those CF₁F₀ that have remained intact on the membrane (Engelbrecht and Junge, 1988). Any ‘stopcock’ action of δ has to be overcome in the operating enzyme in favour of a controlled operation as valve, admitting protons from the channel further onto the coupling site, or as part of the conformational transducer between protons and ATP (Engelbrecht and Junge, 1990).

Following the same line of research we also found subunit β to be effective in throttling proton conduction by CF₀ (monitored by electrochromic absorption transients), and restoring photophosphorylation in CF₁-depleted thylakoids (S. Engelbrecht and W. Junge, in preparation). In this series of experiments, subunit α could not be purified in sufficient amounts. The particular samples of γ, δ and ε were only marginally effective by themselves, but the combination of γ, δ and ε was, as if distorted δ was reshaped by its association with γ and ε (see also Engelbrecht and Junge, 1990). Interestingly, the combination of γ, δ and ε was equally effective on spinach CF₀, when subunit γ was taken either from spinach or from the thermophilic bacterium PS3. The finding that the
small subunits of CF$_1$ act in concerted manner and that subunit $\beta$ by itself specifically interacted with the exposed proton channel, CF$_0$, qualifies the concept of $\delta$ just acting as a plug.

Instead, it seems as if there are several essential contacts between CF$_0$ and CF$_1$. Following the hypothesis of a rotational mechanism of catalysis (Boyer, 1989) one may speculate that both portions of F$_1$, bearings ($\beta$?) and rotating shaft elements (at least $\gamma$ and $\delta$?), may interact with CF$_0$ so as to block and, in CF$_1$F$_0$, so as to control, proton conduction.

**Trapping of protons by CF$_1$F$_0$ without concomitant conduction**

Cooperative transient trapping of protons was first observed in thylakoids treated with very low concentrations of EDTA (Junge et al. 1984). This effect is documented in Fig. 2. Thylakoids isolated from spinach chloroplasts according to standard procedures, but without added magnesium in the medium, were incubated for 2 min in distilled water with very low concentrations of EDTA added (typically 10 $\mu$mol l$^{-1}$ at 10 $\mu$mol l$^{-1}$ chlorophyll, but see below). The exposure to low ionic strength was stopped by addition of NaCl to 10 mmol l$^{-1}$.

![Fig. 2. Voltage transients (left) and pH transients (right) in the thylakoid lumen induced by a single flash of light. Spinach thylakoids prepared without addition of divalent cations were incubated for 2 min at low ionic strength (distilled water with 10 $\mu$mol l$^{-1}$ EDTA). NaCl was then added (10 mmol l$^{-1}$). Voltage and pH transients were recorded twice without and with venturicidin (1 $\mu$mol l$^{-1}$) added. This procedure labelled events attributable to CF$_0$. EDTA treatment induced transient trapping of protons (see text) without equivalent charge transfer across the membrane. Each point represents a time interval of 68 $\mu$s. The lower traces are differences between each pair of top traces. The right-hand one shows the extent of proton intake by CF$_0$. For details, see Griwatz and Junge (1992).](image-url)
Fig. 2 shows voltage transients (left) and pH transients (right) in the lumen for thylakoids that had undergone mild EDTA treatment. Although the extent and the decay of the voltage were unaffected by addition of venturicidin (blocker of F\textsubscript{o} channels binding to the proteolipid, Galanis et al. 1989), the acidification of the lumen was seemingly increased. We attributed this increase to the abolition by venturicidin of a transient trapping of protons at the luminal side of CF\textsubscript{i}F\textsubscript{o} or of CF\textsubscript{o}. The extent of proton intake is shown in the lower part of Fig. 2 (right). It is simply the difference between the two traces at the top. On an enlarged time scale (Fig. 3) proton intake appeared biphasic, rapid trapping preceding a slow phase attributable to proton leakage through a few exposed CF\textsubscript{o} channels. The pH dependence of proton trapping was remarkably steep (see Fig. 3). Whereas rapid trapping was absent at pH 7.2, it was fully expressed at pH 7.7. In contrast, the comparatively slow proton leakage was practically constant over this narrow pH range. The steep pH-dependence of proton trapping conformed with a hexacooperative binding isotherm (Hill coefficient around 6) (Griwatz and Junge, 1992; Junge et al. 1984). It is noteworthy that the proton-trapping capacity was saturated by the first flash of

![Proton intake related to CF\textsubscript{o} in EDTA-treated thylakoids as function of pH. The transients are equivalent to the one in the lower right of Fig. 2. The ordinate scale was normalized with respect to the pH-dependent sensitivity profile of the indicator dye Neutral Red. For details see Griwatz and Junge (1992).](image-url)

*Fig. 3. Proton intake related to CF\textsubscript{o} in EDTA-treated thylakoids as function of pH. The transients are equivalent to the one in the lower right of Fig. 2. The ordinate scale was normalized with respect to the pH-dependent sensitivity profile of the indicator dye Neutral Red. For details see Griwatz and Junge (1992).*
light; further flashes did not produce further trapping. It was inhibited by various inhibitors of proton conduction through CF$_{0}$, namely, venturicidin, DCCD and organotins, and thus was clearly attributable to the ATPase. Trapping was also eliminated by adding very small concentrations of divalent cations (typically $10 \mu$mol l$^{-1}$), concentrations too small to produce rebinding of possibly solubilized CF$_{1}$.

The pH difference which is generated by a single turnover of both photosystems is very small, about 0.05 units (Junge et al. 1979). What was the driving force for proton trapping? We found that rapid trapping could be eliminated if the flash-induced transmembrane voltage was even more rapidly shunted by an ionophore (e.g. valinomycin) (Griwatz and Junge, 1992). This finding led us to interpret these effects in the framework of Mitchell's concept of a proton well (Mitchell, 1977). Consider a proton-specific domain that reaches from the lumenal surface of the membrane into CF$_{1}$F$_{0}$. The lumenal surface still represents an electrical equipotential surface, but the electrochemical equipotential surface stretches out into this proton-conducting domain. Potentially buffering groups at its top sense a pH that is more acid than the one at the lumen surface. The pH decrease is equivalent to the voltage drop between the lumen and the location of these groups in the membrane. This concept for the transformation of electrical into chemical (entropic) force could account for the observed kinetic equivalence of $A_{\text{H}}$ and $A_{\text{pH}}$ for ATP synthesis (Graber et al. 1984; Junesch and Graber, 1987). Without a proton well the kinetic equivalence is not at all trivial.

Proton trapping was highly cooperative. In spinach thylakoids the pK of the trapping groups was 7.3 and the Hill coefficient around 6. This behaviour suggests the presence of at least six identical and strongly interacting groups. The candidates are two essential residues, Glu61 and Arg41 (see Fraga and Fillingame, 1991; Miller et al. 1990, for E. coli) on subunit III. Only this subunit of CF$_{0}$ is present in sufficient numbers (9–12 in E. coli). Derivatization of a single copy by DCCD is sufficient to block ATPase activity in E. coli (Hermolin and Fillingame, 1989).

Transient trapping of protons has only been observed with distorted CF$_{1}$F$_{0}$. Is the trapping state an intermediate of the operational cycle of this enzyme? In the same type of flash spectrophotometric experiments described above trapping has not been detected during ATP synthesis by CF$_{1}$F$_{0}$ (Junge, 1987) (and see Fig. 1) or proton leakage through exposed CF$_{0}$ (Althoff et al. 1989). In both cases the respective transporter has carried out multiple turnovers. Each experiment was an average over very many enzyme cycles. Time resolution of partial reaction steps was not expected.

Thus, it is conceivable that mild EDTA treatment stabilizes and thereby exposes to observation one particular intermediate of the normal operating cycle of the ATP synthase. The respective residues are placed on the lumenal side, beyond the selectivity filter, but before the main dielectric barrier in CF$_{1}$F$_{0}$. It is tempting to associate this barrier with the main coupling step in CF$_{1}$F$_{0}$.

Another situation where proton binding precedes conduction is related to proton slip through CF$_{1}$F$_{0}$. There are at least four mechanisms of proton conduction by thylakoid membranes: (1) coupled proton translocation by CF$_{1}$F$_{0}$ during ATP synthesis; (2) proton channeling through exposed CF$_{0}$; (3) proton slip through CF$_{1}$F$_{0}$; and (4) any other proton leakage. While the first two are self-explanatory, proton slip differs from proton leakage...
(through the bilayer or through proteins other than CF$_1$F$_o$) in its sensitivity to nucleotides
and/or specific blockers of CF$_1$. In contrast to the proton leak in the cristae membrane of
mitochondria, which is insensitive to blockers of F$_1$F$_o$ (Brown and Brand, 1991), several
authors have reported proton slip in thylakoid membranes (McCarty et al. 1971; Underwood and
Gould, 1980; Gräber et al. 1981; Strotmann et al. 1986; Evron and Avron, 1990). It is particu-
larly evident at very low nucleotide concentrations. Fig. 4 demonstrates this effect. Thylakoids
were incubated in nucleotide-free medium and illuminated by a constant background intensity of
green light. They were excited with a group of three short light flashes creating a voltage trans-
ient (Fig. 4A) and a pH transient (Fig. 4B) in the lumen on top of the steady acidification. The
electrical leakage was greater in the absence of ADP than with ADP added (see Fig. 4A). The attri-
bution of this slip to CF$_1$F$_o$ was corroborated by its inhibition by venturicidin. Fig. 4B demonstrates

![Graph](image)

Fig. 4. Voltage transients (A) and pH transients (B) in the lumen of thylakoids in the absence
of ADP, with $1 \mu$mol l$^{-1}$ ADP added and with $1 \mu$mol l$^{-1}$ venturicidin added. 0.1 mmol l$^{-1}$
phosphate and 3 mmol l$^{-1}$ MgCl$_2$ were present. A group of three short flashes was fired on a
continuous background of green light (10 W m$^{-2}$). In the absence of ADP, electrical leakage
and proton intake are evident. Sensitivity to venturicidin qualifies both as proton slip through
CF$_1$F$_o$. 

\( \Delta V/L_{522} \times 10^3 \)

\( \Delta V/L_{548} \times 10^3 \)

Time (ms)

$0 \mu$mol l$^{-1}$ ADP

$1 \mu$mol l$^{-1}$ venturicidin

$1 \mu$mol l$^{-1}$ ADP

$0 \mu$mol l$^{-1}$ ADP

$1 \mu$mol l$^{-1}$ ADP

$1 \mu$mol l$^{-1}$ venturicidin

$0 \mu$mol l$^{-1}$ ADP
that the slip was due to proton conduction. There was apparently less proton release into the lumen in the absence of venturicidin. As in the context of Fig. 1, this diminution was attributed to increased proton intake. Interestingly, the addition of ADP eliminated charge slip (Fig. 4A) but not proton intake (Fig. 4B). Still venturicidin eliminated both. Proton trapping was more rapid than proton conduction. This discrepancy is evident from a comparison of the respective differences between the traces shown in Fig. 4, which are plotted in Fig. 5. It has still to be determined how this example of proton intake by CF₁F₀ without concomitant proton conduction relates to the result after EDTA treatment.

Other properties of the proton slip, as will be detailed elsewhere (G. Groth and W. Junge, in preparation), are as follows. The inhibition of the electrical slip by ADP occurred with an apparent $K_i(\text{ADP})$ of $200 \text{nmol}\cdot\text{l}^{-1}$. It required inorganic phosphate; $K_i(\text{P}_i)$ of $45 \mu\text{mol}\cdot\text{l}^{-1}$. GDP was also effective. There was an energetic threshold for the slip. More importantly, when a pH difference was created by continuous light, such that the electrical potential difference induced by a single additional flash of light was still below threshold, there was no slip. The slip became evident, however, when the
electrical potential difference was increased (by firing three flashes instead on only one, see Fig. 4). This showed that the slip threshold was defined in terms of proton-motive force. The electrical component, $\Delta \Psi$, was additive to the chemical one, $\Delta \text{pH}$.

A simplistic structural model based on the above kinetic studies on proteolytic reactions is given in Fig. 6. To avoid the impression that we can pin down any of the functional elements to certain protein domains, the enzyme is sketched as a ball and rod. For protons, the $\text{CF}_1\text{F}_0$ entity represents a structure with three elements, an entry channel, a major dielectric barrier and an exit channel. Protons enter $\text{CF}_1\text{F}_0$ through an extremely proton-selective filter, which is part of $\text{CF}_0$. On the cis side of the dielectric barrier, but after or within the filter, there are highly cooperative proton binding groups. They are freely accessible only in structurally distorted $\text{CF}_1\text{F}_0$ (induced by EDTA treatment or, perhaps also, at low nucleotide concentration). Then a small portion of the total transmembrane electrical potential difference is converted into a pH difference at these groups, as postulated in Mitchell’s (1977) concept of a proton well. It is tempting to identify the major dielectric barrier with the coupling step. Whether the proton trapping groups are involved in the reaction cycle of the undistorted ATP synthase remains to be seen. It is hoped that the above speculations are of heuristic value, at least until the atomic structure of $\text{CF}_1\text{F}_0$ is available as a time-resolved movie.

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References


