Biochimica et Biophysica Acta 848 (1986) 257–264
Elsevier

BBA 41926

The slow rise of the flash-light-induced alkalization by Photosystem II of the suspending medium of thylakoids is reversibly related to thylakoid stacking *

Andrea Polle and Wolfgang Junge

Abteilung Biophysik, Fachbereich Biologie / Chemie, Universität Osnabrück, Postfach 4469, 4500 Osnabrück (F.R.G.)

(Received July 30th, 1985)

Key words: Photosynthesis; Proton pump; Photosystem II; Photophosphorylation; Thylakoid stacking; (Pea chloroplast)

We studied the kinetics of flash-induced proton uptake at the reducing site of Photosystem II with Cresol red as indicator for pH transients in the suspending medium. The rise of the alkalization which was observed when Photosystem II was hidden in the stacked regions of the thylakoid membranes was much slower (100 ms) than the reduction of the bound quinones (less than 1 ms). We asked for the delay mechanism. We found that the rise of the alkalization became biphasic if thylakoids were unstacked. This was reversed upon restacking. The portion of the fast phase (half-rise time, 2.7 ms) increased, if the concentration of Mg^{2+} was lowered. After EDTA-treatment we observed solely fast proton uptake. Experiments with dark-adapted chloroplasts showed that the biphasicity was not attributable to the alternating transitions of the bound quinone acceptors through the semiquinone and the hydroquinone stages. The dependence of fast proton uptake on the degree of membrane stacking was on line with our previous proposal (Hong and Junge (1983) Biochim. Biophys. Acta 722, 197–205) that the propagation of a pH pulse in the narrow gaps between stacked membranes was slowed down by multiple reactions of protons with fixed buffering groups. This concept was corroborated by a theory which was given in the subsequent article (Junge, W. and Polle, A. (1986) Biochim. Biophys. Acta 848, 265–273).

Introduction

The linear electron transfer across photosynthetic membranes is coupled with proton translocation from the external phase into the lumen of thylakoids. Photosystem I, one of the sites mediating proton consumption, is mainly located in the region of the stroma lamellae, while Photosystem II, the water-plastoquinone oxidoreductase, is located in the appressed membrane portions of stacked thylakoids [1,2]. The pumped protons serve as driving force for the ATP-synthases which are confined to stroma lamellae and to top, bottom and possibly to fringes of stacked thylakoids [3]. The distance between a particular Photosystem II and the nearest synthase may be as large as 300 nm (for a recent review in lateral protein distribution, see Ref. 4). It has been suggested that some 10% of the proton-motive force may be lost during lateral proton flow from pumps to ATP-synthases [5,6]. In this respect it was interesting that the flash induced proton uptake from the external suspending medium rose by two orders of magni-

* A short report on this subject was presented by us (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. II, pp. 261–264, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands.

Abbreviations: DCCD, dicyclohexylcarbodiimide; EDTA, ethylenediaminetetraacetic acid; PS II, Photosystem II; PS I, Photosystem I.

tude more slowly (60 ms) [7] than the redox reactions supposed to drive proton uptake (typically less than 1 ms). It was puzzling that proton uptake by both photosystems was delayed, although Photosystem II and Photosystem I were located in different membrane domains. This has caused speculation on shields and barriers, possibly of proteinaceous origin, which might cover the sites of proton uptake at both photosystems [7–10]. In a following communication [11] we showed that proton uptake by Photosystem I was drastically accelerated, if the natural acceptor system (ferredoxin/$NADP^+$) was supplemented. This left Photosystem II as the only site where proton uptake from the suspending medium was greatly delayed.

In Photosystem II an electron which is photochemically driven from the inner side towards the outer side of the membrane is stabilized within less than 200 ps [12] on the first of two bound plastoquinone molecules, Q_A. Q_A plus its partner, Q_B, serves as a two-electron gate. Only after having accepted to electrons plus two protons the quinolic form of Q_B, PQH_2, dissociates from its binding site to become part of the mobile plastoquinole pool which reduces the cytochrome b_6-f complex [13–16]. Which of the redox states of the acceptor quinone complex binds protons and whether or not a bound proton can reside on a semiquinone proper is under debate. However, it is generally accepted that each electron transfer via Q_A–Q_B causes the uptake of one proton per electron, at least under repetitive flash excitation (for a review, see Ref. 18).

In this communication we investigated delayed proton uptake at the reducing site of Photosystem II by variation of the stacking conditions. In isolated and broken chloroplasts, the terminal electron acceptor $NADP^+$ was lost. Instead we used ferricyanide – which does not bind protons upon reduction – under conditions where it did not compete with plastoquinone as Photosystem II acceptor. Ferricyanide also deactivated a third site of proton uptake, which is associated with cyclic electron transfer. These experimental conditions left Photosystem II as the only sink for protons from the suspending medium. Cresol red served as indicator for pH transients specifically in the bulk phase (see Refs. 17 and 32).

We showed that the slow alkalization by Photosystem II of the suspending medium is intimately related to thylakoid stacking. This corroborated the previous suggestion [20] that the delay was caused by multiple buffering in the partition domains. A theoretical description of proton diffusion under buffering in the partition gap is presented in the following article [19].

Materials and Methods

Pea seedlings (*Pisum sativum* var. Kleine Rheinländerin) were grown in a 16 h/8 h light-dark cycle. Unstacked thylakoids were prepared according to the procedure described by Steinback et al. [21]: 40 g of 14–18-days-old plants were harvested and washed in ice-cold water. The plant material was homogenized in 100 ml grinding medium that contained sorbitol (400 mM), NaCl (10 mM) and tricine/NaOH (100 mM) (pH 7.8). The slurry was filtered through a nylon mesh (20 μm) and then spun down at $1000 \times g$ for 5 min. The pelleted chloroplasts were resuspended in NaCl (10 mM) and tricine/NaOH (10 mM), pH 7.8 and centrifuged at $10000 \times g$ for 10 min. The final pellet was diluted with sorbitol (100 mM), NaCl (10 mM) and tricine-NaOH (10 mM) (pH 7.8), yielding a stock solution with a chlorophyll concentration of about 5 mM. The stock solution was stored on ice. Aliquots could be used for measurements during 5 h without appreciable ageing. For some experiments the stock solution was dark-adapted for 3 h on ice as indicated. Stacked thylakoids were prepared after the same procedure, but all media contained additionally $MgCl_2$ (5 mM).

EDTA-treated thylakoids were prepared as follows: chloroplasts obtained from the grinding medium were incubated with EDTA (1 mM) and tricine (2 mM) (pH 7.8) in a volume of 15 ml for 10 min on ice. The suspension was spun down for 10 min at $20000 \times g$ and the EDTA-treatment was repeated once. The resulting pellet was diluted with NaCl (5 mM) and tricine (2 mM) (pH 7.8) to yield a final stock solution with approx. 3–4 mM chlorophyll.

Some experiments were performed with frozen and rethawed thylakoids, which were prepared as in Ref. 22.

Stacked thylakoids (10 μM chlorophyll) were added to an assay medium containing NaCl (10 mM), $MgCl_2$ (5 mM) and cresol red (15 μM) (pH 7.9). Destacked thylakoids were suspended in the same assay medium, but without $MgCl_2$. To destack or restack thylakoids, the membranes were stirred slowly for 15 min in the respective assay medium and at room temperature prior to measurement.

Absorption changes of cresol red were measured in the same apparatus as described by Förster et al. [23]. The samples was contained in an optical cell with 2 cm pathlength. Absorption changes of cresol red at 575 nm were obtained by subtration of the signals recorded in the absence of the dye from those recorded in the presence of the dye. An automatic dc-offset and -averaging circuit was applied to avoid subtraction artefacts. The absorption changes of cresol red were solely indicative of pH-transients in the external medium and they were free from contributions of other events and other compartments. This was apparent from quenching by added buffers (e.g., bovine serum albumin, phosphate, etc.) which had no access to the thylakoid lumen in these particular chloroplast preparations. For detailed studies on buffer access to the lumen, see Refs. 20, 24; and for the theoretical basis of the selectivity of cresol red for pH transients in the suspending medium, see Ref. 19.

Samples were excited with saturating light flashes (xenon flash lamp; wavelength, larger than 610 nm, 15 μs FWHM, 1 mJ/cm^2). The measuring light was gated open only during the actual sampling interval plus 20 ms to adapt the photomultiplier. The energy of the measuring light per repetition was kept below 10 μJ/cm^2. Typically, 20 or 40 samples were averaged. Optical signals were detected by a photomultiplier (EMI9558) with only six dynodes in operation. The repetition rate of the exciting flashes was 0.1 Hz, unless otherwise indicated.

The flash-detection spectrophotometer was also used for measurements of the variable fluorescence. In this case a photomultiplier (RCA 31000B) was installed at right angle to the exciting light source. Thylakoids were excited by continuous blue light (BG28). Fluorescence induction was measured at wavelengths greater than 665 nm (RG665).

Results

Ferricyanide, when used at low concentrations, accepts electrons from Photosystem I, only, but not from Photosystem II. Thereby, it deactivates proton uptake by Photosystem I, but it leaves proton uptake by Photosystem II intact [25]. We measured the extent of flash-induced proton uptake as function of the ferricyanide concentration for three different types of thylakoid preparation. The result is shown in Fig. 1. In freshly prepared thylakoids, regardless whether stacked or unstacked, the alkalization dropped to half of the maximal extent already at very low concentrations of ferricyanide (some 10 μM). It remained stable at this level over a wide range of ferricyanide concentrations (10–1000 μM). There was only little influence of the stacking conditions. Different behaviour was observed after storage of chloroplasts under liquid nitrogen and after thawing. Then proton uptake was strongly dependent on the concentration of ferricyanide. Since the electron transfer was not affected (full amplitude of pH signal in Fig. 1 in the absence of ferricyanide), the reducing site of PS II was more accessible to ferricyanide after freezing. The experiments which are described in the following were carried out with fresh

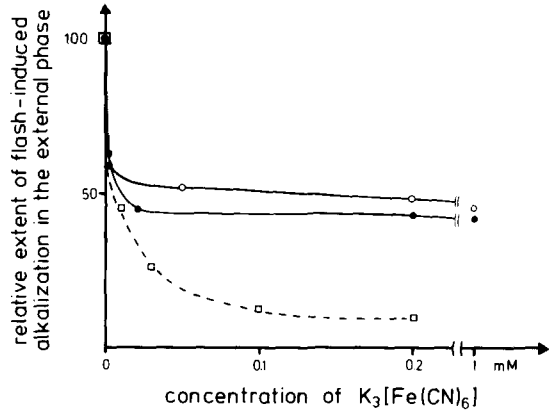

Fig. 1. Relative extent of the flash-induced alkalization of the suspending medium of thylakoids as function of the concentration of ferricyanide. The points refer to three different types of material: (○) freshly prepared in stacking medium, (■) freshly prepared in unstacking medium and (□) frozen/thawed and suspended in stacking medium. For details see Materials and Methods. When ferricyanide was omitted, methyl viologen (10 μM) was used as electron acceptor.

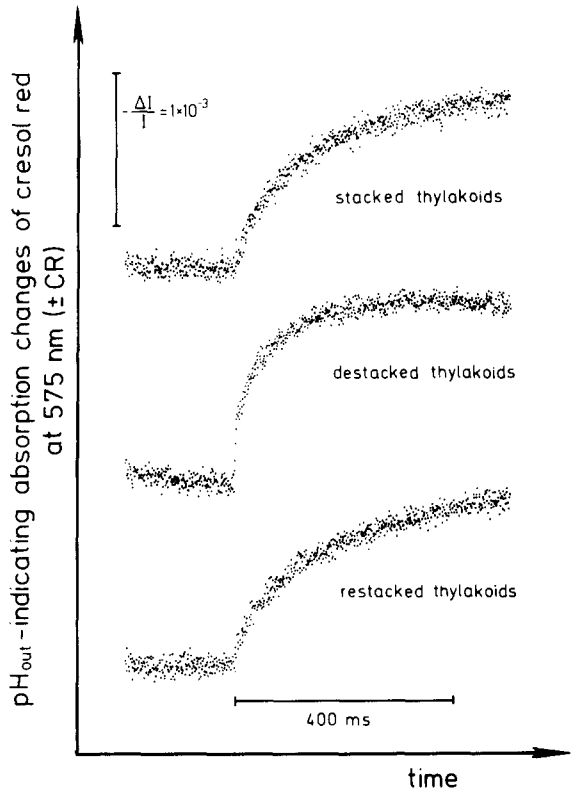

Fig. 2. Time-course of the pH$_{out}$-indicating absorption changes of cresol red under different stacking conditions. Flash-induced proton uptake was measured in the presence of ferricyanide (200 μM). Further assay conditions are given under Materials and Methods.

thylakoids and with ferricyanide concentrations in the plateau range according to Fig. 1, where proton uptake was entirely due to PS II.

Fig. 2 shows proton uptake by Photosystem II under variation of the stacking conditions. The upper trace was obtained with stacked thylakoids. The rise of the external alkalization was slow with an average half-rise time of approx. 100 ms. When thylakoids were incubated in the unstacking medium the rise of the alkalization was accelerated to become biphasic (middle trace in Fig. 2). We analyzed the rise kinetics by means of a curve-fitting programme (SIMPLEX). Analysis for three exponentials revealed that the signal was composed of only two major components with the following half-rise time and relative extent: 2 ms (40%) and 60 ms (60%). The effect was reversible. When Mg^{2+} (5 mM) was added to unstacked thylakoids, the original slow rise of the alkalization

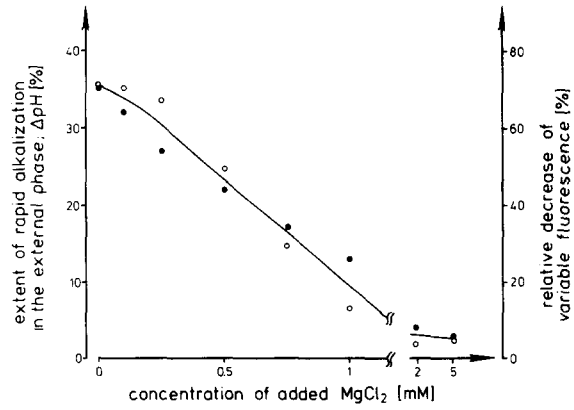

Fig. 3. The relative extent of rapid proton uptake (●) and variable fluorescence (○) as function of the Mg^{2+}-concentration. The experiments were performed with unstacked thylakoids which were restacked in the presence of the respective Mg^{2+}-content. The rapid component of the alkalization was read out from traces as in Fig. 2 by computer analysis. Fluorescence induction curves were measured at 680 nm in the presence of DCMU (5 μM). The indicated extent of variable fluorescence was related to the magnitude of variable fluorescence of a stacked thylakoid preparation.

was reproduced again (lower trace in Fig. 2) and also, when thylakoids were restacked in presence of high concentrations of NaCl (50–100 mM).

We investigated whether the extent of rapid phase of alkalization was correlated with the degree of unstacking. As known from the detailed work from the laboratory of Barber [26], the increase of thylakoid unstacking is accompanied by decrease of variable Photosystem II fluorescence. We used fluorescence only as qualitative indicator for stacking. It is apparent from Fig. 3 that the delay of proton uptake was associated with thylakoid stacking. There was one obstacle to this ready conclusion. The extent of proton uptake by Photosystem II which could be accelerated by low salt conditions was always below 50% of the total. This could be indicative of incomplete unstacking (see further down) or, alternatively, due to different rates of proton uptake during the two successive reduction steps of the acceptor pair Q_A-Q_B. We scrutinized these possibilities, starting with the latter.

Fig. 4 shows the alkalization of the external phase in response to a series of light flashes given to dark-adapted chloroplasts. With ferricyanide present the Q_A-Q_B system was synchronized in the oxidized state in the dark [27]. On the other hand,

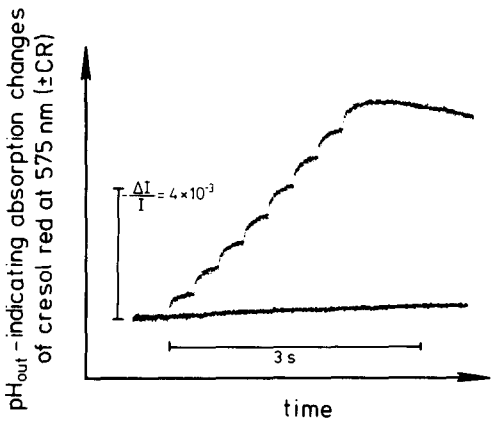

Fig. 4. Pattern of the pH_{out}-indicating absorption changes of cresol red from dark adapted thylakoids under excitation with a series of red xenon flashes ($\lambda \geq 610$ nm, 2 μs FWHM, > 2 mJ), spaced at 150 ms, time resolution: 2 ms/point). The stock of unstacked thylakoids was dark-adapted for 3 h. The assay medium contained chlorophyll (20 μM); NaCl (10 mM), $K_3[Fe(CN)_6]$ (50 μM) and cresol red (15 μM), pH 7.9. The cuvette was automatically refilled with new dark-adapted material for each sample. 20 sweeps were averaged. Upper trace: in the absence of DCMU and lower trace: in the presence of DCMU (5 μM).

the ferricyanide concentration was too low to cause rapid reoxidation of the semiquinone stage by ferricyanide between flashes. (The evidence for this is documented in Fig. 1. Proton uptake at 50% of the total extent is clear evidence that ferricyanide accepted at Photosystem I, only.) It was assumed that starting from the dark and under successive flashes the quinone system cycled through its two oxidoreduction transitions ($QQ \rightarrow QQ^-$ and $QQ^- \rightarrow QQ^{2-}$, QQ). The experiment was carried out with destacked thylakoids and with a biphasic rise of the alkalization under repetitive flashes (as in the middle trace of Fig. 2). It was apparent from Fig. 4 that each flash induced a biphasic rise of the alkalization. This demonstrated that biphasicity was not related with the two different redox transitions of the acceptor system. Moreover, there was no oscillation of the extent of the alkalization. Apparently, both reduced stages of the plastoquinone acceptors bound one proton per electron. The lower trace shows the outcome of this experiment in the presence of DCMU. Here no proton uptake was observed at all. Since DCMU prevents further electron transfer from Q_A to Q_B [13], this demonstrated that the semiquinone stage of Q_A^- could not bind one proton, at least in the presence of DCMU.

TABLE I

THE EFFECT OF EDTA-TREATMENT ON THE ELECTRON-TRANSFER RATE

The electron-transfer rate was measured as oxygen evolution with a Clark-type membrane electrode under continuous illumination with saturating white light. The assay medium contained: chlorophyll (50 μM), NaCl (10mM), tricine (2 mM), pH 7.8 and $K_3[Fe(CN)_6]$ (2 mM) as electron acceptor. Nigericin (1 μM) was used as uncoupler.

Condition	Electron-transfer rate (mmol e^-/mol chlorophyll per s)	
	without uncoupler	with uncoupler
Stacked thylakoids	22	56
EDTA-washed thylakoids	26	28

We concluded that the biphasic rise of the alkalization in unstacked thylakoids was not attributable to the two alternating redox transitions of the bound quinones. We questioned whether it was caused by incomplete unstacking. Electron microscopic studies by Mustardy [28] and by Haehnel (personal communication) show that the proportion of nonappressed thylakoid membranes could be enhanced by EDTA-washing. EDTA treatment is commonly applied to extract membrane proteins, i.e., the coupling factor of the ATP-synthase, CF1 [29]. Fig. 5 shows the effects of such a treatment on the proteolytic reactions. Proton uptake, indicated by the small uprising peak, was superimposed by rapid efflux ($t_{1/2} = 10$ ms) of internally produced protons. Moreover, the extent of both signals (upward and downward) was much too low. In addition to unstacking and uncoupling, partial inhibition of electron transfer occurred.

This was also evident from measurements of oxygen evolution with ferricyanide as electron acceptor under continuous saturating light. Table I shows that the electron-transfer rate of EDTA-treated thylakoids was inhibited by 50%, and that the thylakoids were uncoupled. In agreement with these results, the magnitude of the electrochromic absorption change (Fig. 6, lower trace) which indicates photosystem I and II activity was only 50% of controls ($\Delta I/I_0 = 1.1 \cdot 10^{-3}$ instead of $2 \cdot 10^{-3}$

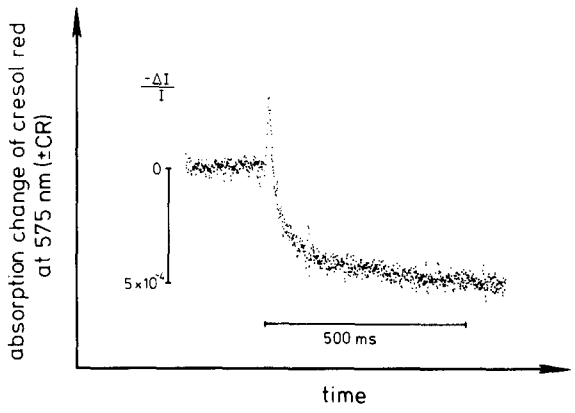

Fig. 5. Time-course of the pH_{out}-indicating absorption changes of cresol red in EDTA-treated chloroplasts. Assay conditions: chlorophyll (10 μM), NaCl (5 mM), $K_3[Fe(CN)_6]$ (200 μM), cresol red (15 μM), pH 7.8. 40 samples were averaged, time resolution: 1 ms/point.

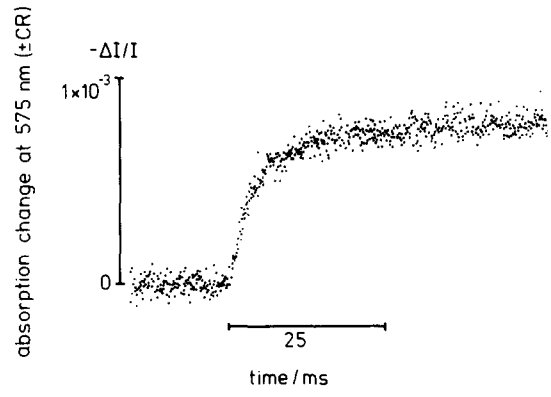

Fig. 7. Time-course of the pH_{out}-indicating absorption changes of cresol red in EDTA and DCCD-treated thylakoids. Assay conditions as in Fig. 5. Thylakoids were incubated for 10 min with DCCD (20 μM). 40 samples were averaged, time resolution: 100 μs/point.

in the control) and decayed rapidly ($t_{1/2}$ = 2–5 ms). Fig. 6 (upper trace) shows that the fast decay of the electric field was prevented by addition of DCCD, a reagent which closes the proton channel CF0 [30]. Apparently, the electron transfer was only slightly affected by incubation with DCCD.

We questioned whether the rapid release of internally produced protons into the external phase was blocked under these conditions. Fig. 7 shows a time-resolved measurement of proton uptake when the thylakoids were unstacked by EDTA-treatment in the presence of DCCD. Obviously, short-circuting of pumped protons was prevented. There was a uniphasic rise of the external alkalization with a half-rise time of 2.7 ms.

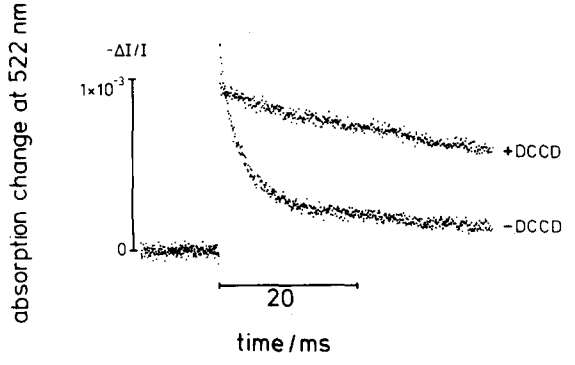

Fig. 6. Time-course of the electrochromic absorption changes at 522 nm in EDTA-treated thylakoids. Assay conditions as in Fig. 5, when indicated thylakoids were incubated with DCCD (20 μM) for 10 min. 20 samples were averaged, time resolution: 50 μs/point.

Discussion

We observed that the alkalization of the suspending medium of stacked thylakoids which is caused by flashing light rose rather slowly (100 ms). This was in agreement with previous observations [7,31]. It seemed particularly odd that the half-rise time of the alkalization was by more than one order of magnitude longer than the typical turnover time of the linear electron-transport chain and of photophosphorylation under saturating and continuous illumination. Because sand grinding or mild sonication of thylakoids accelerated the rise of the alkalization [7], it had been inferred that the delay was caused by some gross structural element. It was unknown, however, whether it was caused by some proteinaceous shield which is known to cover the reducing side of Photosystem II, at least [8–10,33] or by the superstructure of stacked thylakoids. We investigated this matter.

We used cresol red to monitor pH transients. Owing to its low lipid solubility and owing to the small size of the inner volume (and the partition volume) of thylakoids relative to the outer volume (10^3–10^4), this dye is selective for pH transients, occurring in the external bulk phase (see Fig. 6 in Ref. 32). Under our experimental conditions flash

excitation of thylakoids caused proton uptake from the outer medium at the reducing site of Photosystem II, only.

If thylakoids are well stacked, the membrane proteins show a pronounced lateral asymmetry with Photosystem II hidden in the partition domains of grana stacks [1–4,34,35]. It is generally accepted that proton uptake occurs at the level of protein bound plastoquinone acceptors in the reaction center of Photosystem II. The slow rise of the external alkalization showed that the acceptor sites for protons are not directly accessible from the external bulk phase, the main compartment of cresol red. Consequently, four types of proteolytic reactions have to be considered: (1) a reduced quinone binds one proton per electron from neighbouring buffers; (2) the proton deficiency diffuses (among acidic groups within proteins and between proteins along the membrane); (3) it crosses the interface between the membrane and bulk water; and (4) the indicator dye reacts in response to the pH transient in the bulk medium. It was surely not the indicator dye which delayed the response. This was obvious from experimental evidence (see Fig. 7) and also from the known behaviour of weak acids and bases in aqueous environment. If the protonation rate of cresol red was diffusion controlled, as expected, and for the given concentration of cresol red one calculates a rise-time of some 2 μs in response to a small and step-shaped pH perturbation ($t_{rise} = k c_{base}^{-1}$, see Eigen [36]). Gutman and his colleagues [37] have conducted detailed model studies with photolysable proton donors, buffers and pH-indicator dyes which are distributed between micellar interfaces and bulk water. They found that the transition of protons between bulk water and a micellar interface and vice-versa is very rapid (submicroseconds). This left only two types of partial protolytic reaction as possible reasons for the observed delay between redox reactions at Photosystem II and the alkalization of the suspending medium, namely (1) a slow protonation of the reduced product and (2) a slow diffusion of an alkalization pulse from the site of proton uptake and along the membrane towards the bulk phase.

For Photosystem II our results showed that the rate of the external alkalization was dependent on the degree of thylakoid stacking. Although the diffusion barrier for electron acceptors like ferricyanide [8] persisted under unstacking conditions (cf. Fig. 1), proton uptake was partially accelerated (Fig. 2). Under gradual variation of the degree of unstacking we found a gradual increase of the relative extent of accelerated alkalization (see Fig. 3). This, however, did not exceed 50% of the total extent. We excluded the possibility that the limitation was caused by a different protolytic behaviour of the two redox reactions which, in alternation, drive proton uptake at Photosystem II, namely the production of the singly- and the two-fold-reduced forms of the quinone acceptor pair. Therefore, the imppossibility to accelerate more than 50% of the total alkalization had to be attributed either to partial unstacking or to heterogeneity of Photosystems II.

More complete unstacking was induced by EDTA-treatment (compare EM pictures by Mustardy [28]). This also caused proton leakage from the lumen of the thylakoids into the external medium (Fig. 5). The leaks were closed by DCCD (Figs. 6 and 7). DCCD is classically applied to close the proton channel CF0, but it has been found to inhibit a number of other enzymes involved in proton translocation (see Refs. 38 and 39). Sane et al. [40] reported inhibition of photosynthetic electron flow by DCCD and suggested that it was a proton flow inhibitor. We showed, however, that in EDTA-treated thylakoids and in the absence of Mg^{2+} the electron flow was only slightly inhibited by DCCD (Fig. 6). Under these conditions we observed monophasic and rapid alkalization only (Fig. 7: half-rise time was 2.7 ms). The extent, however, was diminished. This was mainly caused by EDTA (Table I). Rigorously speeking, we could not discriminate whether 50% of the electron transport chains were unspecifically inhibited or whether this treatment deactivated selectively those Phososystem II which had a long delay in their proton uptake under unstacking conditions.

The conservative conclusion was that at least one portion of the Photosystem II-generated alkalization of the suspending medium of thylakoids was delayed by the slow propagation of an alkalization pulse along the narrow partition gap of stacked thylakoids. Based on standard theory of diffusion we have previously proposed that the

diffusion constant for protons was drastically (10^4–10^5-fold) diminished by multiple buffering of protons by fixed proteinaceous groups in the partitions [20]. In the following communication [19] we develop a theory for quantitative description of pulsed proton flow in partition.

Acknowledgements

We thank Dr. U. Kunze and H. Lill for computer software, H. Kenneweg for drawings and photographs and G. Möhrke for help with typing. Financial support has been provided by the Deutsche Forschungsgemeinschaft (JU97/11-7 and Sonderforschungsbereich 171, Projekt A2).

References

1 Andersson, B. and Anderson, J.M. (1980) Biochim. Biophys. Acta 593, 427–440
2 Andersson, B. and Haehnel, W. (1982) FEBS Lett. 146, 13–17
3 Berzborn, R. (1968) Z. Naturforsch. 23b, 1096–1104
4 Haehnel, W. (1984) Annu. Rev. Plant Physiol. 35, 659–693
5 De Kouchkovsky, Y. and Haraux, F. (1981) Biochem. Biophys. Res. Commun. 99, 205–212
6 Haraux, F. and De Kouchkovsky, Y. (1982) Biochim. Biophys. Acta 679, 235–247
7 Ausländer, W. and Junge, W. (1974) Biochim. Biophys. Acta 357, 285–298
8 Renger, G. (1976) Biochim. Biophys. Acta 440, 287–300
9 Renger, G. (1977) in Bioenergetics of Membranes (Packer, L., Papageorgiou, G.C., and Trebst, A., eds.), pp. 339–350, Elsevier/North-Holland/Biomedical Press
10 Mattoo, A.K., Pick, U., Hoffman-Falk, H. and Edelmann, M. (1981) Proc. Natl. Acad. Sci. USA 78, 3, 1572–1576
11 Polle, A. and Junge, W. (1986) Biochim. Biophys. Acta 848, 274–278
12 Trissl, H.-W., Kunze, U. and Junge, W. (1982) Biochim. Biophys. Acta 682, 364–377
13 Velthuys, B.R. (1981) FEBS Lett. 126, 277–281
14 Lavergne, J. (1982) Biochim. Biophys. Acta 682, 345–352
15 Oettmeier, W. and Soll, H.J. (1983) Biochim. Biophys. Acta 724, 287–290
16 Crofts, A.R. and Wraight, C. (1983) Biochim. Biophys. Acta 726, 149–185
17 Junge, W., McGeer, A. and Ausländer, W. (1978) in Frontiers of Biological Energetics (Dutton, L. et al., eds.), pp. 275–283, Academic Press, New York
18 Junge, W. and Jackson, J.B. (1982) in Photosynthesis. Energy Conversion by Plants and Bacteria (Govindjee, ed.), Vol. 1, pp. 589–646, Academic Press, New York
19 Junge, W. and Polle, A. (1986) Biochim. Biophys. Acta 848, 265–273
20 Hong, Y.C. and Junge, W. (1983) Biochim. Biophys. Acta 722, 197–208
21 Steinback, K.E., Burke, J.J. and Arntzen, C.J. (1979) Arch. Biochim. Biophys. 195, 546–557
22 Reeves, S.G. and Hall, D.O. (1973) Biochim. Biophys. Acta 314, 66–78
23 Förster, V., Hong, Y.C. and Junge, W. (1981) Biochim. Biophys. Acta 638, 141–152
24 Junge, W., Ausländer, W., McGeer, A. and Runge, T. (1979) Biochim. Biophys. Acta 546, 121–141
25 Junge, W. and Ausländer, W. (1973) Biochim. Biophys. Acta 333, 59–70
26 Barber, J. (1980) Biochim. Biophys. Acta 594, 253–308
27 Robinson, H.H. and Crofts, A.R. (1983) FEBS Lett. 153, 221–226
28 Mustardy, L. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. III, pp. 75–78, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
29 McCarty, R. (1971) Methods Enzymol. 23, 251–253
30 Linnett, P.E. and Beechey, R.B. (1979) Methods Enzymol. 25, 472–518
31 Hope, A.B. and Matthews, D.B. (1983) Aust. J. Plant Physiol. 10, 363–372
32 Junge, W. (1982) in Electrogenic Ion Pumps (Slayman, C.S., ed.), pp. 431–464, Academic Press, New York
33 Renger, G. and Tiemann, R. (1979) Biochim. Biophys. Acta 545, 316–324
34 Staehelin, L.A. (1976) J. Cell Biol. 71, 136–158
35 Kyle, I.J. (1983) Eur. J. Biochem. 137, 205–213
36 Eigen, M. (1963) Ang. Chem. 75, 489–588
37 Gutman, M., Huppert, D., Pines, E. and Nachliel, E. (1981) Biochim. Biophys. Acta 642, 15–26
38 Solioz, M. (1984) TIBS, July, 309–312
39 Azzi, A., Casey, R. and Nalecz, M. (1984) Biochim. Biophys. Acta 768, 209–226
40 Sane, P., Johanningmeier, U. and Trebst, A. (1979) FEBS Lett. 108, 136–140