Flow–Force Relationships in Lettuce Thylakoids. 1. Strict Control of Electron Flow by Internal pH

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ABSTRACT: The regulation by the proton gradient of the electron flow from water to ferricyanide was investigated in thylakoids extracted from lettuce leaves. When the transmembrane proton current was varied by an uncoupler or by the ATP synthase activity, a unique relationship was found between the rate of ferricyanide reduction and the proton gradient, restricted here to its ΔpH component. This behavior was conserved in CF_1-depleted thylakoids where the passive proton flow was varied by the concentration of an F_o inhibitor or by the concentration of an uncoupler after 100% inhibition of F_o. This shows that under our experimental conditions no direct proton transfer exists in steady state between the site of regulation of the redox chain and the ATPase. Studies at two different pH's indicate that the internal pH, and not the transmembrane pH difference, controls the electron transfer between PS2 and PS1. Modeling the data suggests that a single deprotonation step is kinetically limiting.

In energy-transducing systems, H^+ ions are translocated through the membrane by redox carriers, generating an electrochemical proton gradient, and then cross back through the membrane through the F_oF_1-ATPase, giving rise to ATP synthesis (Mitchell, 1961, 1977). Many other energetic processes are actually coupled to transmembrane proton fluxes. A question which has been debated for many years is whether the proton gradient is fully delocalized, i.e., if its magnitude is the same across all the domains of the membrane. In the other case, a "microchemiosmotic" scheme can be proposed to describe the energy-transducing processes [for reviews, see Westerhoff et al. (1984), Ferguson (1985), and Haraux (1985)]. Microchemiosmotic models were often invoked to explain anomalous or nonunique relationships between the proton gradient, $\Delta\tilde{\mu}_{H^+}$,[1] and phenomena that it is expected to strictly control. These phenomena were essentially (1) the steady-state redox flow, modulated by the membrane H^+ conductance (Padan & Rottenberg, 1973; Azzone et al., 1978a); (2) the "phosphate potential", ΔG_p, at equilibrium (Azzone et al., 1978b; Woelders et al., 1985), adjusted by the redox flow or by uncouplers; and (3) the steady-state rate of ATP synthesis (Casadio et al., 1978; Haraux et al., 1983; Mandolino et al., 1983; Sigalat et al., 1985; Zoratti & Petronilli, 1985; Petronilli et al., 1991), modulated by energy input or leaks. In some cases, however, these flux–force or force–force relationships were found to be consistent with the delocalized chemiosmotic model [Diolez & Moreau, 1985; Sorgato et al., 1985; Woelders et al., 1985, 1988; Davenport & McCarty, 1986; see also Cotton et al. (1987) for the case of transhydrogenase]. So the problem remains open.

Different modes of localized coupling have been imagined. In the most popular model, a proton loop directly links redox carriers and ATPases, more or less short-circuiting the aqueous compartments facing the membrane (Van Dam et al., 1978; Westerhoff et al., 1984). This is actually a moderate version of the "direct coupling" hypothesis (Williams, 1961, 1978). This mode of coupling requires a close contact between redox pumps and ATPases, the limiting case being that the two kinds of enzymes are embedded in the same supercomplex. A model not too far from this was especially proposed for thylakoid membranes. Mainly based on kinetic experiments, it postulates that a small proton pool, not equilibrating with the lumen, connects the internal sites of the redox carriers and of the ATPases (Dilley & Schreiber, 1984). Bound calcium and, in steady state, a high ATP synthase activity would maintain this pool operative (Chiang & Dilley, 1987; Renganathan et al., 1991). For our part, we have proposed an alternative model (Haraux & de Kouchkovsky, 1983), where the proton circulation from $\Delta\tilde{\mu}_{H^+}$ producers and consumers is somewhat limited by the conductance of the compartments delimited by the membrane [see also Hong and Junge (1983)]. At variance with the preceding views, the energetic coupling would thus generally not be optimal, its efficiency depending on the statistical distance between the redox carriers and the ATPases (Haraux et al., 1983; Sigalat et al., 1985). Although elaborated for the case of thylakoids, it could in principle be extrapolated to other biomembranes, provided one takes into account the specificity of each system and in the first place the geometrical properties of that model.

One of the most widely invoked arguments for a direct coupling between the redox and phosphorylating proton flows is that in some cases the thermokinetic control exerted on the electron transport seems not to be a strict function of the proton gradient. Indeed, it was sometimes found, in mitochondria, that the acceleration of the proton flow through the ATPase stimulates respiration more than an equivalent increase of membrane leaks (Padan & Rottenberg, 1973; Azzone et al., 1978a; O'Shea & Chappell, 1984). Other reports, however, have shown a strict control of the respiration rate by the electrochemical proton gradient (Diolez & Moreau, 1985; Woelders et al., 1986). Until now, this problem was not investigated in thylakoid membranes. So we have

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[1] Abbreviations: $\Delta\tilde{\mu}_{H^+}$, transmembrane difference in electrochemical potential of protons; $\Delta\psi$, transmembrane difference in electrical potential; ΔpH, transmembrane pH difference; pH_e or pH_i, external or internal pH; ΔG_p, Gibbs free energy of phosphorylation, or "phosphate potential"; J_e, rate of electron transfer; PS1 and PS2, photosystems 1 and 2; (C)F_o and (C)F_1, membranous and soluble, catalytic sectors of (chloroplast) F_oF_1-ATPase; BSA, bovine serum albumin; DCCD, *N,N'*-dicyclohexylcarbodiimide; EDTA, ethylenediaminetetraacetic acid; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; b_6f, cytochrome b_6f.

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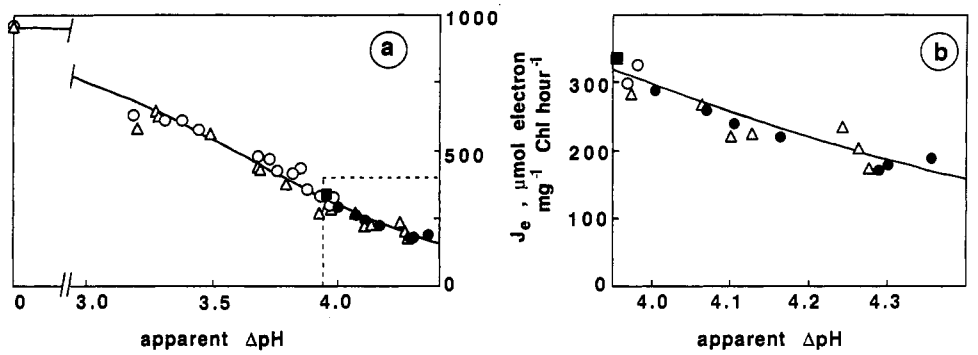

FIGURE 1: Rate of ferricyanide photoreduction (J_e) versus ΔpH in standard thylakoids. Conditions were as described under Materials and Methods, except that 20 mM KCl, 10 mM K_2HPO_4, and 250 nM valinomycin were present and pH was 8.2: (■) no venturicidin, no FCCP, highest phosphorylating activity; (●) variable venturicidin concentration (40–670 nM), no FCCP; (▲) 670 nM venturicidin, variable FCCP concentration (1–500 nM); (○) no venturicidin, variable FCCP concentration (1–500 nM). (a) All data; (b) enlarged area of overlapping data with 670 nM venturicidin and variable FCCP and with no FCCP but variable venturicidin [corresponding to data framed by the dashed rectangle in (a)]. Continuous line: theoretical curve according to $J_e = J_{max}/[1 + 10^{n(\Delta pH - \Delta pH_{0.5})}]$, with J_{max} = 952 μmol of electrons (mg of Chl)$^{-1}$ h^{-1}, n = 0.91, and $\Delta pH_{0.5}$ = 3.62. See text and Appendix for details.

examined, in this system, how $\Delta\tilde{\mu}_{H^+}$ controls the electron flow under different conditions. The results do not support the idea of a direct route for protons between redox pumps and ATP synthases. The photosynthetic redox chain is strictly controlled by the proton gradient, or more precisely by the internal pH.

MATERIALS AND METHODS

Extraction of Thylakoids. Green leaves of lettuce (*Lactuca sativa* L.) from a local market were chopped twice for a short time (less than 1 s) in medium containing 0.4 M sorbitol, 10 mM Tricine (pH 7.8), 10 mM NaCl, 20 μM BSA, and 40 mM sodium ascorbate. The crude extract was filtered through a nylon sieve (mesh size = 25 μm) and then centrifuged at 2000*g* for 10 min. The pellet was briefly resuspended in a hypotonic medium containing 10 mM Tricine (pH 7.8) and 10 mM NaCl and then recentrifuged at 2500*g* for 10 min. All these steps were carried out at 5 °C and under dim light. The new pellet was resuspended in the assay medium at a chlorophyll concentration of 1 mM, as determined by Mackinney's method (1941), and stored on ice and in darkness before use.

Ca^{2+}-Treated Thylakoids. When indicated, 1 mM $CaCl_2$ was added to the storage medium. In this case, thylakoids were incubated for at least 2 h, on ice and in darkness, before use.

EDTA-Treated Thylakoids. Crude extract of lettuce leaves was prepared and centrifuged as above. The pellet was resuspended for 3 min in a hypotonic medium containing 30 mM sorbitol, 2 mM Tricine (pH 7.8), and 1 mM EDTA and then centrifuged at 2500*g* for 10 min [adapted from Strotmann et al. (1973)]. The new pellet was titrated for chlorophyll content, diluted, and stored in the assay medium as previously.

Electron-Transfer and ΔpH Measurements. Native or EDTA-treated thylakoids were diluted to 10 μM chlorophyll concentration in an assay medium containing, unless otherwise stated, 0.2 M sorbitol, 10 mM Tricine (pH 7.8), 10 mM KCl, 6 mM $MgCl_2$, 2 mM K_2HPO_4, 10 μM diadenosine pentaphosphate (to inhibit possible adenylate kinase), 0.5 mM ADP, 100 nM valinomycin (to collapse $\Delta\psi$), 4 μM 9-aminoacridine, and 0.8 mM potassium ferricyanide. The 1.5-mL sample was stirred and thermostated at 20 °C in a 1 × 1-cm spectroscopic cuvette. Ferricyanide photoreduction was followed by the absorbance decrease at 420 nm, and the ΔpH was simultaneously monitored by the quenching of the 9-aminoacridine fluorescence in a setup described elsewhere (de Kouchkovsky et al., 1982). ΔpH was calculated according to the classical formula (Schuldiner et al., 1972), assuming an internal thylakoid volume of 8 L (mol of Chl)$^{-1}$. The intensity of the saturating red actinic light was 1.5 kW m^{-2}.

The rates vs ΔpH relationships were fitted using a nonlinear iterative method based on Marquart's algorithm (Enzfitter, Elsevier Biosoft).

ΔG_p and ΔpH Measurements. Thylakoids were assayed for ΔpH at 10 μM chlorophyll concentration in the same medium as above, except that 50 μM pyocyanine replaced ferricyanide, to ensure a cyclic electron flow around PS1, and ADP and phosphate concentrations were 30 μM and 0.3 mM, respectively. The sample was illuminated as previously described, and once the 9-aminoacridine fluorescence reached a constant level, small aliquots were taken up at different times for ATP titration. This was achieved in a laboratory-built luminometer (de Kouchkovsky et al., 1982), using the luciferin–luciferase technique (Lemasters & Hackenbrock, 1978). The time of illumination required to reach a constant ATP level was between 5 and 30 min, depending on the light intensity, which was adjusted with neutral-density filters. ΔG_p was computed from the amounts of formed ATP and remaining ADP and phosphate. The values of the Gibbs standard free energy, $\Delta G^{\circ\prime}$, used in this calculation were 30.7 kJ mol^{-1} at pH 7.8 and 32.8 kJ mol^{-1} at pH 8.2 (Rosing & Slater, 1972).

Reagents. All chemicals were of analytical grade. Venturicidin, valinomycin, diadenosine pentaphosphate, ADP, and ATP (disodium salt, less than 1% ADP) were purchased from Sigma, and FCCP was from Fluka. DCCD from Serva was purified according to Stevens et al. (1967). Venturicidin, DCCD, and FCCP were added to the thylakoid suspensions in small volumes of concentrated ethanolic solutions (no effect of ethanol alone was noticed). Pyocyanine was prepared from phenazine methosulfate (McIlwain, 1937).

RESULTS

Regulation of Electron Flow in Thylakoids. Figure 1 shows the relationship between the transmembrane pH difference and the rate of ferricyanide reduction. Starting from the control situation (filled square, Figure 1), i.e., the maximum rate of ATP synthesis, the proton flow through ATPases was progressively blocked by increasing concentrations of venturicidin, an inhibitor of the F_o moiety. Thus ΔpH increased and, as a consequence, electron flow decreased. Once the

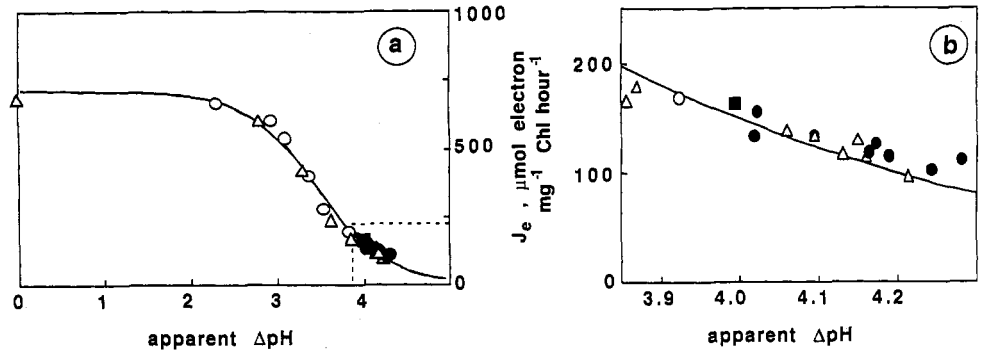

FIGURE 2: Rate of ferricyanide photoreduction (J_e) versus ΔpH in thylakoids incubated with calcium. Conditions were as described under Materials and Methods, and pH was 7.8: (■) no venturicidin, no FCCP, highest phosphorylating activity; (●) variable venturicidin concentration (20–670 nM), no FCCP; (▲) 670 nM venturicidin, variable FCCP concentration (70–2000 nM); (○) no venturicidin, variable FCCP concentration (0.1–2000 nM). (a) All data; (b) enlarged area of overlapping data corresponding to data framed by the dashed rectangle in (a). Continuous line: theoretical curve, drawn as in Figure 1, with J_{max} = 711 μmol of electrons (mg of Chl)$^{-1}$ h^{-1}, n = 1.06, and $\Delta pH_{0.5}$ = 3.46.

maximal ΔpH was reached, which was achieved with 0.67 μM venturicidin, the membrane was made leaky for protons by adding increasing concentrations of FCCP. As expected, ΔpH was decreased and the electron flow was accelerated. It is clear, especially by looking at Figure 1b, that a unique curve is followed, regardless of the way the membrane H^+ conductance was adjusted. So the control exerted by ΔpH does not depend on whether the protons flow through ATPases or artificial proton leaks. When ΔpH was depressed by FCCP below the value corresponding to the maximum rate of ATP synthesis, the proton flow was mainly due to leaks, venturicidin being present or not. In this ΔpH range (Figure 1a, open triangles and circles), the existence of a single flow–force relationship simply means that venturicidin has no inhibitory effect on the redox chain, a prerequisite for the validity of such an experiment.

The conditions of this experiment were slightly different from those previously published (Sigalat et al., 1985, 1988). KCl and valinomycin concentrations were 20 mM and 250 nM, respectively, instead of 10 mM and 100 nM, to be sure to fully dissipate Δψ. Also pH was 8.2 instead of 7.8, and phosphate concentration was 10 instead of 2 mM, to increase the contribution of the phosphorylating proton flux to the total transmembrane H^+ current. Under these new conditions, the coupled electron flow (control) represented 35% of the maximum rate, and the minimum electron flow (+venturicidin), around 17%. With the previous conditions, we also have obtained a single flow–force relationship, as in Figure 1, but the coupled electron flow represented only 25% of the maximum, whereas the relative value of minimal electron flow was unchanged (not shown). In the next experiments, where the contribution of the phosphorylating proton flow is not an essential problem, we have used standard conditions to allow cross-checking with previous conditions.

Regulation of Electron Flow in Ca^{2+}-Treated Thylakoids. From the data of Figure 1, no privileged interaction between the site of coupling of the redox chain and the ATPase could be detected. According to Chiang and Dilley (1987), the presence of calcium in the storage medium is a key factor to observing a completely localized mode of coupling in thylakoids. We have thus performed an experiment similar to that reported in Figure 1, but using thylakoids stored for at least 2 h in the presence of 1 mM $CaCl_2$. The results displayed in Figure 2 show that the electron flow is controlled regardless of the way ΔpH is adjusted, as in Figure 1.

Regulation of Electron Flow in EDTA-Treated Membranes. Although the electron flow is 2 times greater under phosphorylating conditions than with saturating venturicidin

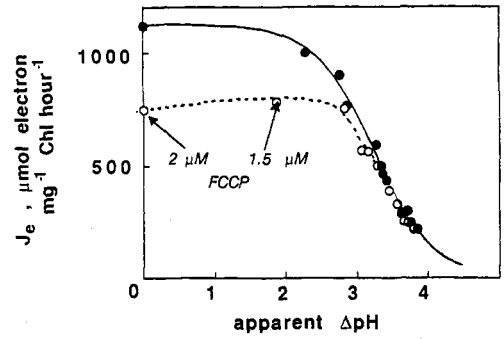

FIGURE 3: Rate of ferricyanide photoreduction (J_e) versus ΔpH in EDTA-treated thylakoids. Conditions were as described under Materials and Methods: (●) variable venturicidin concentration (0–670 nM), no FCCP; (○) 670 nM venturicidin, variable FCCP concentration (1–2000 nM). The two highest FCCP concentrations are indicated by arrows. Continuous line: theoretical curve, drawn as in Figure 1, with J_{max} = 1123 μmol of electrons (mg of Chl)$^{-1}$ h^{-1}, n = 1.05, and $\Delta pH_{0.5}$ = 3.24.

(Figure 1), one might not consider this fact as sufficient to demonstrate that the proton gradient exerts strict control on the electron flow in all circumstances. Indeed, under phosphorylating conditions, the redox chain runs only at one-third of its maximum rate (Figure 1a). So it would be interesting to increase the proton flux through the ATPases with respect to the proton leaks. Unfortunately, this is not possible for the phosphorylating flow itself, but one can strongly increase the H^+ flow through F_o, the membranous sector of the ATPase, by removing the F_1 moiety. This was achieved by incubating the membranes with EDTA. After such a treatment, the same experiments as in Figures 1 and 2 were carried out, with the results shown in Figure 3. The main difference is that we now started with a negligible ΔpH, allowing the uncoupled redox chain to run at its maximum rate. When venturicidin was added, we could scan a very large range of ΔpH, from about 0 to almost 4, and of electron flow, which could be reduced by approximately 80%. When the membrane conductance was increased again by adding FCCP at concentrations below a few hundredths nanomolar, the same flow–force curve was followed, in the opposite direction. At higher FCCP concentrations, the two curves seriously diverged. However, this divergence had nothing to do with localized H^+ currents. Indeed, Figure 4 shows that when the FCCP concentration is further increased, in the absence of venturicidin, the electron transfer decreases in a monotonous way. If one corrects the data of Figure 3 for this inhibitory effect, the two flow–force curves become identical within the entire ΔpH range.

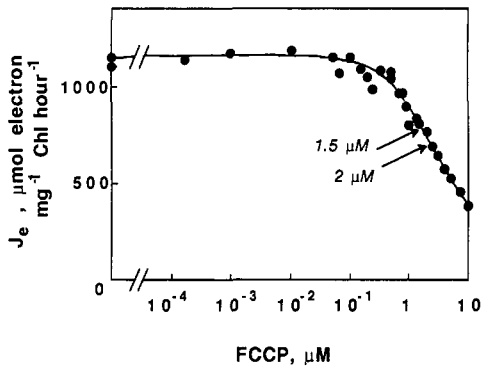

FIGURE 4: Rate of ferricyanide photoreduction (J_e) as a function of FCCP concentration in EDTA-treated thylakoids. The two FCCP concentrations mentioned in Figure 3 are indicated by arrows. Conditions were as in Figure 3, but without venturicidin.

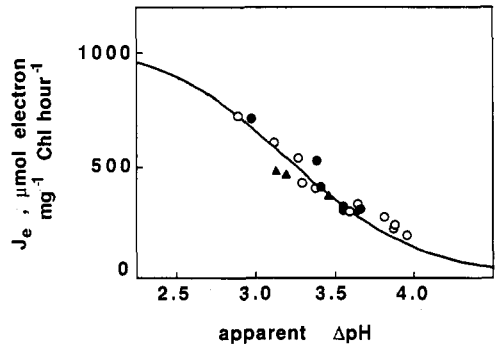

FIGURE 5: Rate of ferricyanide photoreduction (J_e) versus ΔpH in EDTA-treated thylakoids. Conditions were as in Figure 3: (●) variable DCCD concentration (1–25 μM), no FCCP; (▲) variable DCCD concentration (30–60 μM), no FCCP; (○) 25 μM DCCD, variable FCCP concentration (1–2000 nM). The theoretical curve was drawn as in Figure 1, with J_{max} = 1056 μmol of electrons (mg of Chl)$^{-1}$ h^{-1}, n = 1.04, and $\Delta pH_{0.5}$ = 3.21.

The same experiment could be performed by replacing venturicidin by DCCD, the most classical F_o inhibitor. Figure 5 shows the results of such an experiment carried out on EDTA-treated membranes. They are comparable to the results obtained in Figure 3 in an identical ΔpH range, and thus the same conclusion has to be drawn. In addition, one can notice that in this experiment DCCD had no significant inhibitory effect on the electron transfer itself, at variance with a previous report (Sane et al., 1979). However, between 30 and 60 μM, it decreased ΔpH and increased J_e, i.e., it had the opposite effect than at lower concentrations, but the effect always followed the same flow–force curve. This is a simple uncoupling effect.

Regulation of Electron Flow at Two Different pH's. An important question for understanding the functioning of the redox chain is to determine if the rate of the coupled electron transfer is controlled by ΔpH, internal pH, or a complex combination of external and internal pH. The general problem of the pH dependency of proton pumps, and its mechanistic implications, has been discussed in detail (Hansen et al., 1981). Regarding the photosynthetic redox chain, with its two photosystems, it was very early assumed to be controlled by internal pH, and the pH dependence of the fully uncoupled electron flow was proposed to be used to calibrate the internal pH under coupled conditions (Rumberg & Siggel, 1969). When ΔpH probes became available (Schuldiner et al., 1972), a complex study made at different pH's suggested that the redox chain in thylakoids is controlled by a pH intermediate between pH_e and pH_i. This led to imagining the regulating site embedded in the membrane (Bamberger et al., 1973).

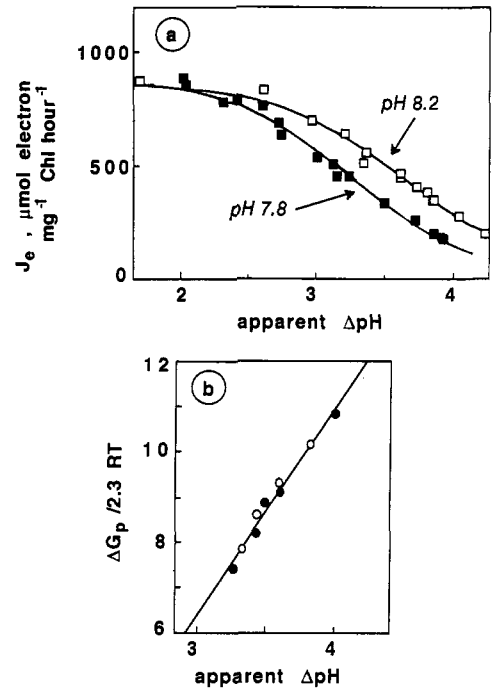

FIGURE 6: Rate of ferricyanide photoreduction, J_e (a), and "static head" ΔG_p (b) versus ΔpH with standard thylakoids at two different pH's. Conditions were as described under Materials and Methods for each experiment, except that external pH was adjusted to 7.8 (■, ●) or 8.2 (□, ○). Experiments a and b were performed with two different thylakoid preparations: (a) variable FCCP concentration (20–2000 nM) and 0.67 μM venturicidin; (b) variable light intensity (3–15 W m^{-2}), no venturicidin. Theoretical curves in (a) were drawn as in Figure 1, with J_{max} = 890 μmol of electrons (mg of Chl)$^{-1}$ h^{-1}, n = 0.94, and $\Delta pH_{0.5}$ = 3.25 at external pH 7.8, and J_{max} = 864 μmol of electrons (mg of Chl)$^{-1}$ h^{-1}, n = 0.91, and $\Delta pH_{0.5}$ = 3.65 at external pH 8.2. Data in (b) were fitted with the linear relation $\Delta G_p/2.3RT$ = 4.57ΔpH −7.32.

Further experiments favored an electron transfer regulated by pH on the two sides of the membrane, with optimal values for external and internal pH (Schönfeld & Schickler, 1989). On the other hand, the limiting step of the cyclic electron transfer in chromatophores of *Chromatium vinosum* was found to be pH_i-dependent (Hashimoto & Nishimura, 1979). To clarify the situation, we have thus investigated the ΔpH dependency of the electron flow at two pH's routinely used in our experiments, i.e., pH 7.8 and 8.2. The experiment was performed on standard thylakoids (not CF_1 depleted), with variable FCCP, and the results are displayed in Figure 6a. Compared to that obtained at pH 7.8, the flow–force curve drawn at pH 8.2 is translated to the higher ΔpH values by exactly 0.4 pH unit, and the maximum rate of electron transfer is not modified. This shows that the rate of electron transfer is actually controlled by internal pH, and not by ΔpH.

However, to be sure that this result is not due to some pH dependency of the 9-aminoacridine response, as may occur under some experimental conditions (Bizouarn et al., 1991), we have also established, at pH 7.8 and 8.2, the relationship in "state 4" (Chance & Williams, 1955) between the apparent ΔpH and the phosphate potential, ΔG_p. In this "static head" situation of equilibrium between ATP synthesis and hydrolysis, ΔG_p indeed is expected to be a strict function of $\Delta\tilde{\mu}_{H^+}$, i.e., $\Delta G_p = q\Delta\tilde{\mu}_{H^+}$, where q is the mechanistic H^+/ATP stoichiometry. When $\Delta\psi = 0$ (our conditions), $\Delta G_p = 2.3qRT\Delta pH$, where R and T have their usual meaning. This relationship was even used in some cases to calibrate the probe (Strotmann & Lohse, 1988; Bizouarn et al., 1991). Figure 6b shows the results of an experiment where ΔpH and ΔG_p, measured in

Table I: Kinetic Parameters of pH_i-Regulated Electron Transport from Water to Ferricyanide, Determined in Various Conditions with 12 Different Membrane Preparations at Two External pH's[a]

pH_e	EDTA treatment	special conditions	n	$\Delta pH_{0.5}$	J_{max}, μmol of electrons (mg of Chl)$^{-1}$ h^{-1}
7.8	yes	DCCD instead of venturicidin	1.04 ± 0.20	3.21 ± 0.07	1056 ± 52
		standard	1.05 ± 0.06	3.24 ± 0.03	1123 ± 22
			1.37 ± 0.05	3.15 ± 0.01	1340 ± 14
			1.28 ± 0.15	3.24 ± 0.04	1532 ± 40
	no	Ca^{2+}-treated	1.06 ± 0.05	3.46 ± 0.03	711 ± 18
		standard	1.09 ± 0.05	3.37 ± 0.03	768 ± 21
			0.99 ± 0.08	3.10 ± 0.05	723 ± 25
			0.94 ± 0.06	3.25 ± 0.03	890 ± 19[b]
			0.91 ± 0.09	3.65 ± 0.04	864 ● 28[b]
8.2	no	KCl, 20 mM,	0.91 ± 0.03	3.62 ± 0.02	952 ± 19
		K_2HPO_4, 10 mM, and	1.40 ± 0.10	3.75 ± 0.03	916 ± 23
		valinomycin, 250 mM	1.31 ● 0.08	3.70 ± 0.02	944 ± 19
			1.15 ± 0.07	3.66 ± 0.03	832 ± 16

[a] Standard conditions were as described under Materials and Methods. Data were fitted with the equation $J_e = J_{max}/[1 + 10^{n(\Delta pH - \Delta pH_{0.5})}]$; one may also calculate $pH_{0.5}$ as $pH_e - \Delta pH_{0.5}$ (see Appendix). With native thylakoids, all data were considered to calculate the kinetic parameters, with or without venturicidin (all data points in Figure 1). With EDTA-treated membranes, where FCCP was inhibitor, only data gathered under conditions of variable venturicidin concentration were taken into account (● in Figure 3). [b] These values were derived from the same thylakoid preparation.

state 4, were varied by the light input. A unique relationship was then found, at pH 7.8 and 8.2, between ΔpH, as probed by the 9-aminoacridine fluorescence quenching, and ΔG_p. This proves that, in the present medium and at these concentrations of probe and membranes, external pH has no effect on the response of 9-aminoacridine, thus confirming the validity of the results of Figure 6a.

Modeling the Flow–Force Relationship. Sophisticated models have been proposed to describe the steady-state functioning of the redox chain [by Siggel (1976) for thylakoids]. We have developed in the Appendix a simplified model, based on reasonable approximations, to account for the regulatory effect of external and internal pH on the electron flow. This gives a very simple relationship between pH_i and the rate of electron transfer, J_e: $J_e = J_{max}/[1 + 10^{pH_{0.5}-pH_i}]$, where J_{max}, the maximal rate of electron transfer, is a function of the light input and of the kinetic constants of different reaction steps, including the diffusion of mobile carriers. $pH_{0.5}$ is the internal pH which inhibits half of the reaction rate, so it is a kind of operational pK. However, this $pH_{0.5}$ depends not only on the pK of the quinones at the internal site of b_6f but also on the same parameters as J_{max}. We could fit all our data by this model, in a very satisfactory way (see Figures 1–3, 5, and 6). Although the process is strictly pH_i-dependent, we have preferred, for the sake of simplicity, to express J_e as a function of ΔpH: $J_e = J_{max}/[1 + 10^{n(\Delta pH - \Delta pH_{0.5})}]$, where $\Delta pH_{0.5} = pH_e - pH_{0.5}$. To evaluate better the validity of the model, we have chosen not to fix the value of n (theoretically equal to 1, as in the first equation above), which was then determined by the fitting procedure. Table I summarizes the parameters obtained in a number of experiments carried out on standard or EDTA-treated thylakoids.

First, one should notice that n, the number of protons involved in the limiting step(s), is found very close to the theoretical value of 1 (1.12 ± 0.18, all conditions). Second, the results of Figure 6, i.e., the strict pH_i dependence of the redox flow, is statistically confirmed: (1) J_{max} is approximately the same at external pH 7.8 and 8.2 (794 ± 86 and 901 ± 52 μmol of electrons (mg of Chl)$^{-1}$, respectively, for standard thylakoids). (2) Close values of $pH_{0.5}$ are found at pH 7.8 ($pH_{0.5}$ = 4.58) and 8.2 ($pH_{0.5}$ = 4.52); that is, $\Delta pH_{0.5}$ = 3.22 ± 0.09 at pH 7.8 (standard and EDTA-treated thylakoids), and $\Delta pH_{0.5}$ = 3.68 ± 0.05 at pH 8.2. (3) Even though the maximal rate of electron transfer is about 50% higher with EDTA-treated material than in standard thylakoids, $pH_{0.5}$ still does not significantly differ in the two kinds of preparation (4.59 ± 0.04 and 4.54 ± 0.08, respectively).

The ΔpH values presented here were computed according to the simple model of Schuldiner et al. (1972), which does not take into account the probe–membrane interactions [not to mention the problem of the determination of the lumen volume, and the seasonal factor: see Sigalat (1988)]. This has surely affected the values of ΔpH, and thus the scale units of the figures are only relative; the same is probably true for $pH_{0.5}$, and maybe also for n. In principle, the data of Figure 6b, which represents the state 4 equilibrium between the apparent ΔpH and the phosphate potential, ΔG_p, could be used to correct the ΔpH values, provided the H^+/ATP stoichiometry is known. Indeed, at equilibrium, ΔG_p is related to the true value of ΔpH by the equation $\Delta G_p/2.3RTq$ = (true ΔpH), where q is the H^+/ATP stoichiometry. The best estimates of this stoichiometry are between 3 (Rathenow & Rumberg, 1980; Davenport & McCarty, 1984; Strotmann & Lohse, 1988) and 4 (Rumberg, 1990). Thus, with ΔG_p in kJ mol^{-1}, (true ΔpH) = $\Delta G_p/16.8$ or $\Delta G_p/22.4$ at 20 °C for q = 3 or 4, respectively. From the experimental linear relation of Figure 6b, replacing $\Delta G_p/2.3RTq$ by (true ΔpH), the ΔpH value should be corrected according to the formula (true ΔpH) = 1.50(apparent ΔpH) − 2.44 if q = 3 or (true ΔpH) = 1.13(apparent ΔpH) − 1.83 if q = 4. The same formula applies for apparent and true $\Delta pH_{0.5}$, from which one draws $pH_{0.5} = pH_e - \Delta pH_{0.5}$. The number of protons, n, involved in the regulatory step also has to be corrected for the difference between the slopes of apparent and true ΔpH. One has (true n) = (apparent n)/1.50 if q = 3 or (true n) = (apparent n)/1.13 if q = 4. The first correction, assuming H^+/ATP = 3, then gives n = 0.75 and $pH_{0.5}$ = 5.39, instead of 1.12 and 4.55. The second correction, with H^+/ATP = 4, gives n = 0.99 and $pH_{0.5}$ = 5.98. In our ΔG_p calculations, we have taken the value of ΔG_o from the most classical reference (Rosing & Slater, 1972), but the recently reevaluated values of ΔG_o (Krab & Van Wezel, 1992) should introduce only very minor changes.

DISCUSSION

Unique Flow–Force Relationship: Principle. It was claimed that the relationship between the electron flow and the $\Delta\tilde{\mu}_{H^+}$ should always depend on the nature of the downhill proton flux, even in the case of a delocalized proton gradient (O'Shea & Chappell, 1984). This troublesome proposal was

based on the fact that the proton backflow, which exactly balances the redox pump activity in steady state, is the product of $\Delta\tilde{\mu}_{H^+}$ and the membrane conductance (generalized Ohm law). The membrane conductance itself would be a function of the $\Delta\tilde{\mu}_{H^+}$, which depends in addition on the nature of the leaks: ionophore, ATPase, etc. So no univocal relationship should be expected between the electron flow and the $\Delta\tilde{\mu}_{H^+}$. This reasoning is actually wrong, since it is incomplete: it neglects the fact that if the same $\Delta\tilde{\mu}_{H^+}$ can be obtained with a protonophore or with a proton flow through F_oF_1, this is precisely because the proton gradient was adjusted at the unique value where the two kinds of conductances are the same [see Ghazi (1985) for a serious discussion of this problem]. So the principle used here is fully valid to check the delocalized character of the proton gradient. Of course, it is no more true if some of the conditions used to vary the proton gradient have an effect on the redox chain itself (Rigoulet, 1990). This kind of additional regulation, for example, by nucleotides, seems more probable in intact mitochondria, which are relatively complex systems, than in washed thylakoids.

Unique Flow–Force Relationship: Reliability of Measurements. At a given external pH, the electron flow in thylakoid membranes is strictly controlled by ΔpH regardless of whether the protons leave the internal compartment through F_o or F_oF_1 or via artificial proton leaks. This demonstrates that in thylakoids no privileged proton circuit links the regulatory site of the redox chain, here the cytochrome b_6f, and the ATPase. Until now, this question was essentially examined in mitochondria, with variable results. A strict control of the redox flow by the membrane potential, $\Delta\psi$, was observed in mitochondria extracted from potato tuber (Diolez & Moreau, 1985) or rat liver (Woelders et al., 1986), in the absence of any ΔpH. On the contrary, Azzone and co-workers (1978) found in rat liver mitochondria that, for an identical decrease of $\Delta\psi$, the reaction of phosphorylation stimulated respiration more than partial uncoupling by FCCP. But the same authors have also shown that the distribution of cationic probes could depend not only on $\Delta\psi$ but also on the ionophore used to decrease the membrane potential, especially since ionic probes do not obey the Nernst equilibrium when they cross the membrane in a neutral form or against another ion. Additional problems may arise from the simultaneous presence of ΔpH and $\Delta\psi$, these two components being measured by different techniques, on separate samples. Moreover, they are not expected to control the redox flow in an identical way. The weight of membrane–probe interactions in the results was emphasized by Woelders et al. (1988), who have found in mitochondria a unique relationship between $\Delta\psi$ and the rate of ATP synthesis when the former was monitored by K^+ distribution, but not when it was estimated with an organic cation. Lastly, one should mention that the heterogeneity of vesicles, in size and in activity, may be a source of errors in this kind of semiquantitative study (Duszyński & Wojtczak, 1985).

None of these problems occurred in our experiments, but Figures 3 and 4 show a good example of another kind of difficulty which may be encountered. In Figure 3, the flow–force curves obtained with variable venturicidin and variable FCCP diverge for the lower ΔpH, which is solely due to an inhibitory effect of FCCP (Figure 4) and not to any localized proton pathway. The inhibitory effect of FCCP and other uncouplers on PS2 activity is well documented (Renger, 1969; Harth et al., 1974; Johnson et al., 1983). It is particularly clear with EDTA-treated thylakoids (Figure 4). Such inhibitory effects cannot be excluded in various systems. Unfortunately, in many studies (Padan & Rottenberg, 1973; Azzone et al., 1978a; O'Shea & Chappell, 1984; Rigoulet, 1990), a very limited range of activities was investigated, and the maximal rate of electron transfer, at high uncoupler concentrations, was almost never reached. This makes it impossible to check a possible inhibitory effect of uncouplers.

Regulation of Electron Flow by Internal pH and Related Problems. Previous reports using different approaches indicated a linear electron flow regulated by the internal pH (Rumberg & Siggel, 1969; Tikhonov et al., 1981). Another report suggested regulation by a pH intermediate between pH_e and pH_i (Bamberger et al., 1973), but to reach this conclusion, correlations between the electron flow and the pH (internal or external) were established with data obtained at different levels of light energy input, which is meaningless in this context (see Appendix). Another group found a complex relationship between the rate of light-saturated electron transfer and the pH on both sides of the membrane (Schönfeld & Schickler, 1989). At variance with our results, an inhibitory effect of high pH was observed, but gramicidin was there used instead of FCCP. To fit their data, these authors had to use an empirical model involving 4 pK values.

The data presented here (Figure 6 and Table I) clearly indicate that the electron transfer between PS2 and PS1 is regulated by internal pH. In accordance with Rumberg and Siggel (1969), but unlike others (Bamberger et al., 1973; Schönfeld & Schickler, 1989), we have obtained a rate vs pH_i relationship following a simple titration law with a unique apparent pK and a number of protons, n, close to 1: as an average (Table I), n = 1.12, without correction of the 9-aminoacridine signal. If the probe is calibrated by static head ΔG_p, n = 0.75 or 0.99, depending on the assumed H^+/ATP stoichiometry. Concerning the mid-pH of the regulation process (=$pH_{0.5}$), its average value would respectively be 4.6, 5.4, or 6.0, depending on the same choices for ΔpH estimation. On a different basis, a value of approximately 5.5 was proposed for $pH_{0.5}$ (Siggel, 1976). As explicated in the Appendix, $pH_{0.5}$ depends on the pK of the regulatory deprotonation steps but also on diffusional processes and on the extent of energy input. This control exerted by the internal pH is purely kinetic; due to the quasi-irreversibility of the photochemical steps, the $\Delta\tilde{\mu}_{H^+}$ which would be theoretically necessary to block fully the redox chain is very high.

Localized Proton Gradient and Membrane Topography. The inhibition of the electron flow by a higher uncoupler concentration, observed here with EDTA-treated membranes (Figures 3 and 4), was sometimes interpreted as the dissipation of a restricted proton pool protecting PS2 against deactivation (Theg & Homann, 1982; Johnson et al., 1983). Except the inhibition of the uncoupled electron flow, this proton pool did not seem to play a significant role in our experiments. No localized proton pathway could indeed be detected in this work. This does not mean, however, that the lumen space is isopotential for protons. Using similar thylakoid preparations and experimental conditions, we have consistently found that the rate of photophosphorylation was not a univocal function of the $\Delta\tilde{\mu}_{H^+}$, and we have interpreted this fact in a microchemiosmotic way (Haraux et al., 1983; Sigalat et al., 1985). This could seem contradictory with the present report, but we have always thought that the redox carriers and the phosphorylating enzymes, which are located in distinct microenvironments and which pump protons in opposite directions, sense different local values of the proton gradient (Haraux & de Kouchkovsky, 1983; de Kouchkovsky et al., 1984; Haraux,

1985). It is then possible that the ΔpH at the b_6f level is somewhat higher than in other domains of the membrane. However, due to its random distribution in the granal and agranal region of the membrane (Albertsson et al., 1991), this complex would not have any privileged position with respect to F_oF_1-ATPase. So the local ΔpH at the b_6f level would be affected in the same way by proton flow through the ATPases or via artificial leaks. Such a situation could be called "randomized" rather than "delocalized", even though these two cases are not experimentally discriminable. The problem of the local ΔpH across the ATPase is different and will be treated in depth in the forthcoming paper.

APPENDIX

Relationship between Rate of Electron Transfer and External and Internal pH. The linear electron transfer between PS2 and PS1 can be split into the following elementary steps (the possible existence of a Q-cycle has not been considered, but has no importance here):

(*1*) *Photochemical Steps.* $A_{ox} \leftrightarrow A_{red}$, reduction of the acceptor side of PS2 (pheophytin or primary quinone Q_A); and $B_{red} \leftrightarrow B_{ox}$, oxidation of the donor side of PS1 (P_{700}, plastocyanin, or even cytochrome *f*). The different electron carriers downstream of the b_6f complex are considered as a single group for the sake of simplicity, because no pH-dependent reaction is present here. The two "photochemical" reactions are supposed to be at quasiequilibrium, with $A_{red} \gg A_{ox}$ and $B_{ox} \gg B_{red}$ (saturating light). A double-protonated oxidized quinone (QH_2^{2+}) is an improbable species here.

(*2*) *Reduction of Quinones on the External Side of the Membrane.* Two consecutive steps:

$$A_{red} + Q_e \xrightarrow{k_a} A_{ox} + Q_e^-$$

$$A_{red} + Q_e^- \xrightarrow{k'_a} A_{ox} + Q_e^{2-}$$

where k_a and k'_a are second-order rate constants, in $M^{-1}\ s^{-1}$. Both steps are considered as irreversible for the sake of simplicity.

(*3*) *External Protonation of Quinols.* Two consecutive steps:

$$Q_e^{2-} + H_e^+ \xrightarrow{K_e} QH_e^-$$

$$QH_e^- + H_e^+ \xleftrightarrow{K'_e} QH_{2e}$$

K_e and K'_e are acid dissociation constants, in M.

(*4*) *Oxidation of Quinol on the Internal Side of the Membrane.*

$$B_{ox} + QH_{2i} \xrightarrow{k_b} B_{red} + QH_{2i}^+$$

This step is considered as irreversible. k_b is a second-order rate constant.

(*5*) *Internal Deprotonation of Semiquinone.*

$$QH_{2i}^+ \xleftrightarrow{K_i} QH_i + H_i^+$$

K_i is an acid dissociation constant.

(*6*) *Oxidation of Semiquinone on the Internal Side of the Membrane.*

$$B_{ox} + QH_i \xrightarrow{k'_b} B_{red} + QH_i^+$$

This step is considered as irreversible. k'_b is a second-order rate constant.

(*7*) *Internal Deprotonation of Quinone.*

$$QH_i^+ \xleftrightarrow{K'_i} Q_i + H_i^+$$

K'_i is an acid dissociation constant.

(*8*) *Diffusion of Quinol from PS2* to b_6f *Complex and of Quinone from* b_6f *Complex to PS2*

$$QH_{2e} \underset{k}{\overset{k}{\rightleftarrows}} QH_{2i}$$

$$Q_i \underset{k}{\overset{k}{\rightleftarrows}} Q_e$$

where k is a diffusion constant, expressed here in s^{-1}.

The index e refers to the external face of the membrane; i, to the internal face. To simplify, one assumes that the membrane consists of two equal domains, internal and external, with two different quinone pools, exchanging their molecules at the rate constant k (different diffusion constants for QH_2 and Q should not change the result). Lateral transfer between PS2 and b_6f is of course included in this process.

The basic equations to solve the system are the following:

$$[Q_t] = [Q_e] + [Q_e^-] + [Q_e^{2-}] + [QH_e^-] + [QH_{2e}] + [QH_{2i}] + [QH_{2i}^+] + [QH_i] + [QH_i^+] + [Q_i]$$

where $[Q_t]$ is the total quinone concentration in the membrane, in M;

$$J_e = k_a[A_{red}][Q_e] = k'_a[A_{red}][Q_e^-] = k_b[B_{ox}][QH_{2i}] = k'_b[B_{ox}][QH_i] = k([QH_{2e}] - [QH_{2i}]) = k([Q_i] - [Q_e])$$

where J_e is the steady-state rate of electron transfer, in $M\ s^{-1}$; and

$$K_e = \frac{[Q_e^{2-}][H_e^+]}{[QH_e^-]},\quad K'_e = \frac{[QH_e^-][H_e^+]}{[QH_{2e}]},\quad K_i = \frac{[QH_i][H_i^+]}{[QH_{2i}^+]},\quad \text{and}\quad K'_i = \frac{[Q_i][H_i^+]}{[QH_i^+]}$$

the classical acid–base equilibria.

After rearrangement, one obtains the following equation:

$$\frac{[Q_t]}{J_e} = T + \left(\frac{1}{k_b[B_{ox}]} + \frac{1}{k}\right)\frac{K'_e}{[H_e^+]}\left(1 + \frac{K_e}{[H_e^+]}\right) + \left[\frac{1}{K'_i}\left(\frac{1}{k_a[A_{red}]} + \frac{1}{k}\right) + \frac{1}{K_i k'_b[B_{ox}]}\right][H_i^+]$$

with

$$T = \left(\frac{2}{k_a} + \frac{1}{k'_a}\right)\frac{1}{[A_{red}]} + \left(\frac{2}{k_b} + \frac{1}{k'_b}\right)\frac{1}{[B_{ox}]} + \frac{2}{k}$$

which does not depend on pH. J_e is *a priori* a function of pH_e and pH_i, except if $pK_e \gg pH_e$ and $pK'_e \gg pH_e$, which means that the external protonation of quinone is not kinetically

limiting. In this case, one obtains

$$\frac{[Q_t]}{J_e} = \left(\frac{2}{k_a} + \frac{1}{k'_a}\right)\frac{1}{[A_{red}]} + \left(\frac{2}{k_b} + \frac{1}{k'_b}\right)\frac{1}{[B_{ox}]} + \frac{2}{k} + \left[\frac{1}{K'_i}\left(\frac{1}{k_a[A_{red}]} + \frac{1}{k}\right) + \frac{1}{K_i k'_b[B_{ox}]}\right][H_i^+]$$

which may be written as

$$J_e = J_{max}/[1 + 10^{pH_{0.5}-pH_i}]$$

with

$$J_{max} = \frac{[Q_t]}{\left(\frac{2}{k_a} + \frac{1}{k'_a}\right)\frac{1}{[A_{red}]} + \left(\frac{2}{k_b} + \frac{1}{k'_b}\right)\frac{1}{[B_{ox}]} + \frac{2}{k}}$$

and

$$pH_{0.5} = \log\left[10^{pK'_i}\left(\frac{1}{k_a[A_{red}]} + \frac{1}{k}\right) + \frac{10^{pK_i}}{k'_b[B_{ox}]}\right]$$

J_{max} and $pH_{0.5}$ depend on the energy input (*via* $[A_{red}]$ and $[B_{ox}]$) and on a diffusion step (*via* k).

REFERENCES

Albertsson, P. Å., Andreasson, E., Svensson, P., & Yu, S. G. (1991) *Biochim. Biophys. Acta 1098*, 90–94.

Azzone, G. F., Pozzan, T., Massari, S., & Bragadin, M. (1978a) *Biochim. Biophys. Acta 501*, 296–306.

Azzone, G. F., Pozzan, T., & Massari, S. (1978b) *Biochim. Biophys. Acta 501*, 307–316.

Bamberger, E. S., Rottenberg, H., & Avron, M. (1973) *Eur. J. Biochem. 34*, 557–563.

Bizouarn, T., de Kouchkovsky, Y., & Haraux, F. (1991) *Biochemistry 30*, 6847–6853.

Casadio, R., Baccarini-Melandri, A., & Melandri, B. A. (1978) *FEBS Lett. 87*, 323–328.

Chance, B., & Williams, G. R. (1955) *J. Biol. Chem. 217*, 409–427.

Chiang, G., & Dilley, R. A. (1987) *Biochemistry 26*, 4911–4916.

Cotton, N. P. J., Myatt, J. F., & Jackson, J. B. (1987) *FEBS Lett. 219*, 88–92.

Davenport, J. W., & McCarty, R. E. (1984) *Biochim. Biophys. Acta 766*, 363–374.

Davenport, J. W., & McCarty, R. E. (1986) *Biochim. Biophys. Acta 851*, 136–145.

de Kouchkovsky, Y., Haraux, F., & Sigalat, C. (1982) *FEBS Lett. 139*, 245–249.

de Kouchkovsky, Y., Haraux, F., & Sigalat, C. (1984) *Bioelectrochem. Bioenerg. 13*, 143–162.

Dilley, R. A., & Schreiber, U. (1984) *J. Bioenerg. Biomembr. 16*, 173–193.

Diolez, P., & Moreau, F. (1985) *Biochim. Biophys. Acta 806*, 56–63.

Duszyński, J., & Wojtczak, L. (1985) *FEBS Lett. 182*, 243–248.

Ferguson, S. J. (1985) *Biochim. Biophys. Acta 811*, 47–95.

Ghazi, A. (1985) *Biochem. J. 229*, 833–837.

Hansen, U. P., Gradmann, D., Sanders, D., & Slayman, C. L. (1981) *J. Membr. Biol. 63*, 165–190.

Haraux, F. (1985) *Physiol. Veg. 23*, 397–410.

Haraux, F., & de Kouchkovsky, Y. (1983) *Physiol. Veg. 21*, 563–576.

Haraux, F., Sigalat, C., Moreau, A., & de Kouchkovsky, Y. (1983) *FEBS Lett. 155*, 248–252.

Harth, E., Reimer, S., & Trebst, A. (1974) *FEBS Lett. 42*, 165–168.

Hashimoto, K., & Nishimura, M. (1979) *J. Biochem. 85*, 57–64.

Hong, Y. Q., & Junge, W. (1983) *Biochim. Biophys. Acta 722*, 197–208.

Johnson, J. D., Pfister, V. R., & Homann, P. H. (1983) *Biochim. Biophys. Acta 723*, 256–265.

Krab, K., & Van Wezel, J. (1992) *Biochim. Biophys. Acta 1098*, 172–176.

Lemasters, J. L., & Hackenbrock, C. R. (1978) *Methods Enzymol. 57*, 36–50.

Mackinney, G. (1941) *J. Biol. Chem. 140*, 315–322.

Mandolino, G., De Santis, A., & Melandri, B. A. (1983) *Biochim. Biophys. Acta 723*, 428–439.

McIlwain, H. (1937) *J. Chem. Soc. 2*, 1704–1711.

Mitchell, P. (1961) *Nature 191*, 144–148.

Mitchell, P. (1977) *FEBS Lett. 78*, 1–20.

O'Shea, P. S., & Chappell, J. B. (1984) *Biochem. J. 219*, 401–404.

Padan, E., & Rottenberg, H. (1973) *Eur. J. Biochem. 40*, 431–437.

Petronilli, V., Persson, B., Zoratti, M., Rydström, J., & Azzone, G. F. (1991) *Biochim. Biophys. Acta 1058*, 297–303.

Rathenow, M., & Rumberg, B. (1980) *Ber. Bunsen-Ges. Phys. Chem. 84*, 1059–1062.

Renganathan, M., Pan, R. S., Ewy, R. G., Theg, S. M., Allnutt, F. C. T., & Dilley, R. A. (1991) *Biochim. Biophys. Acta 1059*, 16–27.

Renger, G. (1969) *Naturwissenschaften 56*, 370.

Rigoulet, M. (1990) *Biochim. Biophys. Acta 1018*, 185–189.

Rosing, J., & Slater, E. C. (1972) *Biochim. Biophys. Acta 267*, 275–290.

Rumberg, B., & Siggel, U. (1969) *Naturwissenschaften 56*, 130–132.

Rumberg, B., Schubert, K., Strelow, F., & Tran-Anh, T. (1990) in *Current Research in Photosynthesis* (Baltscheffsky, M., Ed.) Vol. III, pp 125–128. Kluwer Academic Publishers, The Netherlands.

Sane, P. V., Johanningmeier, U., & Trebst, A. (1979) *FEBS Lett. 108*, 136–140.

Schönfeld, M., & Schickler, H. (1989) *FEBS Lett. 243*, 218–222.

Schuldiner, S., Rottenberg, H., & Avron, M. (1972) *Eur. J. Biochem. 25*, 64–70.

Sigalat, C. (1988) Thesis, University of Paris Sud.

Sigalat, C., Haraux, F., de Kouchkovsky, F., Phung Nhu Hung, S., & de Kouchkovsky, Y. (1985) *Biochim. Biophys. Acta 809*, 403–413.

Sigalat, C., de Kouchkovsky, Y., Haraux, F., & de Kouchkovsky, F. (1988) *Biochim. Biophys. Acta 934*, 375–388.

Siggel, U. (1976) *Bioelectrochem. Bioenerg. 3*, 302–318.

Sorgato, M. C., Lippe, G., Seren, S., & Ferguson, S. J. (1985) *FEBS Lett. 181*, 323–327.

Stevens, C. L., Singhal, G. H., & Ash, A. B. (1967) *J. Org. Chem. 32*, 2895.

Strotmann, H., & Lohse, D. (1988) *FEBS Lett. 229*, 308–312.

Strotmann, H., Hesse, H., & Edelmann, K. (1973) *Biochim. Biophys. Acta 314*, 202–210.

Theg, S. M., & Homann, P. H. (1982) *Biochim. Biophys. Acta 679*, 221–234.

Tikhonov, A. N., Khomutov, G. B., Ruuge, E. K., & Blumenfeld, L. A. (1981) *Biochim. Biophys. Acta 637*, 321–333.

Van Dam, K., Wiechmann, A. H. C. A., Hellingwerf, K. J., Arents, J. C., & Westerhoff, H. V. (1978) in *Membrane Proteins* (Nicholls, P., Møller, J. V., Jørgensen, P. L., & Moody, A. J., Eds.) Vol. 45, pp 121–132, Pergamon Press, Oxford.

Westerhoff, H. V., Melandri, B. A., Venturoli, G., Azzone, G. F., & Kell, D. B. (1984) *Biochim. Biophys. Acta 768*, 257–292.

Williams, R. J. P. (1961) *J. Theor. Biol. 1*, 1–17.

Williams, R. J. P. (1978) *Biochim. Biophys. Acta 505*, 1–44.

Woelders, H., Van der Zande, W. J., Colen, A.-M. A. F., Wanders, R. J. A., & Van Dam, K. (1985) *FEBS Lett. 179*, 278–282.

Woelders, H., Putters, J., & Van Dam, K. (1986) *FEBS Lett. 204*, 17–21.

Woelders, H., Van der Velden, T., & Van Dam, K. (1988) *Biochim. Biophys. Acta 934*, 123–134.

Zoratti, M., & Petronilli, V. (1985) *FEBS Lett. 193*, 276–282.