J. Membrane Biol. 108, 263–271 (1989)

The Journal of
Membrane Biology

Proton Channel of the Chloroplast ATP Synthase, CF_o: Its Time-Averaged Single-Channel Conductance as Function of pH, Temperature, Isotopic and Ionic Medium Composition

Gerd Althoff, Holger Lill, and Wolfgang Junge
Biophysik, Fachbereich Biologie/Chemie, Universität Osnabrück, 4500 Osnabrück, West Germany

Summary. The proton-driven ATP synthase of chloroplasts is composed of two elements, CF_o and CF_1. The membrane bound CF_o conducts protons and the peripheral CF_1 interacts with nucleotides. By flash spectrophotometric techniques applied to thylakoid membranes from which about 50% of total CF_1 was removed, we have previously determined the protonic (time-averaged) single-channel conductance of CF_o. Being in the order of 1 pS, it was sufficiently large to support the proposed role of CF_o as a low-impedance access for protons to the coupling site in CF_oCF_1. On the other hand, it was too large to be readily reconciled with current concepts of proton supply to and proton conduction through the channel.

We studied the time-averaged single-channel conductance of CF_o under variation of pH, pD, ionic composition, temperature, and water/membrane structure with the following results: (i) CF_o was proton-specific even against a background of 300 mM monovalent or 30 mM divalent cations. (ii) While the conductance of CF_o was pH/pD-independent in the range from 5.6–8.0, in D_2O it was lower by a constant factor of 1.7 than in H_2O. (iii) Addition of glycerol diminished the conductance and abolished the isotope effect. (iv) The Arrhenius activation energy was 42 kJ/mol and thus intermediate between the ones found for the water-filled pore, gramicidin (30 kJ/mol), and the mobile carrier, valinomycin (65 kJ/mol).

The results implied that CF_o is endowed with an extremely proton-specific (10^7-fold) selectivity filter. Its conductance is very high, and its conduction cycle is not necessarily rate limited by a protolytic reaction. The mechanisms of rapid proton supply to the channel mouth and of proton conduction remained enigmatic.

Key Words proton channel · ATP synthase · CF_o · photosynthesis · energy coupling

Introduction

ATP synthesis in photosynthetic bacteria, chloroplasts of green plants, aerobic bacteria and mitochondria is promoted by F_oF_1-type ATP synthases (*see* Senior (1988) for a recent review). They are composed of a membrane-embedded proton channel, F_o, and a peripheral catalytic portion, F_1. Active research is focussed on defining the sites where the electrochemical energy of the proton is transduced into chemical bond energy. For a while it has been disturbing that attempts to determine the protonic conductance of the bare F_o portion, which were mainly based on measurements of proton efflux from F_o reconstituted vesicles, have yielded figures in the range of 10–100 $H^+/F_o \cdot$ sec (recent review by Schneider & Altendorf, 1987). This was too low for the proposed function of F_o as a low impedance access (proton well (Mitchell, 1977)) to the coupling site. As an example, for CF_oCF_1 of chloroplasts one calculates a time-averaged single-enzyme conductance of about 1 fS under maximum turnover rate (based on a rate of 400 ATP/sec (Junesch & Gräber, 1985) and under a stoichiometry of 3 H^+/ATP (Junge, Rumberg & Schröder, 1970; Davenport & McCarty, 1981)). A different experimental approach, however, has revealed that the time-averaged single-channel conductance of CF_o is much higher, more than sufficient for the proposed function, namely in the order of 1 pS (Lill, Althoff & Junge, 1987). It has relied on the spectrophotometric measurement of flash-light induced voltage transients and pH transients in thylakoid membranes from which a fraction of CF_1 molecules had been removed to expose the CF_o counterpart. The conductance of *single CF_o channels* has become accessible, because several lines of evidence have shown that out of 50 exposed CF_o per vesicle only one was highly conducting (Lill et al., 1986, 1987). This has left one fraction of vesicles proton tight, and the complement had one, two or more active channels for which Poisson's distribution was assumed. Perhaps the most direct proof for the rareness of highly conducting channels among many exposed CF_o has come from "biochemical counting" experiments, which showed that the sub-

set of proton leaky vesicles could be resealed by addition of much less molecules of CF_1, than had been removed (H. Lill & W. Junge, 1989).

The discussion on the proton conduction mechanism of F_o has been focussed on hydrogen-bonded chains (Dunker & Marvin, 1978; Nagle & Morowitz, 1978; Brünger, Schulten & Schulten, 1983; Nagle & Tristram-Nagle, 1983; Schulten & Schulten, 1985). We asked whether or not the magnitude of its conductance, the selectivity for protons and the pH-dependence of CF_o was compatible with the theoretical predictions.

Materials and Methods

Broken pea chloroplasts were prepared according to the procedure for "stacked thylakoids" in Polle and Junge (1986) except that Mg^{2+} was omitted in the final suspending medium. CF_1 depletion and vesicle forming was carried out as described in Lill et al. (1986) with an incubation time of 10 min in EDTA solution. The stock suspension of the "unstacked thylakoids" (3–4 mg chlorophyll/ml) and the final vesicle suspension (1–2 mg chlorophyll/ml) were stored on ice before use. Storage duration for up to 6 hr was without effect on the results. Flash spectrophotometric experiments were carried out in a setup as described by Junge (1976) and Förster, Hong and Junge (1981). Measurements at room temperature were performed in a cuvette with 2 cm optical path length and 15 ml volume containing 10 μM chlorophyll, 10 μM methyl viologen as electron acceptor and 10 mM NaCl (*see also* figure legends). The suspension was excited by short [15 μsec (full width at half maximum (FWHM)] and saturating flashes of red light ($\lambda > 610$ nm, 1 mJ/cm^2) at 5 sec intervals. Experiments under variation of the temperature were performed in a thermostatted cuvette with an optical path length of 1 cm. The chlorophyll concentration was raised to 20 μM for identical optical density. Temperature was adjusted with 0.5°C tolerance. In these experiments excitation light was delivered by ruby laser flashes ($\lambda = 694$ nm, Q-switch, 2 mJ/cm^2, 80 nsec FWHM).

When thylakoid membranes are excited with short flashes of light the photochemical reaction centers generate a voltage of 30–50 mV across the membrane, positive in the lumen, plus an acidification by less than 0.1 pH units in the lumen (Junge, 1982). pH transients at the lumenal surface of the thylakoid membranes were measured by absorption changes of neutral red (13 μM) at 548 nm. They were obtained in the presence of bovine serum albumin (2.6 mg/ml) as a selective (i.e., not membrane permeable) buffer for the suspension medium (Ausländer & Junge, 1975). At pH 7.5 the proton efflux to the suspending medium was determined by phenol red (15 μM) at 559 nm (Junge & Ausländer, 1973). At pH 6.2 we used 15 μM bromocresol purple (575 nm) and at pH 7.9 cresol red (15 μM, 575 nm) as indicators. The "pH-indicating absorption changes" of the respective indicator dye were obtained by subtraction of a transient which was measured without added dye from another one with the pH-indicating dye added. This eliminated possible artifacts attributable to other effects than pH changes (Junge et al., 1979). The decay of the electrical potential across the thylakoid membranes was measured by electrochromic absorption changes of intrinsic pigments at 522 nm wavelength (Junge & Witt, 1968; Witt, 1979). The pH of the suspending medium was adjusted by addition of 1 mM buffer. We used MES/NaOH in the range between pH 5.6 and 6.5, MOPS/NaOH between pH 6.5 and 7.5 and tricine/NaOH from pH 7.5 to 8.0. N,N'-dicyclohexylcarbodiimide (DCCD), an inhibitor of F_o-type channels (Sigrist-Nelson, Sigrist & Azzi, 1978), gramicidin and valinomycin were added from ethanolic stock solution. The ethanolic concentration in the measuring cuvette was held below 0.5%. Thylakoids were incubated with DCCD for 10 min incubation at room temperature and in the dark prior to measurement. DCCD, gramicidin and valinomycin were purchased from Sigma, Dextran T 70 (mol wt approx. 70,000) from Pharmacia.

Isotopic substitution experiments were carried out in D_2O instead of H_2O. D_2O was about 99.5% pure and the fraction of H_2O in the final reaction mixture was held below 2%. The pD-value was determined with a glass electrode, which was calibrated against standard H_2O buffers. According to Westcott (1978) the pD is related to the reading of the glass electrode, pH_a, as follows: $pD = pH_a + 0.4$. For glycerol/D_2O mixtures and dextran solutions it was assumed that the highly alkaline pK (over 14) of the alcohols prevented the exchange of deuterons for protons during the 5-min interval of a typical experiment.

In flash spectrophotometric experiments 20–40 signals were recorded with a digitalization time of 20 μsec per address and averaged in order to increase the signal-to-noise ratio. The decay of averaged transients of electrochromic absorption changes was analyzed in terms of the following equation

$$U_{app}(t) = U_o \exp(-\bar{n}) \exp(\bar{n} \exp(-Gt/C)). \quad (1)$$

This equation, which is based on a Poisson statistical theory as described in Schmid and Junge (1975) and Lill et al. (1987) (*see* Eq. (8) therein), has only two essential fit parameters, $\bar{n}$, the average number of active channels per vesicle and G, the time-averaged single-channel conductance of these channels. For the other parameter, C, the capacitance of a vesicle, reasonable estimates were available which were cross-checked by applying the same analysis to the known ion channel gramicidin (Lill et al., 1987). The precision of G was dependent on the precision of C. A factor of two was considered as a reasonable margin. Computer-aided data fitting was performed on a DEC-PDP 11/34. The fit routine followed the simplex algorithm (Caceci & Cacheris, 1984) and it was restricted to the first 10 ms of the decay as in Lill et al. (1987).

Results

Specificity for Protons

The lower part of Fig. 1 shows the time course of charge displacement via CF_o (filled symbol) and of proton displacement via CF_o. The latter was viewed both from the lumen side (open triangles) and from the stroma side (open squares) of the thylakoid membrane. The respective transients were obtained as follows: Thylakoids were partially depleted of CF_1 and then excited with a short flash of light at time zero. The traces in the upper left of Fig. 1 show the pH-indicating absorption changes of neutral red (lumen side) in CF_1-depleted vesicles before and after incubation in 25 μM DCCD. The

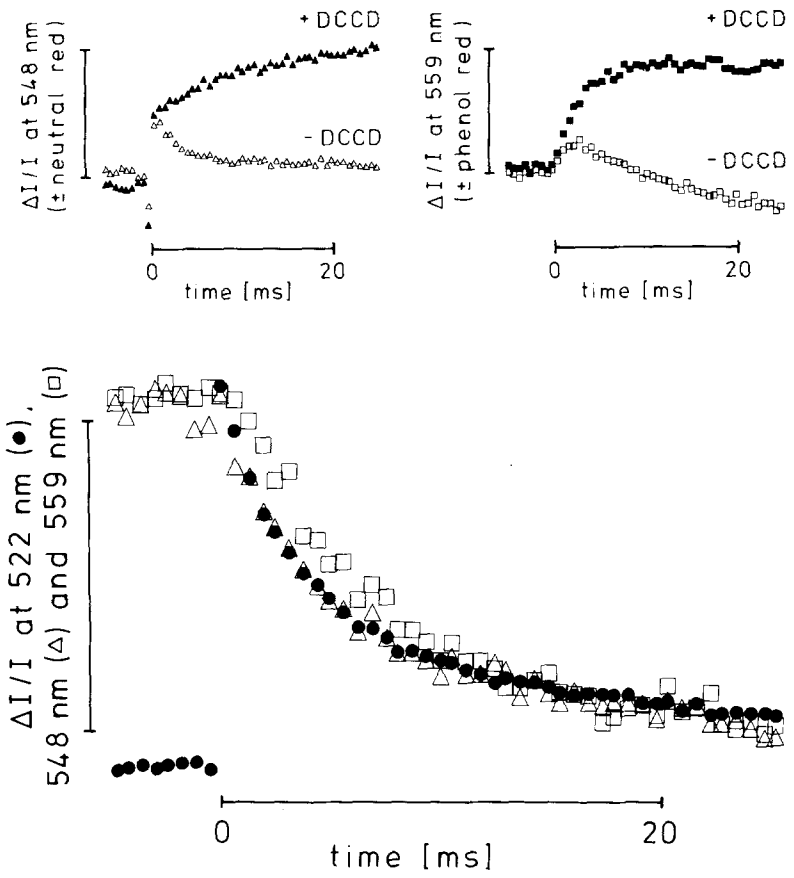

Fig. 1. Complete tracking of proton flow in CF_1-depleted vesicles. *Top, left:* pH-indicating absorption changes of neutral red at 548 nm, indicating acidification at the lumenal side of the thylakoid membrane after a flash of light. Open symbols: CF_1-depleted thylakoids. Filled symbols: same material, but after incubation with DCCD. The bar indicates relative changes of transmitted intensities, $\Delta I/I$ (548 nm) = 5×10^{-4}. *Top, right:* pH-indicating absorption changes of phenol red at 559 nm, indicating pH changes in the suspending medium. Open symbols: transient from a CF_1 depleted vesicle suspension. Filled symbols: Same material, but after incubation with DCCD. The bar indicates $\Delta I/I$ (559 nm) = 1×10^{-3}. *Bottom:* Time course of the electrochromic absorption changes, measured at 522 nm wavelength (filled circles), of CF_o-related proton displacement in the lumen as obtained by absorption changes of neutral red (open triangles) and of CF_o-related proton displacement in the medium as obtained by absorption changes of phenol red (open squares). The bar indicates $\Delta I/I$ (522 nm) = 1.5×10^{-3}, $\Delta I/I$ (548 nm) = 2×10^{-4} and $\Delta I/I$ (559 nm) = 1×10^{-3}

transient without DCCD reflects the superposition of the acidification of the lumen, which is due to the inwardly directed activity of the proton pumps (for kinetic properties (*see* Ausländer and Junge (1975) and Förster and Jung (1985)) and the alkalization due to the passive backflux of protons from the lumen via CF_o. The backflow of protons via CF_o was isolated by subtracting from the transient without DCCD the transient that was obtained in the presence of DCCD (*see* Schönknecht, Junge & Engelbrecht, 1986). This difference was plotted in the lower part of Fig. 1 (open triangles). It represents the *proton displacement by CF_o as viewed from the lumen side.* Measurements with the indicator dye phenol red, which are displayed in the upper right of Fig. 1, yielded pH transients at the stroma side. Similar considerations produced the *proton displacement by CF_o as viewed from the stroma side* which is plotted in the lower part of Fig. 1 as open squares. That the respective displacements were attributable to CF_o was evident from their sensitivity to DCCD (as documented in the upper traces of Fig. 1, and *see below*) and to other blockers of CF_o.

It is evident from inspection of the lower part of Fig. 1 that the charge displacement (filled symbol) followed the same time course as the proton displacement from the lumen (open triangles). Their respective half-decay time was 5 msec. Proton displacement into the medium followed with slight delay (open squares, 6 msec). The delay was possibly attributable to intermediate buffering, an effect most drastically expressed in stacked thylakoids (Junge & Polle, 1986; Polle & Junge, 1986). The approximate coincidence between charge displacement and proton displacement showed that *the proton was the major charge carrier admitted by CF_o.* We asked whether this was also true against higher background of salts and under variations of pH.

Figure 2 shows the (time-averaged) single-channel conductance of CF_o, G, and the average number of active channels per vesicle, $\bar{n}$, as function of salts. G and $\bar{n}$ were obtained by analyzing the decay of the electrochromic absorption changes in terms of Eq. (1). An example: for the trace documented in the lower part of Fig. 1 (filled symbol) the respective figures were G = 0.95 pS and $\bar{n}$ = 1.35. The pH was adjusted to 7.5 units by 1 mM Tris/tricine. The concentration of 1 : 1 electrolytes was varied between 1 and 300 mM and the one of 2 : 1 electrolytes between 0.1 and 30 mM. As shown in Fig. 2, the conductance of CF_o did not depend on the salt con-

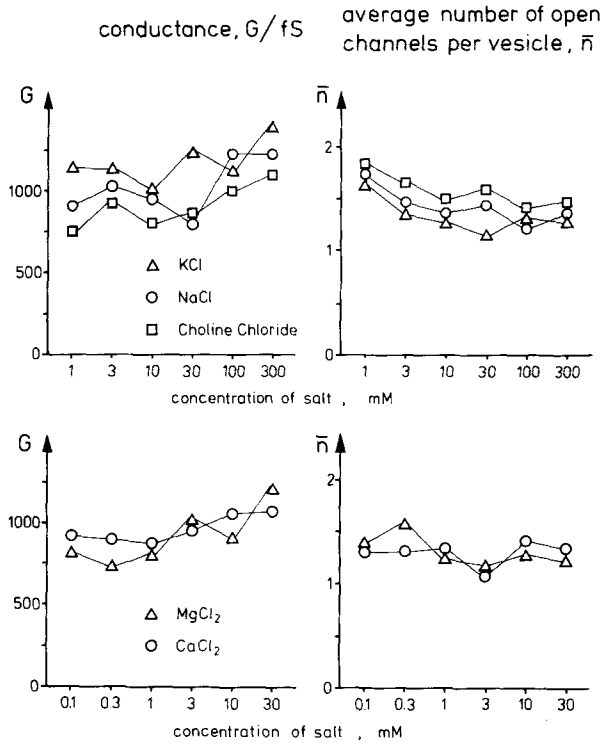

Fig. 2. Time-averaged single-channel conductance and number of active CF_o as function of the concentration of various salts in the suspending medium. The figure shows the parameters G and $\bar{n}$ as obtained by fitting transients of electrochromic absorption changes by Eq. (1). *Top:* 1:1 salts. *Bottom:* 2:1 salts

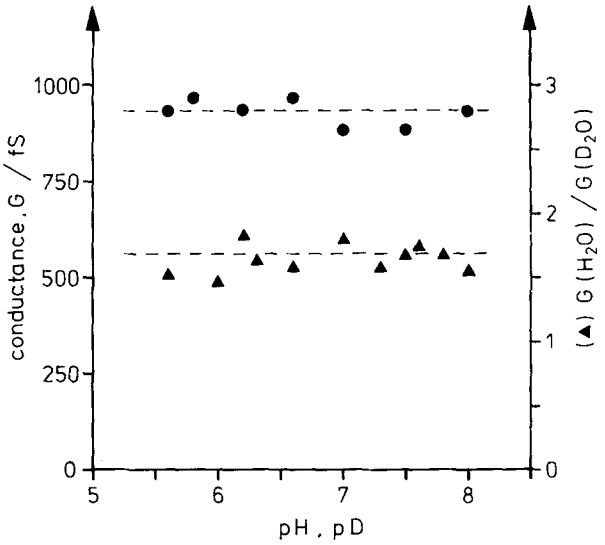

Fig. 3. Time-averaged single-channel conductance of CF_o as function of pH and pD in the suspending medium. Circles indicate the single-channel conductance, G, of CF_o. Triangles show the ratio of G measured in H_2O and in D_2O at equal molal activities, plotted as function of different pD values

centration, nor did there exist a difference between monovalent and divalent cations or between small monovalent cations (Na^+, K^+) and a larger one ($choline^+$). It followed that CF_o was proton specific over the whole concentration range and that its *selectivity for protons over, e.g., Na^+ was greater than 10^7.*

Conductance as Function of pH

Since the conductance of CF_o was proton specific, it was to be expected that its pH dependence reflected the pKs of the major proton carrying groups (*see* Brünger et al., 1983). We studied the single-channel conductance of CF_o as function of the pH in the suspending medium (circles). The salt concentration in the medium was 10 mM NaCl; no buffers were added. Figure 3 shows the results. The single-channel conductance of CF_o was independent of pH between 5.6 and 8.0 (circles). The same holds true in the presence of 300 mM NaCl (to minimize the negative surface potential). The average number of open channels per vesicle also remained constant (*data not shown*). To back up this result, which was obtained through measurements of the charge displacement by electrochromic absorption changes, we measured proton displacement as described in Fig. 1 (lower part, open squares). We used 15 μM bromocresol purple at pH 6.2 and 15 μM cresol red at pH 7.9. In both cases the acidification of the medium showed approximately the same time course as measured at pH 7.5. The respective half-rise times were 6.4 msec at pH 6.2, 6 msec at pH 7.5 and 6.8 msec at pH 7.9. it followed that *the single-channel conductance of CF_o was proton specific but pH independent in the range between 5.6 and 8.*

H/D-Isotope Effect

Since there was no measurable pH dependency in the physiological range that could be taken as indicative of a titratable group, we asked whether or not there was any H^+/D^+-isotope effect. The triangles in Fig. 3 show the ratio of the single-channel conductance of CF_o in H_2O and in D_2O as function of the molal activity expressed as pH and pD, respectively. *The single-channel conductance of CF_o was by a factor 1.7 lower in D_2O than in H_2O.*

Effect of Glycerol

That the conductance of CF_o was independent of pH could be interpreted to indicate that the channel conductance was not limited by a protolytic reaction of a particular group but, perhaps, by the supply of protons to the mouth of the channel. We

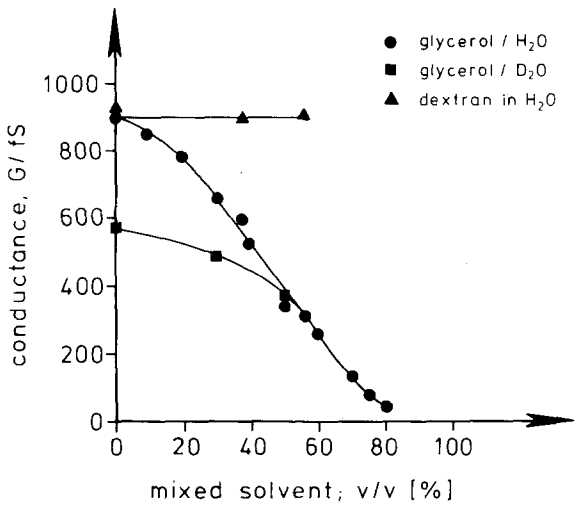

Fig. 4. Effects of increased glycerol concentration on the conductance of CF_o, *G*. Results were obtained by fitting transients of electrochromic absorption changes as recorded in glycerol/H_2O (circles) and glycerol/D_2O (squares) mixtures. The triangles indicate *G* as measured with 5 and 10% dextran (this time wt/wt) in the suspending medium, plotted at points of equal viscosity

studied the effect of glycerol which was expected to disturb bulk water structure and also to alter the surface pressure and the surface potential of the thylakoid membrane (for its effect on lipid monolayers, *see*, e.g., Cadenhead & Bean, 1972).

Figure 4 shows the (time-averaged) single-channel conductance of CF_o as function of the glycerol content in water (circles). The measurements were carried out in the presence of 10 mM NaCl and 1 mM tricine/NaOH at pH 7.5. The conductance decreased with increasing glycerol content, whereas the average number of open channels per vesicle remained constant (*not shown*). With glycerol/D_2O mixtures (squares in Fig. 4) the single-channel conductance decreased less steeply so that the effect of isotopic substitution disappeared at glycerol contents of more than 50%. Because of the very high pK of glycerol (greater than 14) this was certainly not attributable to the exchange of protons between the alcohol and D_2O. Increasing the viscosity of the suspending medium with dextran did not influence the conductance (triangles in Fig. 4, plotted at points of equal viscosity). The data shown in Fig. 4 suggested, that *the conductance of CF_o might be limited by events before or at the mouth of the channel.*

Temperature Dependence

We evaluated the temperature dependence of the conductance of CF_o with regard to two aspects. The Arrhenius activation energy is usually lower for a diffusion controlled reaction than it is for a reaction that involves bond formation/cleavage. The Arrhenius activation energy of carrier-mediated membrane transport often reveals a break point caused by a cooperative transition of membrane structure.

Figure 5 shows an Arrhenius plot of the decay rate of flash-induced electrochromic absorption changes in thylakoids from which about 50% of CF_1 was removed by EDTA treatment. The decay rate was greatly enhanced (i) by removal of CF_1 to expose CF_o (squares), (ii) after closure of the CF_o channels by DCCD, by addition of valinomycin (circles), and (iii) by addition of gramicidin, respectively (triangles). Filled symbols refer to experiments in aqueous suspension; open symbols refer to a medium with 30% ethylene glycol (vol/vol) as cryoprotectant.

Except for valinomycin the graphs were unilinear, i.e., in the range between +20 and −20°C the membrane conductance was to be described by a single activation energy. It was noteworthy that only valinomycin, the mobile potassium ion carrier (Benz et al., 1973), "sensed" a sharp transition of the membrane structure which probably occurred at −10°C, while gramicidin, the pore (*see* Hille, 1984), and CF_o apparently were not affected by this event. The energies of activation are listed in the Table.

The activation energies for gramicidin, 30 kJ/mol, and for valinomycin, 65 kJ/mol, as resulting from experiments with thylakoid membranes were in fair agreement with values published in the literature. For gramicidin Hladky and Haydon (1972) determined an activation energy of 21 kJ/mol and Parsegian (1969) calculated the barrier to ion passage through a hydrophilic pore (pore radius of 0.5 nm) to be 28 kJ/mol. For a carrier of reasonable size Parsegian (1969) calculated an electrostatic barrier of about 70 kJ/mol. *With 42 kJ/mol the activation energy of proton conduction by CF_o was intermediate between the lower one of a pore and the higher one of a mobile carrier.*

Discussion

Flash-spectrophotometric techniques when applied to CF_1-depleted thylakoid membranes give access to the time averaged single-channel conductance of CF_o (1 pS, *see* Lill et al., 1987). For a two-state channel this is the product of the open-channel conductance times the opening probability. Unlike by patch-clamp techniques (Neher & Sakmann, 1976), a discrimination between these parameters is not feasible. On the other hand, the spectroscopic technique allows kinetically resolving not only the electric discharge of the thylakoid membrane after a pulse of light but also the intake of protons by CF_o

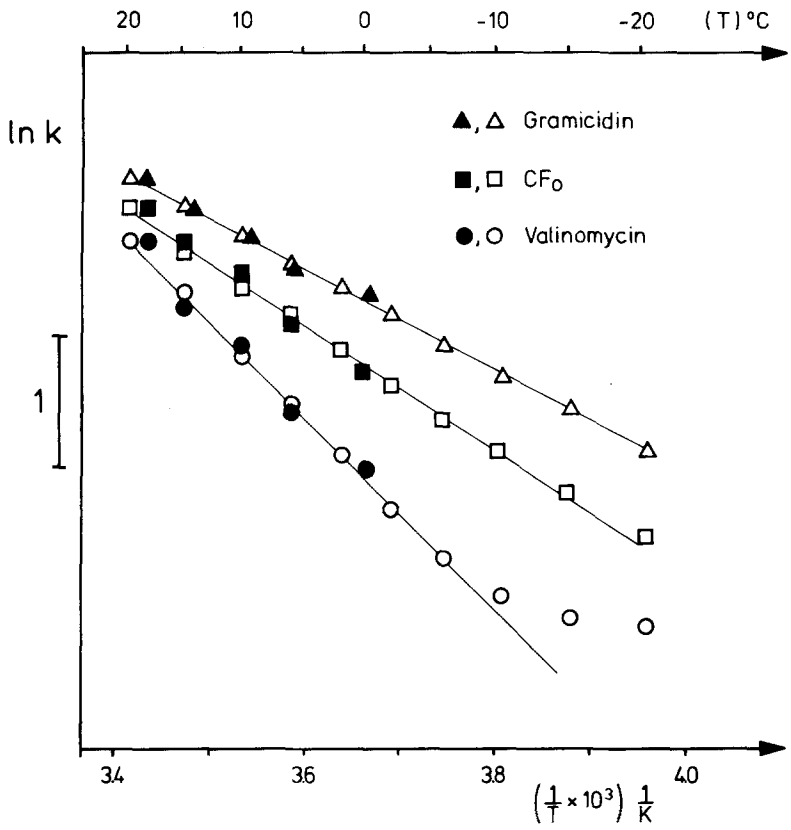

Fig. 5. The temperature dependence of CF_o (squares), gramicidin (triangles) and valinomycin (circles). Arrhenius plot of the decay constants of electrochromic transients. Filled symbols refer to measurements in aqueous suspensions; open symbols refer to experiments with 30% ethylene glycol (vol/vol) in the suspending medium

Table. Arrhenius activation energy E_a of CF_o, gramicidin and valinomycin in EDTA-treated thylakoid membranes (*see* Fig. 5)

Conductor	Solvent	Temp. range (°C)	E_a (kJ/mol)
Gramicidin	H_2O	0 – +25	30
	30% ethylene glycol (vol/vol)	−20 – +20	30
CF_o	H_2O	0 – +25	42
	30% ethylene glycol (vol/vol)	−20 – +20	42
	D_2O	+5 – +25	47
	30% ethylene glycol (vol/vol)	−20 – +20	47
Valinomycin	H_2O	0 – +25	65
	30% ethylene glycol (vol/vol)	−10 – +20	65
	30% ethylene glycol (vol/vol)	−20 – −10	<30

and their deposition at the other side of the membrane ("complete tracking of proton flow," Schönknecht et al. (1986), Junge (1987)). We found that the conductance of CF_o was proton specific even at pH 8 and against a background of 300 mM NaCl. Even though there was an isotope effect of 1.7 for H^+ over D^+, the conductance was seemingly pH independent in the range from 5.6 to 8 pH units. Its temperature dependence followed Arrhenius' equation with an activation energy of 42 kJ/mol, which was intermediate between the one of the pore gramicidin (30 kJ/mol) and the mobile carrier valinomycin (65 kJ/mol) in the same membrane.

The very high selectivity of CF_o for protons over other cations (greater than 10^7) was not obviously required by its physiological function. Under continuous and saturating illumination, the thylakoid lumen is acidified by about 3 pH units relative to the stroma, i.e., the internal pH approaches 5 units. For its function as a proton well in the ATP synthase it would have been sufficient, if it was selective at the more acid pH. Moreover, recent stud-

ies by Dimroth (1987) (*see also* Dibrov et al., 1986) seem to indicate a certain flexibility of the F_oF_1-type ATP synthase of the bacterium *Propionigenium modestum* to operate on protonmotive or sodiummotive driving force. Our results revealed an extreme selectivity of CF_o. This implied that the selectivity filter of the integral CF_oCF_1-complex was located in the CF_o portion.

It was puzzling that the conductance of CF_o was seemingly independent of the pH. As the conductance was inferred from the decay kinetics of electrochromic absorption changes it might be argued that this method has a limited time resolution. This is in contrast with the following observations: excitation with a pulse of a Q-switched ruby laser causes the rise of the electrochromic absorption changes in the range of nanoseconds (Wolff et al., 1969). Their decay, on the other hand, can be accelerated down into the range of microseconds. In the experiments with gramicidin and valinomycin, which are shown in Fig. 5, we added gramicidin to yield a concentration of 60 pM to shorten the half-decay time from some 100 msec in controls to 1.5 msec (20°C, 10 mM NaCl). Doubling of the concentration of gramicidin halved the decay time. This showed that the typical decay time after extraction of CF_1, 5 msec (*see* Fig. 1), was not at all limited by the response time of the electrochromic absorption change.

How does the magnitude, the specificity and the pH independence of the conductance of CF_o compare with the theoretical expectation and with the properties of known proton channels? It is more illustrative to discuss this in terms of rate constant (protons per second) instead of conductance (pico-Siemens). At 33 mV the current driven through a conductor with 1 pS conductance is equivalent to a rate of about 2×10^5 sec^{-1}. The concepts for proton conduction in water and ice, which occurs by a series of defect hoppings and rotations along a hydrogen bonded chain, have been applied to proton transport across biological membranes as mediated by, e.g., bacteriorhodopsin or F_o-type channels (Dunker & Marvin, 1978; Nagle & Morowitz, 1978; Brünger et al., 1983; Nagle & Tristram-Nagle, 1983; Schulten & Schulten, 1985). Nagle and Tristram-Nagle (1983) and Schulten and Schulten (1985) agree that the passage rate *through* a hydrogen bonded chain can range up to the order of 10^5–10^6 sec^{-1}. However the *supply* of protons to the channel mouth and the dissociation of protons may be a problem. As pointed out by Brünger et al. (1983) and experimentally established by Kasianowicz, Benz and McLaughlin (1987), there are two reaction pathways for the protonation of a particular group in the channel mouth: (i) the diffusion controlled binding of a proton and (ii) the hydrolysis of water. While the first dominates in the acid range of pH and pK, the second does so in the alkaline. Likewise, there are two mechanisms for diposal of a proton: (i) the unimolecular dissociation of a proton and (ii) the bimolecular reaction with hydroxyl, the first being rapid in the acid and the second in the alkaline range. It follows from inspection of Eq. (2.6) in Brünger et al. (1983) that there are only two regions in the two-dimensional field of medium pH and group pK where the rate of both on-reaction and off-reaction exceeds a figure of 10^5 sec^{-1}. This is when the pH *and* the pK are both smaller than 5 or both greater than 9. In the neutral pH and pK range, however, the calculated rates are only about 4×10^3 sec^{-1}. It is obvious that the recent proposal that H_3O^+ may be the transported species (Boyer, 1988) does not mend the above supply problems.

It was striking that the turnover rate of CF_o, 2×10^5 sec^{-1} (at 33 mV), was so high in the neutral pH range where proton supply might be a problem. It was also striking that the conductance of CF_o did not reproduce the strong pH dependence as expected from the above arguments and as plotted, for instance, by Brünger et al. (1983) in their Figs. 3 and 6. The turnover rate of CF_o was also much higher than the ones observed for two other proton-conducting channels, namely gramicidin and a synthetic oligopeptide studied by Lear, Wassermann and DeGrado (1988). The proton conductance of gramicidin is higher than the one for K^+ by a factor of 10 (Neher, Sandblom & Eisenman, 1978). However, at pH 7 this antibiotic, while acting as a narrow (about 0.4 nm wide) water accessible pore, has a proton conductance in the order of only 1 fS. A synthetic linear oligopeptide made of only two aminoacids, namely -(Leu-Ser-Leu-Leu-Leu-Ser-Leu)$_3$- was reportedly proton specific (Lear et al., 1988). Linear extrapolation of conductance data, which were obtained at 0.5 M HCl to pH 7, however, led to a unit conductance of less than 0.1 fS.

Several mechanisms for increasing the supply rate of ions to a channel (large mouth, short selectivity filter, counter ion charge in the pore mouth, etc.) have been discussed in the context of potassium maxi-channels (e.g. Latorre & Miller, 1983). They may as well apply to CF_o. For protons additional mechanisms can be inferred that are related to the possibly enhanced diffusion rate of protons at the membrane surface (e.g. Prats, Tocanne & Teissie, 1987). At the moment we face the surprising properties of CF_o and wait for the outcome of ongoing experiments with electrophysiological techniques, which are feasible because of the unexpectedly high conductance. They will allow varying

the lipid composition of the membrane to investigate proton access to the channel mouth.

We would like to thank Mrs. K. Schürmann for technical assistance and Ms. H. Kenneweg for photographs. Financial support by the DFG (SFB 171/B3) is gratefully acknowledged.

References

Ausländer, W., Junge, W. 1975. Neutral red, a rapid indicator for pH-changes in the inner phase of thylakoids. *FEBS Lett.* **59:**310–315

Benz, R., Stark, G., Janko, K., Läuger, P. 1973. Valinomycin-mediated ion transport through neutral lipid membranes: Influence of hydrocarbon chain length and temperature. *J. Membrane Biol.* **14:**339–364

Boyer, D.B. 1988. Bioenergetic coupling to protonmotive force: Should we be considering hydronium ion coordination and not group protonation? *Trends Biochem. Sci.* **13:**5–7

Brünger, A., Schulten, Z., Schulten, K. 1983. A network thermodynamic investigation of stationary and nonstationary proton transport through proteins. *Z. Phys. Chem.* **136:**1–63

Cadenhead, D.A., Bean, K.E. 1972. Selected lipid monolayers on aqueous-glycerol and aqueous-urea substrates. *Biochim. Biophys. Acta* **290:**43–50

Caceci, M.S., Cacheris, W.P. 1984. Fitting curves to data. The simplex algorithm is the answer. *Byte Mag.* **9:**340–362

Davenport, J.W., McCarty, R.E. 1981. Quantitative aspects of adenosine triphosphate-driven proton translocation in spinach chloroplast thylakoids. *J. Biol. Chem.* **256:**8947–8954

Dibrov, P.A., Lazarova, V.P., Skulachev, V.P., Verkhovskaya, M.L. 1986. The sodium cycle: II. Na^+-coupled oxidative phosphorylation in *Vibrio alginolyticus* cells. *Biochim. Biophys. Acta* **850:**458–465

Dimroth, P. 1987. Sodium ion transport decarboxylases and other aspects of sodium ion cycling in bacteria. *Microbiol. Rev.* **31:**320–340

Dunker, A.K., Marvin, D.A. 1978. A model for membrane-transport through α-helical protein pore. *J. Theor. Biol.* **72:**9–16

Förster, V., Hong, Y.Q., Junge, W. 1981. Electron transfer and proton pumping under excitation of dark adapted chloroplasts with flashes of light. *Biochim. Biophys. Acta* **638:**141–152

Förster, V., Junge, W. 1985. Stoichiometry and kinetics of proton release upon photosynthetic water oxidation. *Photochem. Photobiol.* **41:**183–190

Hille, B. 1984. Ionic channels of excitable membranes. Sinauer Associated, Sunderland, Mass.

Hladky, S.B., Haydon, D.A. 1972. Ion transfer across lipid membranes in the presence of gramicidin A. *Biochim. Biophys. Acta* **274:**294–312

Junesch, U., Gräber, P. 1985. The rate of ATP synthesis as a function of Δ pH in normal and dithiothreitol-modified chloroplasts. *Biochim. Biophys. Acta* **809:**429–434

Junge, W. 1976. Flash kinetic spectrophotometry in the study of plant pigments. *In:* Chemistry and Biochemistry of Plant Pigments. Vol. II, pp. 233–333. T.W. Goodwin, editor. Academic, New York

Junge, W. 1982. Electrogenic reactions and proton pumping in green plant photosynthesis. *Curr. Top. Membr. Transp.* **16:**431–465

Junge, W. 1987. Complete tracking of transient proton flow through active chloroplast ATP synthase. *Proc. Natl. Acad. Sci. USA* **48:**7084–7088

Junge, W., Ausländer, W. 1973. The electric generator in photosynthesis of green plants. *Biochim. Biophys. Acta* **333:**59–70

Junge, W., Ausländer, W., McGeer, A.J., Runge, T. 1979. The buffering capacity of the internal phase of thylakoids and the magnitude of the pH-changes inside under flashing light. *Biochim. Biophys. Acta* **546:**121–141

Junge, W., Polle, A. 1986. Theory or proton flow along appressed thylakoid membranes under instationary and under stationary conditions. *Biochim. Biophys. Acta* **848:**265–273

Junge, W., Rumberg, B., Schröder, H. 1970. The necessity of an electric potential difference and its use for photophosphorylation in short flash groups. *Eur. J. Biochem.* **14:**575–581

Junge, W., Witt, H.T. 1968. On the ion transport system of photosynthesis. Investigations on a molecular level. *Z. Naturforsch.* **38:**244–254

Kasianowicz, J., Benz, R., McLaughlin, S. 1987. How do protons cross the membrane-solution interface? Kinetic studies on bilayer membranes exposed to the protonophore S-13 (5-chloro-3-tert-butyl-2′-chloro-4′nitrosalicylanilide). *J. Membrane Biol.* **95:**73–89

Latorre, R., Miller, C. 1983. Conduction and selectivity in potassium channels. *J. Membrane Biol.* **71:**11–30

Lear, J.D., Wassermann, Z.R., DeGrado, W.F. 1988. Synthetic amphiphilic peptide models for protein ion channels. *Science* **240:**1177–1181

Lill, H., Althoff, G., Junge, W. 1987. Analysis of ionic channels by a flash spectrophotometric technique applicable to thylakoid membranes: CF_o, the proton channel of the chloroplast ATP synthase, and, for comparison, gramicidin. *J. Membrane Biol.* **98:**69–78

Lill, H., Engelbrecht, S., Schönknecht, G., Junge, W. 1986. The proton channel, CF_o, of thylakoid membranes. Only a low proportion of CF_1-lacking CF_o is active with a high unit conductance (169 fS). *Eur. J. Biochem.* **160:**627–634

Lill, H., Junge, W. 1989. CF_o, the proton channel of chloroplast ATP synthase. *Eur. J. Biochem.* **179:**459–467

Mitchell, P. 1977. A commentary on alternative hypothesis of proton coupling in the membrane systems catalysing oxidative and photosynthetic phosphorylation. *FEBS Lett.* **78:**1–20

Nagle, J.F., Morowitz, H.J. 1978. Molecular mechanism for proton transport in membranes. *Proc. Natl. Acad. Sci. USA* **75:**298–302

Nagle, J.F., Tristram-Nagle, S. 1983. Hydrogen bonded chain mechanisms for proton conduction and proton pumping. *J. Membrane Biol.* **74:**1–14

Neher, E., Sakmann, B. 1976. Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature (London)* **260:**779–802

Neher, E., Sandblom, J., Eisenmann, G. 1978. Ionic selectivity, saturation, and block in gramicidin A channels. *J. Membrane Biol.* **40:**97–116

Parsegian, A. 1969. Energy of an ion crossing a low dielectric membrane: Solutions to four relevant electrostatic problems. *Nature (London)* **221:**844–846

Polle, A., Junge, W. 1986. The slow rise of the flash-induced alkalization by Photosystem II of the suspending medium is reversibly related to thylakoid stacking. *Biochim. Biophys. Acta* **848:**257–264

Prats, M., Tocanne, J.F., Teissie, J. 1987. Lateral proton conduction at a lipid/water interface. Effect of lipid nature and ionic content of the aqueous phase. *Eur. J. Biochem.* **162:**379–385

Schmid, R., Junge, W. 1975. Current-voltage studies on the thy-

lakoid membrane in the presence of ionophores. *Biochim. Biophys. Acta* **394:**76–92

Schneider, E., Altendorf, K. 1987. Bacterial adenosine 5'-triphosphate synthase (F_1F_0): Purification and reconstitution of F_0 complexes and functional characterization of their subunits. *Microbiol. Rev.* **51:**477–497

Schönknecht, G., Junge, W., Lill, H., Engelbrecht, S. 1986. Complete tracking of proton flow in thylakoids—the unit conductance of CF_0 is greater than 10 fS. *FEBS Lett.* **203:**289–294

Schulten, Z., Schulten, K. 1985. A model for the resistance of the proton channel formed by the proteolipid of ATPase. *Eur. Biophys. J.* **11:**149–155

Senior, A.E. 1988. ATP synthesis by oxidative phosphorylation. *Physiol. Rev.* **68:**177–231

Sigrist-Nelson, K., Sigrist, H., Azzi, A. 1978. Characterization of the dicyclohexylcarbiodiimide binding protein isolated from chloroplast membranes. *Eur. J. Biochem.* **92:**9–14

Wescott, C.C. 1978. pH Measurements. Academic, New York

Witt, H.T. 1979. Energy conversion in the functional membrane of photosynthesis. Analysis by light pulse and electric pulse methods. The central role of the electric field. *Biochim. Biophys. Acta* **505:**355–427

Wolff, C., Buchwald, H.E., Rüppel, H., Witt, K., Witt, H.T. 1969. Rise time of the light induced electrical field across the function membrane of photosynthesis. *Z. Naturforsch.* **24:**1038–1041

Received 20 September 1988